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TOTAL LIPIDS OF THE INTRAMUSCULAR TISSUE OF FALLOW DEER
Non-isothermal, non-oxidative and oxidative TG

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In this study non-isothermal non-oxidative and oxidative TG analysis was applied as a method for determining the thermal stability of the total lipids extracted from raw and maturing intramuscular tissue of fallow deer (Cervus Dama Dama L.). The total lipids were extracted from intramuscular tissue, according to the Folch method and stored for nine months at +4 and –18°C. The changes in the thermal stability of the total lipids during non-oxidative and oxidative TG analysis were correlated with the lipid composition, i.e. fatty acid composition. The Flynn–Wall method was used to determine the values of the activation energy of thermal degradation of the total lipids in a defined mass loss range.

Keywords: *fallow deer, fatty acid composition, intramuscular lipids, thermal degradation, thermogravimetry*

Introduction

It has been recognized that venison provides, in comparison with domestic ruminant meat, lower amounts of intramuscular fatty acids, the composition of which is typically richer in poly-unsaturated (PU) fatty acids and poorer in mono-unsaturated (MU) fatty acids and saturated (S) fatty acids [1–3]. The chemical composition of lipids of animal origin depends on the animal species and within the species it depends on age, gender, degree and type of nutrition, environmental [4]. The effect of animal diet or age on the fatty acid composition of fallow deer was investigated by Vollpeli *et al.* [5]. Many authors [6–8] have recognized that deer is a type of game whose meat is considered as a dietary food because of the high protein content and very low levels of fats and cholesterol. Thus, meat from game has become an important source of raw materials of animal origin.

Thermogravimetry (TG) is used as a standard procedure to control the quality of raw materials and final products of natural and synthetic origin. With TG analysis it is possible to estimate vegetable oil resistance to oxidation, measure the mass gain due to oxygen caption of the oil sample during oxidation, and the initial and final oxidation temperature [9]. TG has been used by Hassel [10] as an alternative method for measuring the stability of oil. Buzas and Kurucz [11] developed a simple and fast method, convenient for estimating the storage time, i.e. the oxidative stability and oxidative state of edible oils. Cross [12] also studied problems related to the oxidative stability of edible oils by applying TG. Bastić *et al*. [13] investigated the rate of oxidation and the evolution of volatile compounds of intramuscular lipids of white meaty hog by non-isothermal non-oxidative and oxidative TG analysis. The kinetics of the oxidation of intramuscular lipids were determined on the basis of the results obtained by this method and conclusions were made about their behavior during thermal treatment and storage. Saičić [14] investigated the total lipids of smoked Zlatibor bacon and confirmed that TG might be applied to evaluate the quality of meat products. TG analysis was applied to detect changes in the lipids of muscle and fatty tissue of different game tissues [15]. The authors concluded that the TG analysis of lipids is a suitable analytical method that enables the correlation of kinetic parameters of thermal degradation (activation energy) and lipid composition. There have also been investigations of the thermal stability of cholesterol and cholesteryl esters [16].

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 analysis of this type of product arises.

The purpose of this study was to present the application of non-oxidative and oxidative TG analysis as a method for determining changes that occur in the total lipids extracted from the intramuscular tissue of fallow deer (doe and deer) stored at different temperatures and during maturing under controlled conditions. The fatty acid composition of the total lipids of the intramuscular tissue of fallow deer was determined in order to better understand the TG effects.

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Experimental

Samples of intramuscular tissue (M. Semimembranosus) of fallow deer were used in all of the investigations. The game originated from Serbia and Montenegro, 'Karadjordjevo': five does 4–5 years old (sample A) and five deers 2.5 years old (sample B). During the processing of the meat, after cutting warm halves and deboning, the samples of muscle tissue were separated. All samples were vacuum-packed into PVC bags and kept at 2±2°C until analyzed. Part of the muscle tissue was subjected to ageing for 42 days in a dry and ventilated storeroom at 8°C. The total lipids were extracted from these samples (fresh and matured) and stored for nine months at +4 and –18°C.

