

Evaluation of lipid composition and fatty acid content of minced beef*

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Abstract: Meat and meat products quality depends on content and composition of the main meat components, proteins, lipids, minerals and water.

In this paper we investigated the kinetics of lipid extraction and lipids and fatty acid composition of minced beef from Leskovac region. Lipids fractions were determined by HPLC. Free fatty acids content in the tested samples ranged from 8.7% to 52.6%, monoacylglycerols ranged from 0.4 to 2.6%, and diacylglycerols from 0.7 to 3.0%, while the content of triacylglycerols was the highest and ranged from 36.9% to 89.6%. The content of fatty acids in acylglycerols was determined by GC. Oleic, palmitic and stearic fatty acid were present in the highest content and ranged from 37.1% to 41.8%, 23.5 to 30.4% and 15.7% to 19.0%, respectively. Some quantities of myristic, pentadecanoic, palmitoleic, margaric, linoleic and phtalic acid were detected in investigated samples too. Based on statistic analysis, samples were classified in two groups, one with high oleic acid content, associated with low palmitoleic acid content and the second one with high stearic acid content associated with low palmitic acid content. Based on *t*-test, content of oleic, stearic and linoleic acid of $39.59\% \pm 1.86\%$, $17.69\% \pm 1.28\%$ and $2.33\% \pm 1.03\%$, respectively, can be used for evaluating the lipid composition of the minced beef.

Key words: minced beef, lipid composition, HPLC, acylglycerols, fatty acids, GC.

Introduction

Deposits of fat in meat are adipose and intramuscular fatty tissue. Lipids in adipose tissue consist, primarily, of triacylglycerols, while in intramuscular tissues of both, triacylglycerols and membrane-bonded fats, such as phospholipids and lipoproteins. Fatty acids associated with these tissues are saturated or unsaturated (Pegg and Shaidi, 2005). Fat is the most variable component of meat and its content varies more than amino acids content. Fat content in meat depends on seasonal variations (Shirai et al., 2002), animal species, diet and meat storage conditions (Melton, 1990; Fennema, 1996) as well as breeding system, weaning and sex (Cividini et al., 2008). Lipids, or more precisely their fatty acid composition, contribute in a wide range to meat quality (Wood et al., 2004) influence on color stability, drip loss and development of oxidative rancidity (Enser et al., 1996a; Enser et al., 1996b;

Enser et al., 1998). The lipid thermal reactions of degradation (Mottram, 1994) and polyunsaturated fatty acids oxidation reactions (Elmore et al., 1999; Elmore et al., 2000), besides the Maillard reaction which occurs between amino acids and sugars, are the main source of volatiles in cooked meat. After oxidation of unsaturated fatty acids, the meat quality (flavor, color, nutritive value, protein functionality etc.) is changed (Pegg and Shaidi, 2005). Hornstein et al. (2006) extracted lipids from beef and pork muscle and fractionated them into triacylglycerols, cephalins and mixture of lecithin and sphingomyelins.

The aim of this paper was to investigate kinetics of lipid extraction, lipid and fatty acid composition of minced beef from region of Leskovac, as well as to determine the efficiency of lipid extraction and the time of total lipid extraction. Chemical composition of fat was given as content of free fatty acids (FFA), monoacylglycerols (MAG), diacylglycerols

***Note:** This work was supported by the project „Development of the formulations and technologies for pharmaceutical products and cosmetics based on liposomes, microspheres and inclusion complexes“ No 19048 of the Ministry of Science and Technological Development of Republic of Serbia.

*This abstract has been published in the Book of Abstracts from the International 55th Meat Industry Conference held on Tara mountain, 15–17th June 2009.

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(DAG) and triacylglycerols (TAG) and as free fatty acids composition. In order to evaluate the investigated samples and to determinate the correlation between lipid parameters and differences between means of samples' fatty acid content, the statistic analysis by determining the Euclidean linkage distances, correlation matrices and t-test was performed.

Material and method

Material

Samples (S1-S5) of minced beef, from Leskovac region were purchased in local stores.

