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Thermogravimetric analysis of the total lipids extracted from the fatty tissue of fallow deer (*Cervus Dama dama* L)

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Abstract: Thermogravimetry, TG, of lipids is an appropriate analytical method, commonly used to correlate the kinetic parameters of the thermal degradation of lipids and their composition. Thus, samples of total lipids extracted from the raw fatty tissue of fallow deer were subjected to non-oxidative and oxidative TG analysis. The samples were previously stored for nine months at +4 °C and –18 °C. The material stability was investigated in order to establish the most favorable conditions for the production and storage of different foods. The total lipids were extracted according to the Folch method. The identification and quantitative analysis of the methyl esters of the fatty acids of the total lipids were performed by capillary gas chromatography. Based on the non-oxidative and oxidative TG results, the activation energies of the thermal degradation and oxidation of the total lipids extracted from different samples of fatty tissue were determined. The Doyle–Gorbachev method was used for the calculations.

Keywords: fallow deer, thermal analysis, oxidation of lipids, thermal degradation of lipids, fatty acid contents.

INTRODUCTION

It is noteworthy that the fat content of fallow deer meat is very low as recognized by many authors.[1–3] Fats can be taken in either as food, in their primary or variable form, or they may be produced biosynthetically in the body. Animal lipids are composed of triglycerides, cholesterol and its derivatives. Phospholipids, glycerides, monoglycerides, diglycerides and free fatty acids also contribute to the content of the total lipids, although to a smaller extent. The study of lipids has assumed considerable importance in recent years with the recognition that they are involed in many vital biological processes in animals. It is well known that lipids

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serve as a major form of energy storage and that they are responsible for maintaining the structural integrity of cells, being the principal components of membranes. The chemical contents of lipids depend on animal type and, within a type, on age, gender, fitness, nutrition and environmental conditions.[4]

It is important to know how and when the total lipids were oxidised. Lipids in biological systems are first oxidised and then degraded. Rancidity and toxic components occur as a result of such reactions. The oxidative degradation of lipids includes primary auto-oxidative reactions, followed by various oxidative and non-oxidative secondary reactions.[5]

It has recently become possible to estimate vegetable oil resistance to oxidation by thermogravimetric analysis (TGA), by measuring the weight gain due to oxygen caption of the oil sample during oxidation, and the initial and final oxidation temperatures. The experimental approach and analytical method developed in this study appear adequate for the same purpose. Compared to older techniques, this approach offers definite advantages.[6]

Thermogravimetric analysis is well established as a routine analytical tool for the study of the thermal behaviour of different materials. Bastić[7] studied the behaviour of lipids during thermal treatment and storage. Non-isothermal thermogravimetric analysis was applied to investigate the oxidation and evolution of volatile components of the intramuscular lipids of white meaty hogs. The kinetics of the oxidation of intramuscular lipids were determined on the basis of the data obtained.[8]

TG may also assist successfully in the quality evaluation of meat products. This applicability was confirmed by investigation of the total lipids samples of smoked Zlatibor bacon taken after different phases of treatment. The non-isothermal TG analysis of the total lipids was performed in nitrogen and air in a selected temperature range. The registered mass losses of the samples were quite similar in both atmospheres, indicating that Zlatibor bacon is a high quality product and, therefore, resistant to environmental effects during chemical treatment and maturity.[9]

The aim of this study was to investigate the thermal stability of the total lipids extracted from the raw fat tissue of both genders of fallow deer and stored at different temperatures by applying oxidative and non-oxidative TG analysis.

The observed TG effects could be better explained by utilizing the previously obtained results of the analytical determination (GC) of the fatty acid composition of the total lipids.

EXPERIMENTAL

Preparation of sample

The total lipids were extracted from samples of fatty tissue of fallow deer (*Cervus Dama dama* L.): five does and stags, labeled A and B, respectively. The game originated from Serbia and Montenegro (stags 2.5 and does 4–5 years old). During the processing of the meat, after cutting the warm halves and deboning, samples of fatty tissue were collected. All the samples were packed under vacuum in poly(vinyl choride) bags and stored at 2 ± 2 °C.