The extraction of total lipids from fresh and matured samples of muscle tissue of fallow deer was performed according to the Folch method [17]. The hydrolysis method was developed by Desmoulin *et al.* [18].

Qualitative analysis of the fatty acid methyl esters was performed using a Varian 3400 gas chromatograph with a FID detector and DB-1 capillary column (30 m length, 0.25 mm inner diameter, 0.25 μm polydimethylsiloxane liquid phase film). Nitrogen was used as the carrier gas. The samples were analyzed in the temperature range 150–350°C at a heating rate of 4°C min^{-1}. The injector temperature was 250°C, while the temperature of the detector was 300°C. The following fatty acid standards were used for identification: saturated C_{10} to C_{24}, unsaturated $C_{(16:1)}$, $C_{(17:1)}$, $C_{(18:1)}$, $C_{(18:2)}$, $C_{(18:3)}$, $C_{(20:4)}$, $C_{(22:1)}$, $C_{(24:2)}$. Quantitative analysis of the fatty acid composition was performed using a Spectra-Physics System I integrator.

TG analysis of the total lipids was performed on a PerkinElmer TGS-2 instrument at heating rates of 2.5, 5 and 10°C min^{-1}. The lipid samples were analyzed in an inert gas atmosphere (nitrogen) and in an oxidative atmosphere (oxygen) at a gas flow rate of 25 cm^3 min^{-1}.

On the basis of the data obtained from non-isothermal TG curves at various heating rates, the Flynn–Wall method [19] was applied to determine the activation energies of thermal degradation and oxidation of the total lipids of the muscle tissue of Cervus Dama dama L.

Results and discussion

The influence of gender, storage conditions and maturing of the intramuscular tissue on the thermal stability of the total lipids of fallow deer was determined from the results of non-oxidative and oxidative TG. Analyses in nitrogen and oxygen were performed in order to compare the rate of mass loss of intramuscular tissue during non-isothermal TG analysis with and without oxidation. The formed peroxides decomposed at lower temperatures in all cases during oxidation and the products evaporated, probably as low molar mass compounds.

The influence of the gender and age (A and B) of fallow deer on the thermal stability of the total lipids

Figure 1 shows the non-oxidative and oxidative TG and differential (DTG) curves of the total lipids extracted from the intramuscular tissue of does (sample A) and deer (sample B), both fresh and matured, at +4 and –18°C, recorded at 2.5°C min^{-1}. The mass losses in the denoted temperature interval ranged from 10 to 15%. The raw and matured sample B exhibited a greater mass loss regardless of the storage conditions. The differences were more expressed in inert atmosphere. The DTG peaks of fresh A and B samples in inert atmosphere appear at about 150°C (Fig. 1a). For the same samples stored at –18°C, the peaks were shifted to lower temperatures, 100–125°C (Fig. 1c). The oxidative DTG curves, especially of matured samples, did not have distinctive peaks.

The influence of storage conditions (+4 and –18°C) on the thermal stability of the total lipids of fallow deer

The non-oxidative and oxidative TG and DTG curves of the total lipids extracted from the intramuscular tissue of fallow deer stored at +4 and –18°C for nine months are presented in Fig. 2. The samples stored at –18°C exhibited greater mass loss and greater thermal instability. This is not an indication of the poorer quality of lipid samples stored at –18°C compared to those stored at +4°C. The lipids stored at +4°C were more exposed to atmospheric oxygen, so oxidation probably took place during storage and thermally more stable compounds were formed. In all cases except A/matured (Fig. 2b), the mass loss in oxygen atmosphere was approximately the same for all the samples.