Chemicals used for extractions were of high quality (carbon tetrachloride, methanol, potassium hydroxide, chloroform and sodium sulfate) and were purchased from Centrohem, Serbia. Chemicals for HPLC and GC analyses were analytical grade (methanol, 2-propanol, and *n*-hexane) and were purchased from Riedel-de Haën, Honeywell Specialty Chemicals, Germany.

Methods

a) The sample of minced meat (50 g) was put into Erlenmeyer flask, 500 ml of carbon tetrachloride was added and extracted during 30 minutes, under reflux, by mixing (200 rot. min⁻¹), at solvent boiling temperature. The extract was separated by Buchner funnel under weak vacuum. The residue of meat was extracted three more times by the same procedure. The extracts were pooled and rinsed with water in the separation funnel (3 x 10 ml H₂O). The volume of the extracts was recorded and an aliquot of 3 ml was taken for dry residue determination test and meat lipid content was calculated. The residue of lipid extract, after dry residue determination test, was evaporated under vacuum and the obtained lipid residue was used for HPLC and GC analyses.

b) Lipid extract (3 ml) was put into the disk plate analyzer (Scaltec SMO 01, Scaltec instruments, Germany) and dried at 110°C to a constant weight. The content of dry residue was read out on the analyzer display. The dry residue content determination test was performed in triplicate.

c) The samples of minced meat (5 g) were put into Erlenmeyer flask. 50 ml of carbon tetrachloride was added and sample was extracted during 10, 30, 45, 60 or 90 minutes, under reflux, by mixing (200 rot. min⁻¹) at solvent boiling temperature. For each extraction time a separate meat sample was used. The extract was separated by using Buchner funnel under weak vacuum. Dry residue content determination test was performed in triplicate and lipid content for each extraction time was calculated.

HPLC analysis

For HPLC analysis, Holčapek *et al.* (1999) modified HPLC method was used. The equipment consisted of Agilent 1100 High Performance Liquid Chromatograph, equipped with a degasser, a binary pump, a Zorbax Eclipse XDB-C18 column (4.4 mm x 150 mm x 5 µm) and a UV/Vis detector. The flow rate of binary solvent mixture (methanol, solvent A, and 2-propanol/*n*-hexane, 5:4 v/v, solvent B) was 1 ml/min, with a linear gradient from 100% A to 40% A+ 60% B in 15 min. Column temperature was held constant, at 40°C. The lipid components were detected at 205 nm. The MAG, DAG and TAG were identified by comparing retention times of lipid components with retention times of standards. Samples were dissolved into a mixture of 2-propanol: *n*-hexane (5:4 v/v) and filtered through 0.45 µm Millipore filters. All measurements were performed in triplicate.

GC analysis

For GC analysis, methyl-esters were prepared as follows: approximately 3 g of lipids were dissolved in 50 ml of anhydrous methanol, with slight stirring, and 1 ml of 1M KOH was added. The content was heated under reflux, by mixing (200 rot. min⁻¹), during 10 minutes. Then, 30 ml of water was added and the content was transferred into a separation funnel. After cooling, esters were extracted with 30 ml chloroform and chloroform extract (lower layer) was separated. The extraction of esters with 20 ml of chloroform was repeated two more times. The pooled chloroform extracts were washed with water, dried with anhydrous sodium sulfate and evaporated to dryness under vacuum.

For GC analysis, the HP 5890 Series II Gas Chromatograph, HP with FID detector, and integrator HP 3396 A was used. Column was ULTRA 2 (25m x 0.32 mm x 0.52 µm), injector temperature 320°C, and injected volume was 0.4 µl. The carrier gas was helium at a constant flow rate of 1 ml/min. Temperature flame ionization detector was set at 350°C and split ratio was 1:20. Oven temperature was initially set at 120°C, with hold time at 120°C of 1 min, then increased by 15°C/min to 200°C, increased by 3°C/min to 240°C, increased by 8°C/min to 300°C and hold at 300°C of 15 min. Fatty acids were identified by comparing retention times of the obtained peaks with the retention times fatty acid peaks in standards. GC analysis of the same lipid sample was performed in triplicate.

