THE COMPOSITION OF HOG INTRAMUSCULAR LIPIDS

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INTRODUCTION

Great efforts have been made lately to investigate and identify components that influence the development of meat flavour /1,2/. The flavour of meat and meat products are one of the most important quality attributes, thus the significance of studying the chemical nature of substances participating in the formation of flavour, as well as the influence of individual factors on their changes. It is considered that flavour depend on a large number of substances belonging to different groups of organic compounds. Thus special importance in forming the specific flavour of meat and meat products is placed on lipid components.

Undesired changes in the flavour, color and nutritional value of meat are considered to be a result of mostly lipid oxidation and their reactions with other meat components. The lipid composition of meat has been studied extensively, as were the influence of type of feeding, breed, sex, and partially processing and storage conditions /3,4,5,6,7,8/.

Unfortunately, beside many publications, the importance of individual lipid components in developing favorable meat flavour, as well as its deterioration, is still not sufficiently known. There are still no data in the available literature as to which compounds form during the various stages of lipid oxidation and the extent

of their contribution to flavour i.e. their changes. Thus we have, in the first phase of our investigations, set the aim of investigating in detail the lipid composition of hog intramuscular lipids by applying latest instrumental methods. This paper gives a review of the results of investigating neutral lipids.

MATERIALS AND METHODS

Sample preparation

In the investigations M. Semimembranosus from 6 white meaty hogs - castrates, 6-7 months old, of mass 95-105 kg, was used. The carcass was cooled to 4°C and the muscle removed 24h upon slaughtering. Each muscle was trimmed of fatty and connective tissue.

Lipid analysis

The extraction of total lipids was performed according to Folch /9/. The lipid extract was fractionated by column chromatography - Silica gel 60 (70-230 mesh) into neutral lipids, phospholipids and glucolipids, according to the procedure described by Johnston /10/. The quantity of these fractions was determined gravimetrically after evaporating the solvent in a stream of N_2 and expressed in % of total lipids.

The further fractionation of neutral lipids by column chromatography (Florisil, 100-200 mesh), according to the procedure applied in the paper of Johnston et al./10/, the following fractions were obtained: hydrocarbons, cholesterol esters, triglycerides, cholesterol, diglycerides, monoglycerides and free fatty acids. The content of each fraction was determined gravimetrically after evaporating the solvent in a stream of N_2 and expressed as % of total lipids, i.e. of the muscle. The purity and identity of each fraction was determined by thin layer chromatography on Silica gel G, by comparing the obtained R_f values with standards that were developed under the same conditions /10/.

The methyl esters of fatty acids of the neutral lipids fraction were prepared with diazomethane (4). The qualitative and quantitative compositions of methyl esters of fatty acids, hydrocarbons and cholesterol were determined by a GC-MS-C combinationn. A capillary column with fused Silica SB-1, 30 m, of inner diameter 0.32 mm and liquid phase film of 100% dimethyl polysiloxane and width of 0.10 μm was used in all analyses. The carrier gas was He (pressure 0.8 bar). The analyses were performed by means of a program from 100°C at 6°C/min.

we used the following standards to identify individual fatty acids: saturated C_{10} C_{20}, C_{22} and C_{24} acids, as well as unsaturated $C_{16}^{1=}$, $C_{18}^{1=}$, $C_{18}^{2=}$, $C_{18}^{3=}$ and $C_{22}^{1=}$ acids. Normal C_{10} to C_{31} paraffins and squalene were used as standards for identifying components in the hydrocarbon fraction, while cholesterol was identified on the basis of an authentic sample.

RESULTS AND DISCUSSION

The results present a mean value of the analysis of 6 individual muscle samples. The content of total intramuscular lipids is approximately 4.0%. The relative mass percentages of the three major lipid classes isolated by column chromatography are given in Table 1. Neutral lipids eluted with chloroform present app. 87.2%, glucolipids separated by acetone app. 1.4% and phospholipids eluted with methanol present app.11.4% of the total lipids. The obtained results generally agree with the results of Luddy et al./3/, except that the denoted authors did not present the glucolipid content in the muscle.

The mean composition of lipids isolated from M.Semimembranosus*

Table 1.