The influence of ageing (raw and matured) on the thermal stability of the total lipids of fallow deer

Figure 3 shows the non-oxidative and oxidative TG and DTG curves of the total lipids extracted from fresh and matured intramuscular tissue of Cervus Dama dama L at +4 and –18°C. The total lipids extracted from fresh intramuscular tissue, regardless of gender and storage conditions, exhibited greater thermal instability and higher mass losses.

As expected, the mass losses in oxidative atmosphere were generally higher. Two initial peaks usually appeared in the non-oxidative DTG curves, the first at about 50°C and the second in the range 95–140°C. These peaks decreased or disappeared in oxidative atmosphere, probably as a consequence of the combined degradation and oxidation reactions.

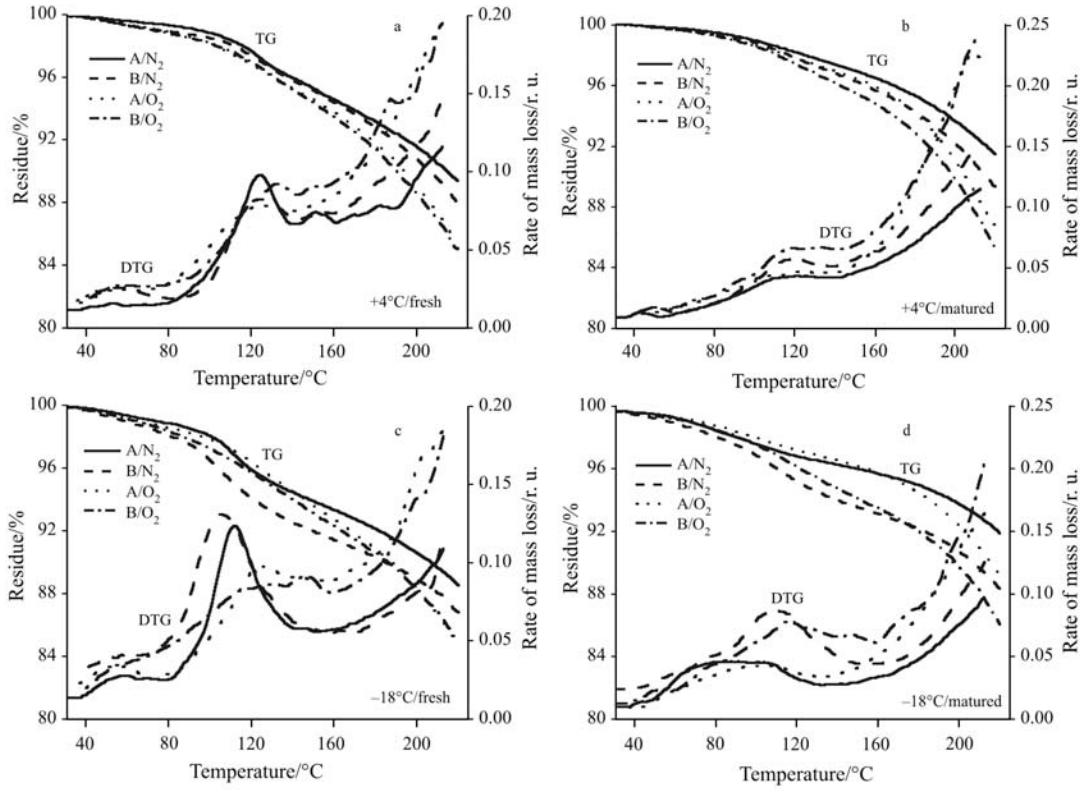

Fig. 1 The non-oxidative and oxidative TG and DTG curves of the total lipids extracted from the intramuscular tissue of fallow deer does (sample A) and deer (sample B) stored for nine months at: a – +4°C (fresh samples), b – +4°C (matured samples), c – –18°C (fresh samples) and d – –18°C (matured samples), heating rate 2.5°C min^{-1}, gas flow rate 25 cm^3 min^{-1}