Statistical analysis

The mean, standard deviation, Euclidean distances (clustering method with single linkage) and

the correlation matrices were determined by program STATISTICA, version 5.0. Obtained data were tested by a single factor ANOVA, and the differences between means were determined by two samples assuming equal variances t-test, using EXCEL software. Significance of differences was defined at $p < 0.05$.

Results and discussion

The obtained results of the kinetics of lipids extraction for minced beef are presented in Figure 1 and Table 1. The results we obtained show that over 90% of the lipids from all samples were extracted

after 30 minutes. The efficiency of extraction (EE) was expressed as ratio of lipid content extracted from the sample after 30 minutes and lipid content in the sample. The lipids content in investigated samples had a wide range, from 5.06 (S1) to 21.02% (S4).

Table 1 shows the HPLC results of FFA, MAG, DAG and TAG content in five samples of baby beef meat.

Figure 2 and Table 1 present HPLC profile of lipids in the tested samples. The content of lipid fractions was determined by measuring peak area at 1.76 min for FFA; peaks area in the range of 3.44-4.58 min, for MAG; peaks area in the range of 5.28-8.68 min, for DAG and peaks area in the range of 10.91-15.81 min, for TAG (Holčapek, 1999). The

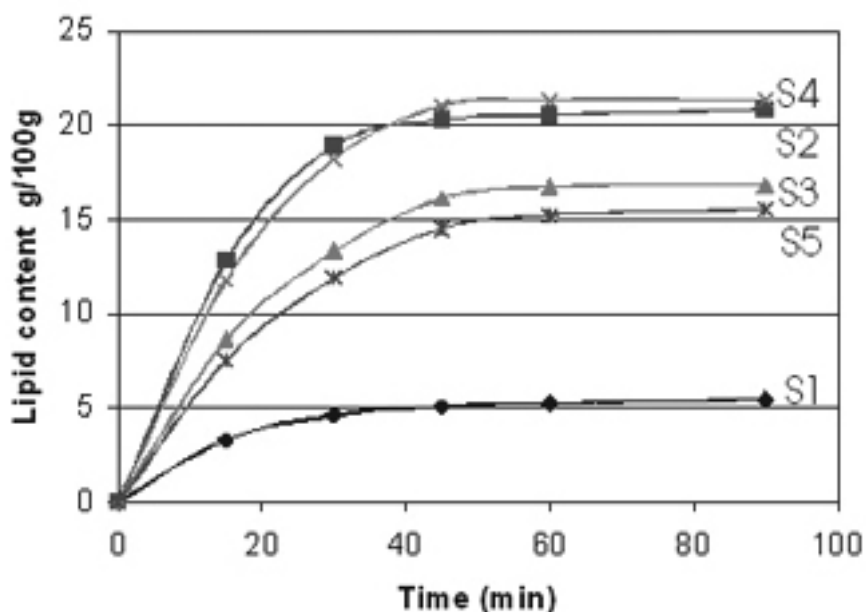


Figure 1. Kinetics of lipids extraction from five samples (S1–S5) of minced meat
Slika 1. Kinetika ekstrakcije lipida iz pet uzoraka (S1–S5) mlevenog junečeg mesa

Legenda/Legend:

Lipid content g/100g/Sadržaj masti g/100g;
Time (min)/Vreme (min)

Table 1. The lipids content (LC), efficiency of extraction (EE) and HPLC results for lipids in five samples of minced meat (S1–S5)

Tabela 1. Sadržaj lipida (LC), efikasnost ekstrakcije (EE) i rezultati HPLC analize lipida iz pet uzoraka mlevenog junečeg mesa (S1–S5)

Sample	LC (%)	EE (%)	FFA (%)	MAG (%)	DAG (%)	TAG (%)
S1	5.06 ± 0.8*	96.5 ± 1.9	40.2 ± 2.2	2.6 ± 0.1	2.0 ± 0.9	55.1 ± 3.4
S2	20.28 ± 2.4	94.7 ± 1.8	52.6 ± 1.9	1.3 ± 0.1	1.6 ± 1.3	64.4 ± 3.1
S3	16.33 ± 1.0	95.7 ± 1.0	61.1 ± 1.7	1.2 ± 0.2	0.8 ± 0.7	36.9 ± 2.7
S4	21.02 ± 1.6	94.9 ± 1.2	8.7 ± 0.4	1.0 ± 0.3	0.7 ± 0.4	89.6 ± 3.8
S5	15.09 ± 1.1	96.2 ± 1.3	18.9 ± 0.6	0.4 ± 0.2	3.0 ± 1.1	77.7 ± 2.8