Extraction

The total lipids were extracted according to the Folch method.[10]

The hydrolysis of the total lipids was performed in sodium methanoate solution, while the separated fatty acids were extracted by diethyl ether. The fatty acids were converted to their respective methyl esters by reaction with diazomethane.

Gas chromatography

Gas chromatography was employed to qualitatively and quantitatively determine the fatty acid composition. All the analyses were performed on a Varian 3400 GC instrument with a DB-5, 30 m long, fused silica capillary column (4 °C min^{-1}, 150–300 °C).

Thermal analysis

The extracted lipids were packed into jars and kept at –18 °C and +4 °C for nine months. After this period, TG of the samples was performed under a constant gas flow rate (oxygen or nitrogen) of 25 cm^3 min^{-1} using a Perkin Elmer TGS-2 instrument at a heating rate of 2.5 °C min^{-1}. The initial sample masses ranged from 11–13.5 mg. The mass changes of the total lipids were monitored in the temperature interval 30–220 °C.

Kinetic analysis of the oxidation and degradation processes of total lipids

In order to investigate the non-oxidative thermal degradation of the total lipids, an analogy between oxidation and homogeneous chemical reactions in the gaseous and liquid phase was introduced. Thus, the oxidation rate may be presented by Equation (1):

$$r_R = k(T) \cdot \varphi(m) \tag{1}$$

where k is the rate constant of thermal degradation defined by the Arrhenius equation, while $\varphi(m)$ is a function dependent on the concentration of the component susceptible to thermal degradation.

The activation energies of thermal degradation and oxidation of the total lipids of the fatty tissue of male and female specimens of fallow deer, were determined on the basis of non-isothermal TG curves at different heating rates under dynamic nitrogen and oxygen atmospheres, by the Doyle–Gorbachev integral method.[11,12] The accuracy of this method depends on the scale of the activation energy investigation and the temperature range. Thus, homogeneous samples, lower sample weights and a relatively slow heating rate were selected to diminish the effects of the experimental conditions.

The kinetic parameters were calculated using Equation (2) according to the Doyle–Gorbachev method:

$$-\ln\left(-\frac{\ln(1-x)}{T^2}\right) = -\ln\left(\left(\frac{A}{q}\right)\cdot\left(\frac{R}{E}+2RT\right)\right)+\frac{E}{RT} \tag{2}$$

If the term $\frac{2RT}{E}$<<1, Equation (2) transforms into:

$$-\ln\left(\frac{-\ln(1-x)}{T^2}\right) = -\ln\left(\frac{A}{q}\right)\cdot\left(\frac{R}{E}\right)+\frac{E}{RT} \tag{3}$$

The value of the activation energy corresponds to the slope of the linear dependence $1/T$ *versus* $-\ln\left(\frac{-\ln(1-x)}{T^2}\right)$

RESULTS AND DISCUSSION

The results shown in Table I were obtained by gas chromatography of the total lipids of fatty tissue. Based on the corresponding standards, the presence of satu-

rated (C_{14} to C_{20}) and unsaturated ($C_{14}^{1=}$, $C_{16}^{1=}$, $C_{17}^{1=}$, $C_{18}^{1=}$ and $C_{18}^{2=}$) fatty acids was confirmed. It may be seen that the content of unsaturated fatty acids was greater in the samples of doe fat than in those of stag fat.