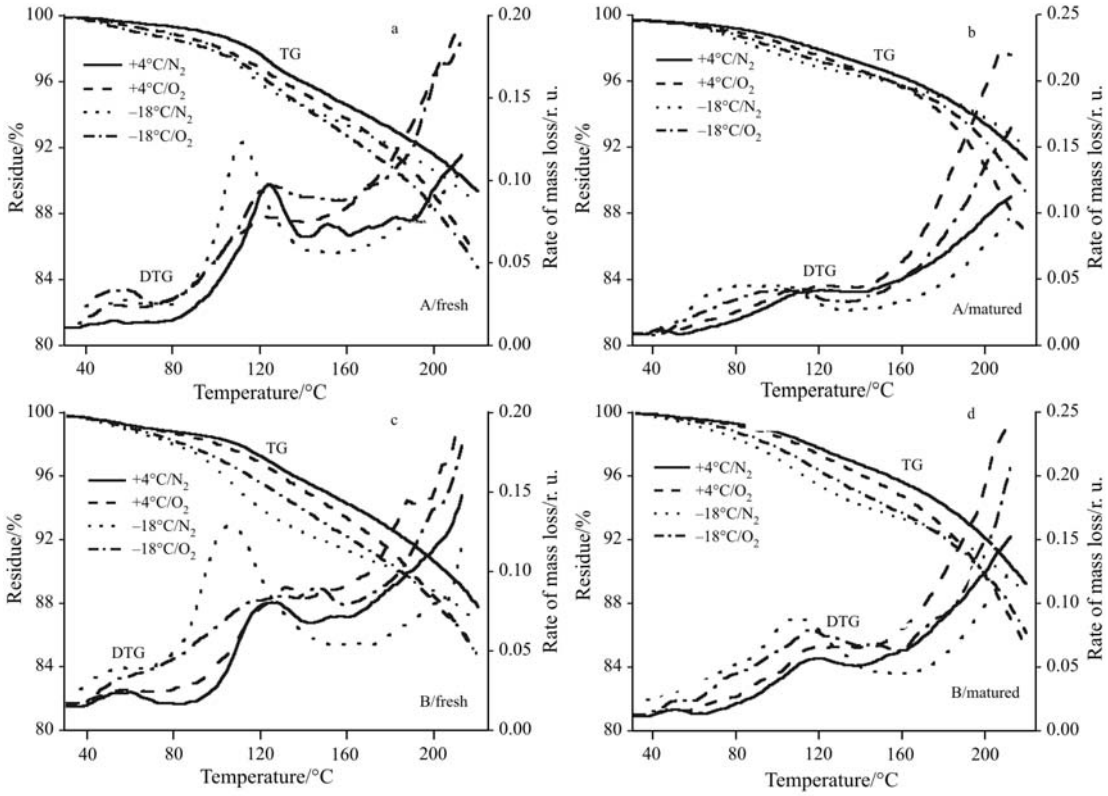

Fig. 2 The non-oxidative and oxidative TG and DTG curves of the total lipids extracted from the intramuscular tissue of fallow deer stored for nine months at +4 and –18°C: a – A (fresh samples), b – A (matured samples), c – B (fresh samples) and d – B (matured samples), heating rate 2.5°C min^{-1}, gas flow rate 25 cm^3 min^{-1}