Class	Mass, %
Total lipids**	4.00±0.65
Neutral lipids	87.23±1.40
Glycolipids	1.37±0.03
Phospholipids	11.40±2.30

*on the total lipids basis

**on the whole muscle basis

For separating the neutral lipid fraction, Florisil was used in column chromatography because the separation of mono- and diglycerides is much better than when using Silica gel /10/. TLC analysis of the neutral lipid fractions separated by column chromatography yielded a clean band for each fraction.

Table 2 presents the mass percentages of individual fractions of neutral lipids.

The results indicate that the major fraction is the triglyceride one which presents more than 70% of the neutral lipids. Their fraction in the investigated muscle is about 3%.

The composition of neutral lipid fractions (mass %) in

Table 2

Lipid fractions	Neutral lipid fraction	Total lipid extract	Muscle tissue
Hydrocarbons	0.57	0.50	0.02
Cholesterol esters	9.08	7.90	0.36
Triglycerides	76.60	66.64	3.06
Cholesterol	6.20	5.39	0.25
Diglycerides	4.20	3.66	0.17
Monoglycerides	1.00	0.92	0.04
Free fatty acids	1.40	1.20	0.05

Table 3 shows the composition of fatty acids of cholesterol esters, while Fig.1 presents a gas chromatogram of this fraction.

Table 3.

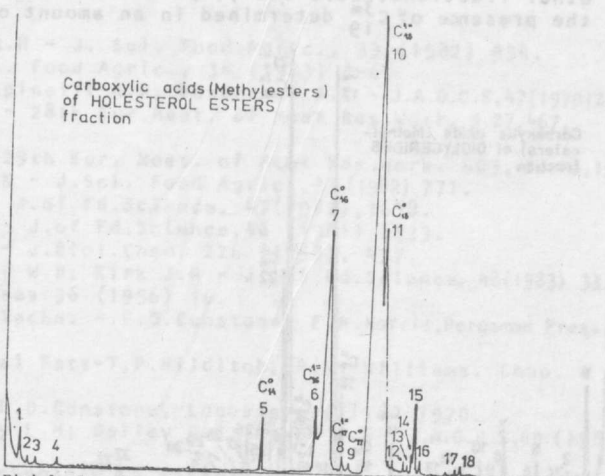
FATTY ACID DISTRIBUTION OF NEUTHAL LIPID SUB-FRACTIONS

	COMPONENT	CHOLESTEROL ESTERS %	TRIGLYCERIDES %	DIGLYCERIDES %	MONOGLYCERIDES %	FREE FATTY ACIDS %
1	iso Valeric acid $(CH_3)_2CHCH_2COOCH_3$				0,6	
2	$CH_2-COOCH_3$ $CH_2-COOCH_3$				0.5	2.0
3	$CH_3-CH-COOCH_3$ $COOCH_3$					0.3
4	$CH_3-CH-COOCH_3$ $CH_2-COOCH_3$			0.6	2.0	1.3
5	$CH_3-CH-COOCH_3$ CH_2 $CH_2-COOCH_3$					0.5
6	C_{10}^{o}					0.1
7	$COOCH_3$ $C_{16}H_{32}$ $COOCH_3$			1.0	2.2	1.0
8	$CH_3-CH-COOCH_3$ $(CH_2)_4$ $COOCH_3$			0.3	0.9	0.3
9	C_{12}^{o}			tr.		0.8
10	$COOCH_3$ $(CH_2)_7$ $COOCH_3$ Azeleinic acid			0.5	1.0	0.9
11	$C_{14}^{1=}$					tr.
12	C_{14}^{o}	1.5	2.0	1.0	1.7	2.2
13	$COOCH_3$ $(CH_2)_8$ $COOCH_3$					0.2
14	C_{15}^{o}			0.2		0.9
15	$C_{16}^{1=}$	2.5	8.0	2.0	4.6	2.1
16	C_{16}^{o}	17.0	22.0	11.0	17.1	21.0
17	$C_{17}^{1=}$	0.2	0.5		tr.	0.1