*mean value followed by standard deviation/srednja vrednost i standardna devijacija

content of FFA is in a very wide range, from 8.7%, in sample 4, up to 61.1%, in sample 3. The content of MAG and DAG in all tested samples is low and the reason for that might be the freshness of meat samples. MAG content is in the range from 0.4%, in sample 5, to 2.6%, in sample 1. DAG content is in range of 0.7% in sample 4 to 3.0% in sample 5. The content of TGA is the highest and it is in range from 36.9%, in sample 3, to 89.6%, in sample 4.

($C_{18:2}$), stearic ($C_{18:0}$) and phtalic (1,2 benzene-dicarboxylic) acid ($C_6H_4(COOH)_2$). Samples 3 and 4, besides these fatty acids, contained 0.82 % and 0.51%, of cholesterol respectively.

The oleic acid was detected in the highest content, which ranged from 37.1 % to 41.8%. It is followed by the content of palmitic acid, ranging from 23.5% to 30.4%, than the content of stearic acid, in the range from 15.7% to 19.0%. The content

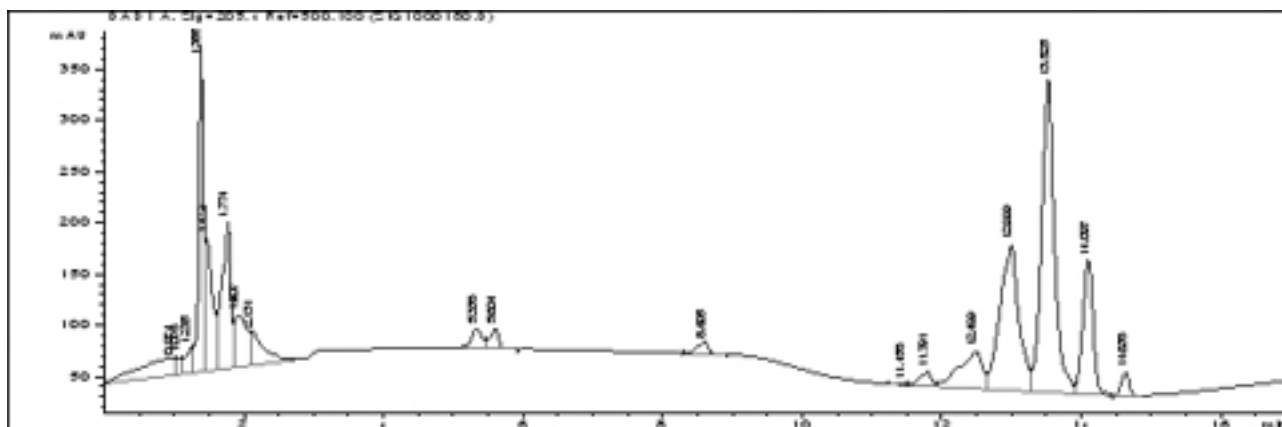


Figure 2. The HPLC profile of lipids in minced beef, sample 5

Slika 2. HPLC profil lipida u junećem mesu, uzorak 5

Results of GC analysis of fatty acid content of lipids in five samples of minced beef are presented in Table 2. Figure 3 shows is GC chromatogram of lipids from sample 1.

of other fatty acids in all samples was less than 5%. Based on these fatty acids content, the content of total saturated fatty acid (TS) in samples 1, 2, 3, 4 and 5 was 50.79%, 50.04%, 48.54%, 46.95% and

Table 2. Results of GC analysis of lipids obtained for five samples of minced beef (S1–S5), (%)

Tabela 2. Rezultati GC analize lipida u pet uzoraka junećeg mesa (S1–S5), (%)