TABLE I. Fatty acid content of the total lipids of raw samples of fatty tissue of fallow deer (% of total peak area)

Fatty acids	Fatty tissue samples	
	A	B
$C_{14}^{1=}$, *cis*-9-Tetradecenoic	0.64	0.445
C_{14}^{0}, Tetradecanoic	4.58	2.92
C_{15}^{0}, Pentadecanoic	1.42	1.15
$C_{16}^{1=}$, *cis*-9-Hexadecenoic	6.06	3.52
C_{16}^{0}, Hexadecanoic	27.2	19.5
$C_{17}^{1=}$, *cis*-10-Heptadecenoic	1.16	0.77
C_{17}^{0}, Heptadecanoic	2.21	1.59
$C_{18}^{2=}$, *cis*-9, 12-Octadecadieanoic	0.45	1.01
$C_{18}^{1=}$, cis-9-Octadecedienoic	17.5	13.9
C_{18}^{0}, Octadecanoic	32.5	43.8
C_{19}^{0}, Nonadecanoic	0.28	0.35
$C_{20}^{4=}$, *cis*-5,8,11,14-Eikosatetraenoic	/	/
C_{20}^{0}, Eikosanoic	0.41	0.5

Hecker *et al.*[13] investigated the influence of animal age on the composition of the fatty tissue and established that the total unsaturation of the fatty acids increased. This increase, in their opinion, originated from the increased activity of desaturase. Leat and Embelton[14] stated that in the case of older cows, stearic acid was converted to palmitoleic and oleic acid. The effect of age on muscle fatty acid composition was an increasing trend in the absolute (mg/100 g) content of many fatty acids and an increase in the total lipid content of the muscle in older deer.[15]

Taking into consideration that the does were twice as old as the stags, the literature data, although referring to cow fatty tissue, were confirmed in this investigation.

The ratios of polyunsaturated to saturated fatty acids of the total lipids of the fatty tissue of fallow deer (Table II) were very small for both samples A and B. According to the low value of the polyunsaturated/saturated fatty acid ratio, PUS/S, a high stability of lipids during storage is to be expected. The aforementioned ratio could also be related to the small mass loss during thermal treatment in an inert atmosphere.

TABLE II. The ratios of saturated (S), mono-unsaturated (MUS) and polyunsaturated (PUS) fatty acids of the fatty tissue of fallow deer

Sample	MUS/S	PUS/S	PUS/MUS
A	0.360	0.010	0.024
B	0.260	0.014	0.053

The effects of fallow deer gender on the thermal stability of the total lipids are shown in Figs. 1 and 2. It can be concluded that the gender of fallow deer had a minor effect on the thermal stability of the total lipids extracted from the fatty tissue, regardless of the atmosphere (non-oxidative or oxidative) applied in the analysis.

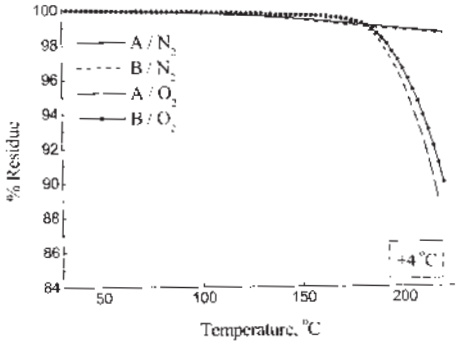

Fig. 1. TG curves of the total lipids of samples A and B, stored for nine months at + 4 °C (heating rate 2.5 °C/min, gas flow rate (N_2, O_2) 25 cm^3/min).

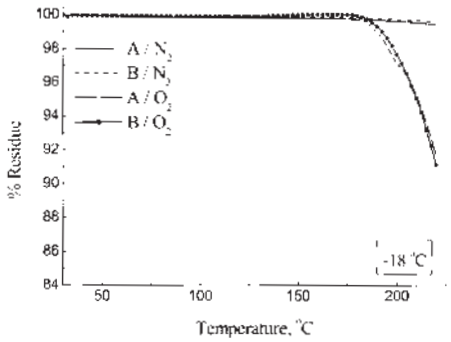

Fig. 2. TG curves of the total lipids of samples A and B, stored for nine months at –18 °C (heating 2.5 °C/min, gas flow rate (N_2, O_2) 25 cm^3/min).