	COMPONENT	CHOLESTEROL ESTERS %	TRIGLYCERIDES %	DIGLYCERIDES %	MONOGLYCERIDES %	FREE FATTY ACIDS %
18	C_{17}^{o}	0.1	0.3	0.2	tr.	1.7
19	$C_{18}^{2=}$ 9.12 (cis,cis)		1.0	0.8		1.7
20	$C_{18}^{1=}$	68.5	49.0	44.0	38.2	36.5
21	C_{18}^{o}	8.5	10.0	8.0	9.6	10.7
22	$(CH_2)_{12}COOCH_3$					2.0
23	$C_8H_{17}-C\equiv C-C_7H_{14}COOCH_3$ Sterculic acid			0.7	1.7	0.8
24	$(CH_2)_{12}COOCH_3$ Chaulmoogric acid			1.0	1.8	3.0
25	$C_{19}^{3=}$					0.3
26	$C_{20}^{4=}$	0.2	0.3			0.2
27	$C_{20}^{3=}$	0.1	tr.			0.3
28	$C_{20}^{2=}$	0.3	0.3			0.9
29	$C_{20}^{1=}$	2.7	1.0	0.5	1.4	0.4
30	C_{20}^{o}	0.1	0.2			0.4
31	$C_{21}^{1=}$		tr.			
32	C_{21}^{o}		tr.			
33	$C_{22}^{1=}$	tr.				
34	C_{25}^{o}					0.2

The numbers in the gas chromatogram which denote particular peaks correspond to those in the legend. Seventeen components were found in this fraction of which 4 were not identified. There are 27.2% saturated acids, 69.9% unsaturated acids with $1^{=}$ and 0.6% with 2 or more double bonds, among these the major components are oleic acid, palmitic acid and stearic acid. The ratio of polyunsaturated to saturated acids is 0.02.

By comparing the composition of this fraction and the triglyceride fraction (Table 3, Fig.2) it may be stated that the same carbonic acids are formed except that in the triglyceride fraction $C_{18}^{2=}$(1%), C_{21}^{o} and $C_{21}^{1=}$ were found in traces, while in cholesterol esters $C_{22}^{1=}$ in traces. Seventeen carbonic acids were found in triglycerides of which 2 were not identified. The fraction consists of 34.5% saturated acids, 58.5% unsaturated with $1^{=}$, and 1.6% with 2 or more double bonds. The ratio of polyunsaturated to saturated acids is 0.04.



Figi 1. Gas chromatogram of carboxylic acids (methylesters) from the chlolesterol ester fraction of neutral lipids on SB-1 (30 m fused capillary column) from 100°/6°/min.

1. Uniden.; 2. Unident.; 3. Unident.; 4. Unident.; 5. C_{14}^{o}; 6. $C_{16}^{1=}$; 7. C_{16}^{o}; 8. $C_{17}^{1=}$; 9. C_{17}^{o}; 10. $C_{18}^{1=}$; 11. C_{18}^{o}; 12. $C_{20}^{4=}$; 13. $C_{20}^{3=}$; 14. $C_{20}^{2=}$; 15. $C_{20}^{1=}$; 16. C_{20}^{o}; 17. Unident.; 18. Phthalic ester.

The diglyceride, monoglyceride and free fatty acid fractions beside linear carbonic acids also contain dicarbonic acids with 4 to 11 C atoms with normal and branched chains, 2 isomers of C_{18} unsaturated acid containing a cyclopentene ring. These acids were not found in triglycerides which leads to the conclusion that only mono- and diglycerides are formed by metabolism but not triglycerides. Table 3 gives the % composition of these fractions, while the gas chromatograms are presented in Fig.3, 4 and 5. From the available numerous literature data the denoted acids, sterculic, chaulmoogric and dicarbonic acids containing 4 to 11 C atoms have not vet been iden-

tified in intramuscular lipids or in meat lipids /12, 13, 14, 15/. Their identification in intramuscular lipids is not insignificant especially keeping in mind their determined existence in lipids. Sterculic acid, found in an amount of 0.7-1.7% in these fractions, was identified up to now in lipids isolated from plants. Chaulmoogric acid was found in an amount of 1-3%, it has rarely been identified in lipids of plant origin and has, so far, been unidentified in meat. The identification of these acids was performed on the basis of obtained mass spectra. The diglyceride fraction, among other substances, contains 2.4% dicarbonic acids, 0.7% cyclopentene acid (sterculic acid) and 1% cyclopropene acid (chaulmoogric acid). Sixteen components were identified in this fraction, while 14 remained unidentified.