Fatty acids	RT (min)	Sample/(%)				
		S1	S2	S3	S4	S5
Myristic acid ($C_{14:0}$)	12.83	4.75 ± 0.24*	2.40 ± 0.23	2.34 ± 0.39	2.74 ± 0.31	2.26 ± 0.28
Pentadecanoic acid ($C_{15:0}$)	14.29	0.74 ± 0.22	0.37 ± 0.04	0.33 ± 0.05	0.38 ± 0.07	0.13 ± 0.06
Palmitoleic acid ($C_{16:1}$)	15.48	4.17 ± 0.21	3.72 ± 0.25	3.13 ± 0.23	3.93 ± 0.63	2.88 ± 0.32
Palmitic acid ($C_{16:0}$)	15.86	23.46 ± 2.65	30.44 ± 1.37	26.00 ± 1.06	25.53 ± 2.04	25.61 ± 2.54
Margaric acid ($C_{17:0}$)	16.61	2.84 ± 0.31	1.11 ± 0.17	1.57 ± 0.23	1.10 ± 0.11	0.62 ± 0.07
Linoleic acid ($C_{18:2}$)	18.06	1.41 ± 0.11	1.69 ± 0.15	2.58 ± 0.13	1.97 ± 0.39	4.00 ± 0.43
Oleic acid ($C_{18:1}$)	18.21	37.07 ± 2.38	38.85 ± 3.37	41.81 ± 1.57	39.25 ± 1.85	40.99 ± 1.99
Stearic acid ($C_{18:0}$)	18.36	19.00 ± 1.95	15.72 ± 1.63	18.30 ± 0.81	17.21 ± 1.75	18.25 ± 0.83
Phtalic acid ($C_{14:0}$) (1,2 benzene-dicarboxylic acid)	18.55	0.06 ± 0.02	1.01 ± 0.18	0.26 ± 0.07	0.17 ± 0.03	1.20 ± 0.19
Cholesterol	23.47	–	–	0.82 ± 0.19	0.51 ± 0.12	–
TS (total saturated)		50.79 ± 2.65	50.04 ± 1.37	48.54 ± 1.06	46.95 ± 2.04	46.87 ± 2.54
TUS (total unsaturated)		42.65 ± 2.38	44.26 ± 3.37	44.52 ± 1.57	45.15 ± 1.85	45.12 ± 1.99

*mean value followed by standard deviation/ srednja vrednost i standardna devijacija

Lipids from all samples contained myristic ($C_{14:0}$), pentadecanoic ($C_{15:0}$), palmitoleic ($C_{16:1}$), palmitic ($C_{16:0}$), margaric ($C_{17:0}$), oleic ($C_{18:1}$), linoleic

46.87%. The content of total unsaturated fatty acids (TUS) was 42.65%, 44.26%, 44.52%, 45.15% and 45.12%, respectively. As the content of saturated

and unsaturated fatty acids is similar, it indicates that there is a balance between these groups of fatty acids. The content of saturated fatty acid was in the range from 46% to 51%, and the content of unsaturated fatty acids was in the range of 42% to 45%.

The dendrogram shows that lipids from sample 4 and sample 5 are joined at the shortest distance level of 17.0 and make the first meat group. The lipids from sample 1 and sample 3 were joined at the distance level of 18.0. On a very close distance level