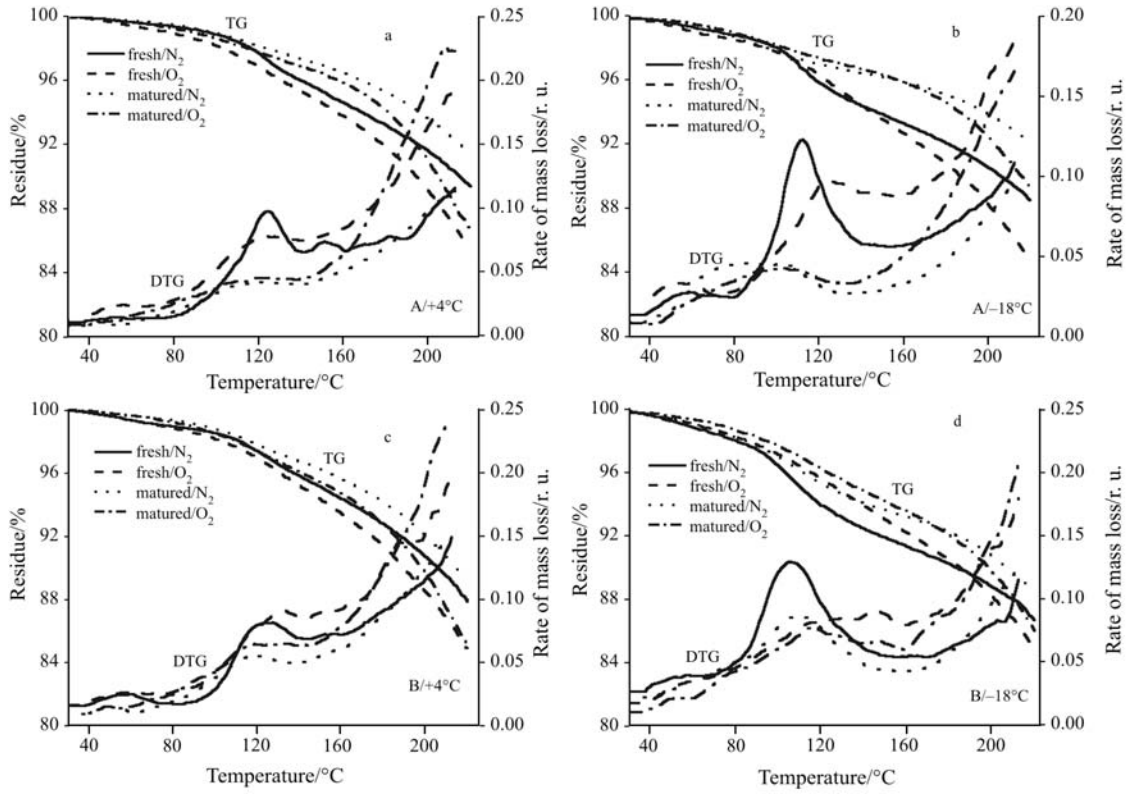

Fig. 3 The non-oxidative and oxidative TG and DTG curves of the total lipids extracted from the fresh and matured intramuscular tissue of fallow deer stored for nine months at: a – +4°C (A), b – –18°C (A), c – +4°C (B) and d – –18°C (B), heating rate 2.5°C min^{-1}, gas flow rate 25 cm^3 min^{-1}

Influence of the lipid composition on the TG curves

The results shown in Table 1 were obtained by GC analysis of the total lipids isolated from fresh and matured samples of the intramuscular tissue of fallow deer, A- does and B-deer. The composition of fatty acids is presented as the percent of the total peak area of the gas chromatogram.

Table 2 shows the ratios of mono-unsaturated to saturated (MU/S), poly-unsaturated to saturated (PU/S) and poly-unsaturated to mono-unsaturated (PU/MU) fatty acids of the total lipids of fresh and matured samples of intramuscular tissue of does (A) and deer (B).

The PU/S ratio for sample B is higher than for sample A, regardless of whether it is a fresh or matured (Table 2). This result is in accordance with the investigations of Santos *et al.* [20] that showed how commercial edible oils degraded in three steps, due to the decomposition of poly-unsaturated, mono-unsaturated and, finally, saturated fatty acids. TG showed that matured A and B samples were more stable than fresh ones. The ratio of poly-unsaturated to saturated fatty acids, as well as the ratio of mono-unsaturated to saturated fatty acids of aged A and B samples were lower than those of fresh samples. Maturing is a process during which the sample of intramuscular tissue is exposed to the influence of atmospheric oxygen. The content of unsaturated fatty acids decreases leading to the increased thermal stability of the total lipids extracted from matured A and B samples.