The changes during non-oxidative TG analysis were insignificant as a consequence of the fatty acid composition (Table I), *i.e.,* a low content of polyunsaturated fatty acids.

The oxidative TG curves show a small increase in mass in the temperature range from 30 to 180 °C. Above 180 °C, which is a "critical" temperature, a sudden mass loss of up to 10 % was observed. Such a behaviour of the total lipids of the fatty tissue was probably caused by oxygen bonding to specific unsaturated bonds, resulting in peroxide formation even at temperature below 180 °C. At temperatures higher than 180 ºC, thermal degradation, which was accompanied by larger mass loss due to the decomposition of the formed peroxide bridges, commenced.

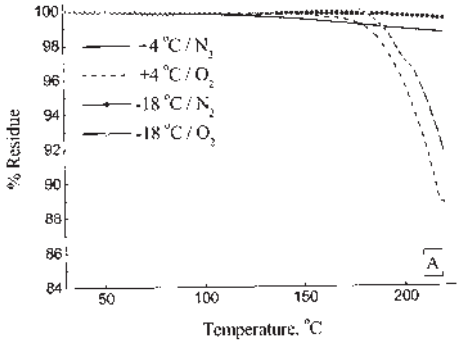

Fig. 3. TG curves of the total lipids stored for nine months at + 4 °C and –18 °C, sample A (heating rate 2.5 °C/min, gas flow rate (N_2, O_2) 25 cm^3/min).

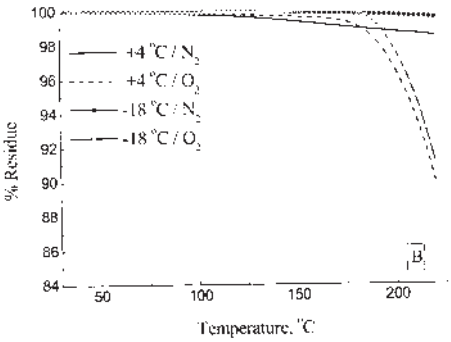

Fig. 4. TG curves of the total lipids stored for nine months at + 4 °C and –18 °C, sample B (heating rate 2.5 °C/min, gas flow rate (N_2, O_2) 25 cm^3/min).

The TG curves of the total lipids stored for nine months at +4 °C and –18 °C, are shown in Figs. 3 and 4 for sample A and B, respectively.

The non-oxidative TG curves indicate a greater stability of the lipids stored at –18 °C, regardless of the animal gender. Although the oxidative TG curves were quite different from the corresponding ones obtained by non-oxidative TG, the results of oxidative TG confirmed that the storage conditions influenced the thermal stability of the total lipids.

Determination of the activation energy of thermal degradation of the total lipids

The Doyle–Gorbachev method was employed to calculate the kinetic parameters of the non-oxidative and oxidative thermal degradation of the total lipids extracted from fatty tissue. The activation energies of non-oxidative and oxidative thermal degradation of the total lipids were determined in the mass loss range from 0.35 to 1.5 %, as shown in Table III. The activation energies of non-oxidative thermal degradation of the total lipids, E_a, ranged from 25 to 50 kJ/mol. These results agree well with literature data[9] of non-isothermal, non-oxidative TG analysis of the total lipids in smoked Zlatibor bacon. The activation energies, calculated according to the same integral method as used in this study, ranged from 35 to 48 kJ/mol, while the E_a values of the samples heated in air were slightly higher (39–50 kJ/mol).