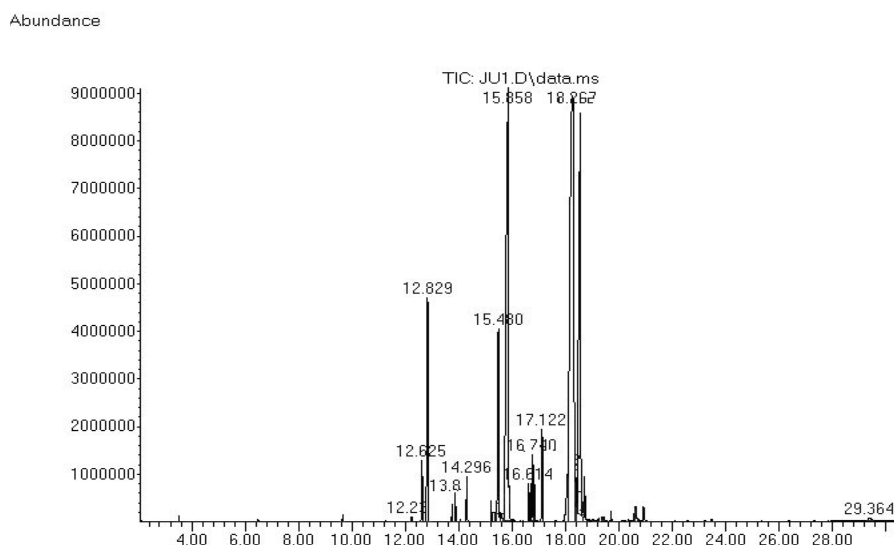


Figure 3. GC chromatogram of minced beef fatty acids pattern, sample 1
Slika 3. GC hromatogram profila masnih kiselina u mlevenom junećem mesu, uzorak 1

By cluster analysis, based on multiple variables, beef meat lipids of five samples were classified. Number of variables was five (five samples) and number of cases was eight: LC, FFA, TAG, palmitoleic, palmitic, linoleic, oleic and stearic acid content. Euclidean linkage distances were obtained and presented by dendrogram in Figure 4.

of 18.1 to these samples was sample 2, so they make the second meat group. Thus, based on similarity of set eight parameters, the investigated samples are different but classified only into two groups.

The correlation coefficients between fatty acids in acylglycerol lipid fraction of beef are presented in Table 3. The sample size was five (N=5, samples S1-S5). Only correlations above the absolute value of 0.8 are taken into consideration. Two correlations were determined and they were opposite: high oleic acid content, is associated with low palmitoleic acid content, while high stearic acid content goes with low palmitic acid content.

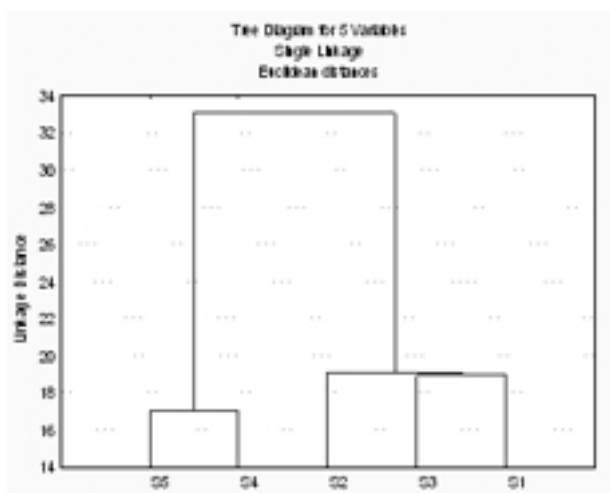


Figure 4. Dendrogram for lipids from five samples of minced beef meat (S1-S5)
Slika 4. Dendrogram lipida iz pet uzoraka mlevenog junećeg mesa (S1-S5)

Table 3. Correlation matrix for fatty acids acylglycerol lipid fraction of beef (N = 5)
Tabela 3. Korelaciona matrica za masne kiseline u frakciji lipida (acigliceroli)

	LC	TG	Palmitoleic acid	Palmitic acid	Oleic acid	Stearic acid
TG	0.40					
Palmitoleic	-0.26	0.14				
Palmitic	0.69	0.29	-0.13			
Oleic	0.47	-0.17	-0.91	0.14		
Stearic	-0.78	-0.32	-0.12	-0.92	0.46	
Linoleic	0.48	0.16	-0.72	0.66	0.51	-0.46

According to ANOVA results, the variances between groups were 13.03, 0.58, 3.54, 78.95, 8.62, 12.71, 41.71, 19.54 and 3.31 for myristic, pentadecanoic, palmitoleic, palmitic, margaric, linoleic, oleic, stearic and phtalic acid, versus variances within groups of 0.88, 0.58, 1.32, 41.19, 0.39, 0.49, 52.77, 21.72 and 0.16, respectively.