The composition of fatty acids of neutral lipids is also affected by the age of the animal. The effect of age on muscle fatty acid composition had an increasing trend in the absolute content of many fatty acids and an increase and in the total lipid content of the muscle in the older deer. The percentage composition of intramuscular fat showed an increase in the mono-unsaturated fatty acid content in older deer [5]. The age of the animal affected the conversion [21] established that in the case of older beef stearic acid transformed to palmitooleic and oleic acids.

The results of Palanska, Mojto and Ondrijačka [22] indicate that the total lipids of European stags contain more poly-unsaturated fatty acids than those of does, thus making them more susceptible to change.

E_a of the non-oxidative and oxidative thermal degradation of the total lipids of intramuscular tissue

The Flynn–Wall method [19] was applied to determine the activation energy of the non-oxidative and oxidative thermal degradation of the total lipids of fallow deer intramuscular tissue. This is a simple and rapid method for determining the activation energies. The mean values of the non-oxidative and oxidative

Table 1 Fatty acid composition of the total lipids isolated from the intramuscular tissue of fresh and matured samples of does (A) and deer (B) (% of the total GC peak area)

Fatty acids	Fresh samples		Matured samples	
	A	B	A	B
$C_{14}^{1=}$ *cis*-9-tetradecanoate	0.47	0.42	0.35	0.27
C_{14}^{0} tetradecanoate	3.42	2.68	4.58	4.84
C_{15}^{0} pentadecanoate	1.19	0.98	2.00	1.42
$C_{16}^{1=}$ *cis*-9-hexadecanoate	6.14	4.72	4.96	3.88
C_{16}^{0} hexadecanoate	28.4	26.3	29.2	25.2
$C_{17}^{1=}$ *cis*-10-heptadecanoate	1.66	1.54	1.42	1.21
C_{17}^{0} heptadecanoate	1.78	1.06	2.92	1.52
$C_{18}^{2=}$ *cis*-9,12-octadecanoate	10.3	15.0	8.33	13.2
$C_{18}^{1=}$ *cis*-9-octadecanoate	25.5	23.0	23.8	22.2
C_{18}^{0} octadecanoate	18.7	20.4	21.3	24.0
C_{19}^{0} nonadecanoate	/	/	/	/
$C_{20}^{4=}$ *cis*-5,8,11,14-eicosatetranoate	2.44	3.81	1.07	2.23
C_{20}^{0} eicosanoate	/	/	/	/

/ – undetected

Table 2 Ratio of saturated (S), mono-unsaturated (MU) and poly-unsaturated (PU) fatty acids of the fresh and matured intramuscular tissue of doe (A) and deer (B)

Sample	MU/S	PU/S	PU/MU
A, fresh	0.63	0.24	0.38
B, fresh	0.61	0.39	0.64
A, matured	0.51	0.16	0.31
B, matured	0.48	0.27	0.56

Table 3 Activation energy of non-oxidative and oxidative thermal degradation of the total lipids extracted from intramuscular tissue, calculated according to the Flynn–Wall method

Sample	E_a/kJ mol^{-1}	
	N_2	O_2
A/+4°C/fresh	–	55±7.1
B/+4°C/fresh	62±9.5	50±1.2
A/–18°C/fresh	–	28±1.5
B/–18°C/fresh	35±6.4	39±5.6
A/+4°C/matured	79±1.6	50±4.4
B/+4°C/matured	59±5.5	52±4.7
A/–18°C/matured	78±2.7	51±5.3
B/–18°C/matured	65±2.1	59±6.1

thermal degradation energies, $<E_a>$, are presented in Table 3. Bastić [23] applied TG data to derive the activation energies of the oxidation of intramuscular lipids of white hogs. A mean value of 61 kJ mol^{-1} was obtained when TG was performed in pure oxygen, which is the same order of magnitude as the $<E_a>$ values of the oxidative thermal degradation of the total lipids of the intramuscular tissue of fallow deer.