TABLE III. The activation energies of the non-oxidative and oxidative thermal degradation of the total lipids

Sample	N_2		O_2	
	+4 °C	Deep freeze	+4 °C	Deep freeze
	E_a/kJ mol^{-1}	E_a/kJ mol^{-1}	E_{aII}/kJ mol^{-1}	E_{aII}/kJ mol^{-1}
A	50	38	126	190
B	34	25	130	168

E_{aII} – The activation energies of the second stage of oxidative TG curves (refer to the temperature range 180–220 °C)

The assumption that the Doyle–Gorbachev method could be applicable to the oxidative thermal degradation of total lipids within the entire investigated temperature range did not lead to the expected results. The oxidative TG curves indicated that the total lipids were very stable (mass loss 0.02–0.85 %) up to temperatures close to 180 °C. Rapid mass loss began in the temperature interval from 180 to 220 °C, reaching values of 7.8 to 11.3 %, depending on the sample type and storage conditions. Application of the Doyle–Gorbachev method was possible in this case if the changes which occurred during oxidative TG were divided into two stages. The first stage covered the interval from 30 to 180 °C and the second one from 180 to 220 °C. The activation energy of oxidative thermal degradation in the first stage was not calculated due to the very small mass loss. In the case of samples stored at –18 °C even a slight mass gain was recorded. When the Doyle–Gorbachev method

was applied in the second stage, extermely high activation energies were obtained. The E_a values, ranging from 130–190 kJ/mol, were 3–5 times higher in comparison to those found in the case on non-oxidative thermal degradation. Such large values of the activation energy of oxidative thermal degradation were attributed to the high starting degradation temperature (180 °C) of the total lipids.

Saičić[9] also studied the activation energy as a parameter which might indicate the susceptibility to thermal changes of a sample. The author claimed that the higher value of the calculated activation energy indicated a higher rate of mass loss with heating. Bastić[7] reported that higher temperatures (above 130 °C) promoted rapid thermal degradation and auto-oxidation of the total lipids, especially if the sample was heated in the presence of oxygen, when the sample was also susceptible to oxidation.

Hence, it may be concluded that the high values of the oxidative activation energy found in this study for the temperature range from 180 to 220 ºC indicate the high thermal susceptibility of the samples, namely, the thermal instability of the samples at high temperatures.

CONCLUSIONS

TG analysis (both non-oxidative or oxidative), on the basis of the presented results, can serve as a standard procedure for determining the influence of gender and storage conditions on the thermal stability of the total lipids of the fatty tissue of fallow deer (*Cervus Dama dama* L.). The results indicate that gender has a negligible influence on the thermal stability of the total lipids, as opposed to the storage conditions. Samples stored at –18 °C exhibited greater thermal stability in comparison to those stored at +4 °C. The TG effects can be explained utilizing the results of the fatty acid composition of the total lipids. The TG analysis of lipids offers the possibility of correlating the kinetic parameters of non-oxidative and oxidative thermal degradation (activation energy) and the fatty acid composition of the total lipids.

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ИЗВОД

ТЕРМОГРАВИМЕТРИЈСКА АНАЛИЗА УКУПНИХ ЛИПИДА ЕКСТРАХОВАНИХ ИЗ МАСНОГ ТКИВА ЈЕЛЕНА ЛОПАТАРА (*Cervus Dama dama* L.)

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Термогравиметријска (TG) анализа липида је погодна аналитичка метода на основу чијих података се могу корелисати кинетички параметри процеса термичке деграда-

ције липида са њиховим саставом. С обзиром на ову врсту примене, изведена је неоксидативна и оксидативна TG анализа на узорцима укупних липида екстрахованим из свежег масног ткива јелена лопатара. Узорци укупних липида су претходно чувани девет месеци на температури +4 °C и –18 °C. Овом методом се утврђује стабилност различитих материјала, што помаже да се утврде најповољнији услови производње и складиштења разних животних намирница. Претходно одређен масно-киселински састав укупних липида јелена лопатара (*Cervus Dama dama* L.) је допринео тумачењу ефеката уочених при TG. Укупни липиди су екстраховани методом по Фолху (Folch). Идентификација и квантитативна анализа метил-естара масних киселина укупних липида изведена је на капиларном гасном хроматографу. На основу резултата неоксидативне и оксидативне TG анализе, применом методе Дојл–Горбачова (Doyle–Gorbachev) одређена је енергија активације процеса термичке разградње и термичке оксидације укупних липида екстрахованих из различитих узорака масног ткива.

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