Results of t-test for means of all investigated fatty acids are shown in Table 4, where the same letter within a row indicates the means which do not significantly differ, i.e. observed t-value was less than t-critical and null hypothesis was accepted.

The highest number of means which do not significantly differ among samples of 20 is for oleic and stearic acid. For linoleic acid this number was 18. So, the five samples of beef meat for these fatty acids were from the same population and their content can be used for evaluating the lipid composition of minced beef samples. The mean values for oleic, stearic and linoleic acid content, based on means of five samples, are $39.59\% \pm 1.86\%$, $17.69\% \pm 1.28\%$ and $2.33\% \pm 1.03\%$, respectively.

Table 4. Results of t-test with hypothesis assuming equal variances for fatty acids in five samples of minced beef (S1–S5)

Tabela 4. Rezultati t-testa sa hipotezom o jednakosti varijanse za masne kiseline iz pet uzoraka junećeg mesa (S1–S5)

Components	Sample					Number of t-test with $t < t$ critical
	S1	S2	S3	S4	S5	
Myristic acid (C _{14:0})	–	abc*	ade	bdf	cef	12
Pentadecanoic acid (C _{5:0})	–	a	ade	bdf	cef	10
Palmitoleic acid (C _{16:1})	ab	ac	de	bcd	ef	11
Palmitic acid (C _{16:0})	abcd	a	bef	ceg	dfg	14
Margaric acid (C _{17:0})	–	ab	a	b	–	4
Linoleic acid (C _{18:2})	abcd	aefg	beh	cfi	dghi	18
Oleic acid (C _{18:1})	abcd	aefg	beh	cfhj	dgij	20
Stearic acid (C _{18:0})	abcd	aefg	beh	cfhj	dgij	20
Phtalic acid (C _{14:0})	abcd	ae	bfg	cfh	degh	16

* the same letter within a row indicates the means among samples which do not significantly different/isto slovo u nizu ukazuje na srednje vrednosti koje se značajno ne razlikuju

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Karakterizacija lipidnog sastava i sadržaja masnih kiselina u mlevenom junećem mesu

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R e z i m e: Kvalitet mesa i proizvoda od mesa zavisi od sadržaja i sastava glavnih komponenti mesa kao što su proteini, lipidi, mineralne materije i voda.

U ovom radu ispitana je kinetika ekstrakcije lipida kao i sastav lipida i masnih kiselina u mlevenom junećem mesu sa teritorije Leskovca. Lipidne frakcije su određivane HPLC tehnikom. Sadržaj slobodnih masnih kiselina u ispitivanim uzorcima se kretao od 8,7 posto do 52,6 posto, sadržaj monoacilglicerola od 0,4 posto do 2,6 posto, sadržaj diacilglicerola od 0,7 posto do 3 posto, dok je sadržaj triacilglicerola bio najviši, od 36,9 posto do 89,6 posto. Sadržaj masnih kiselina u acilglicerolima je određivan gasnom hromatografijom. Najzastupljenije su oleinska, palmitinska i stearinska kiselina čiji je sadržaj varirao od 37,1 posto do 41,8 posto, od 23,5 posto do 30,4 posto i od 15,7 posto do 19,0 posto respektivno. Takođe su utvrđene male količine miristinske, pentadekanoične, palmitoleinske, margarinske, linolne i ftalne kiseline. Na osnovu statističke analize, uzorci su klasifikovani u dve grupe – grupu sa visokim sadržajem oleinske kiseline i niskim sadržajem palmitoleinske kiseline, i grupu sa visokim sadržajem stearinske kiseline i niskim sadržajem palmitinske kiseline. Na osnovu t-testa, sadržaj oleinske, stearinske i linolne kiseline od $39,59\% \pm 1,86\%$, $17,69\% \pm 1,28\%$ i $2,33\% \pm 1,03\%$, respektivno, može da se uzme u obzir za karakterizaciju lipidnog sastava uzoraka mlevenog junećeg mesa.

Ključne reči: juneće meso, lipidi, acilgliceroli, masne kiseline.

Paper received: 11.05.2009.

Paper revised: 31.07.2009.

Paper accepted: 18.08.2009.