Figure 4 shows the dependence of the non-oxidative thermal degradation activation energy on the

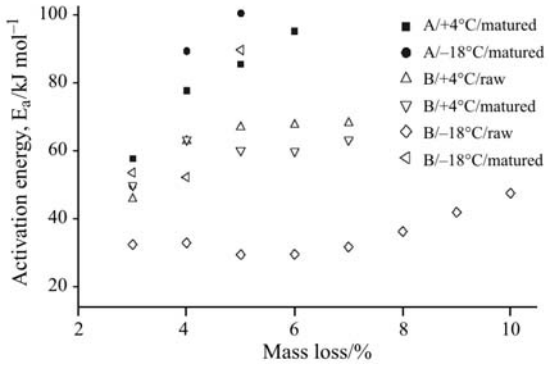

Fig. 4 Dependence of the non-oxidative thermal degradation activation energy on the mass loss of the total lipids extracted from fresh and matured A and B samples stored at +4 and –18°C

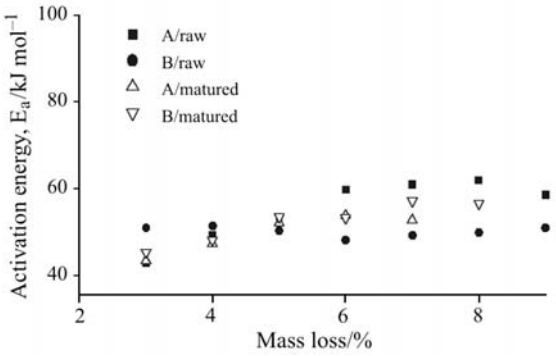

Fig. 5 Dependence of the oxidative thermal degradation activation energy on the mass loss of the total lipids extracted from fresh and matured A and B samples stored at +4°C

mass loss of the total lipids. Figures 5 and 6 show the oxidative thermal degradation activation energies stored at various temperatures as a function of the mass loss of the total lipids. The values of E_a of sam-

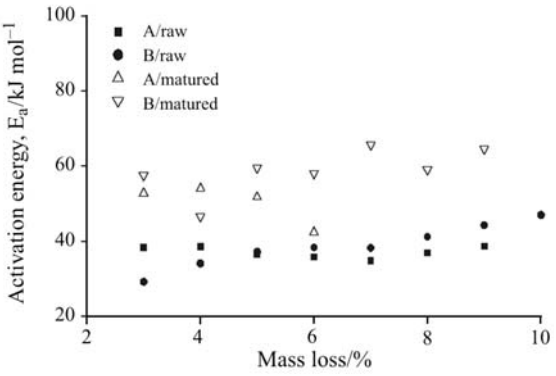

Fig. 6 Dependence of the oxidative thermal degradation activation energy on the mass loss of the total lipids extracted from fresh and matured A and B samples stored at –18°C

ples stored at +4°C are very similar. The E_a values of matured samples stored at –18°C are higher than those of fresh samples. These results clearly indicate that only the fresh samples stored at –18°C were not oxidized during storage.

Conclusions

TG analysis was used to determine the influence of gender, age, storage conditions and maturing on the thermal stability of the total lipids of intramuscular tissue of fallow deer. All of these factors affected the ratio of the poly-unsaturated to saturated and mono-unsaturated to saturated fatty acids of the total lipids of the intramuscular tissue. Even small changes in the PU/S and MU/S ratios caused changes in the thermal stability. As these ratios decreased in a sample, its thermal stability increased. Only fresh lipid samples stored at –18°C did not oxidize during storage, i.e. they maintained their PU/S and MU/S ratios, which, consequently, made them thermally unstable. As the differences in the PU/S and MU/S ratios arising from the investigated factors were small, it may be concluded that TG is an appropriate method for detecting fine structural changes in lipids.

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