Monoglycerides contain isovaleric acid in an amount of 0.6%, dicarbonic acids 6.6%, sterculic acid 1.7% and chaulmoogric acid 1.8%. An isomer of chaulmoogric acid containing a double bond in the ring at position 3 was also identified amounting to 2.0%. The structure od 17 acids present was determined, while 9 remained unidentified. In the monoglyceride and diglyceride fractions the presence of C_{20} and C_{21} acids was not determined although they are present in the triglyceride and cholesterol ester fractions.

As is denoted in Fig.5, free fatty acids contain the greatest number of components, 29 were identified, while 11 remained unidentified. The major components, as in

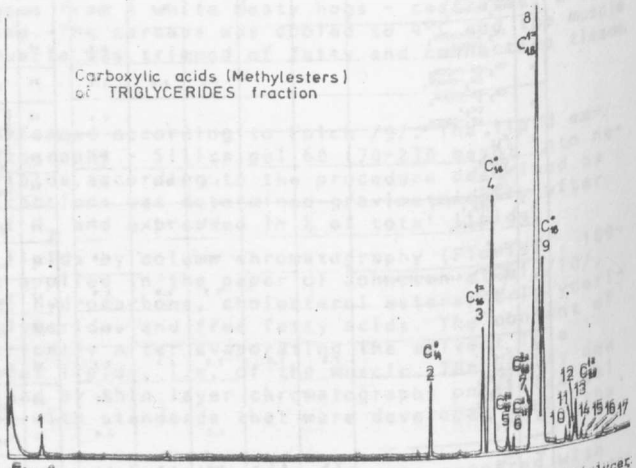


Fig. 2.
Gas chromatogram of carboxylic acids (Methylesters) from the triglyceq fraction of neutral lipids on SB-1 (30 m fuseed capillary column) from 100°/6°/min.

1. Unident.; 2. C^{o}_{14}; 3. $C^{1=}_{16}$; 4. C^{o}_{16}; 5. $C^{1=}_{17}$; 6. C^{o}_{17}; 7. $C^{2=}_{18}$; 8. $C^{1=}_{18}$; 9. C^{o}_{18}; 10. $C^{4=}_{20}$; 11. $C^{3=}_{20}$; 12. $C^{2=}_{20}$; 13. $C^{1=}_{20}$; 14. C^{o}_{20}; 15. $C^{1=}_{21}$; 16. C^{o}_{21}; 17. Unident..

other fractions, were oleic, palmitic and stearic acid. Only in this fraction was the presence of $C^{3=}_{19}$ determined in an amount of 0.3%. It also contained 6.5% dicarbonic

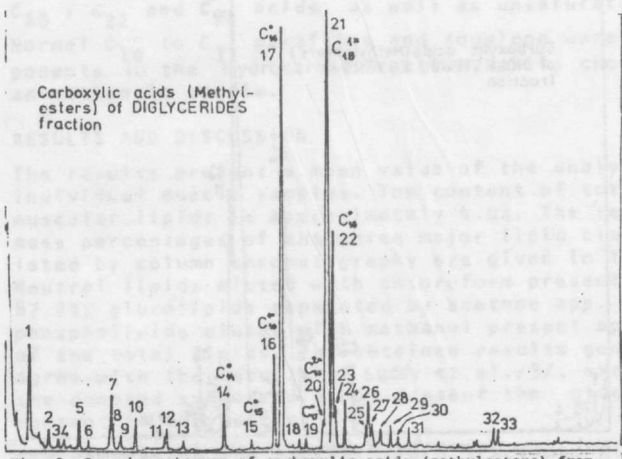
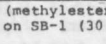


Fig. 3. Gas chromatogram of carboxylic acids (methylesters) from the DIGLYCERIDE fraction of neutral lipids on SB-1 (30 m fuseed silica capillary column) from 100°/6°/min.

1. From 1-4 Unident.; 5. $CH_3OOC-CH_2CH(CH_3)COOCH_3$; 6. Unident.; 7. $CH_3OOC-C_6H_{12}-COOCH_3$; 8. Unident.; 9. $CH_3OOC(CH_2)_4CH(CH_3)COOCH_3$; 10. Phthalic ester; 11. C^{o}_{12}; 12. $CH_3OOC(CH_2)_7COOCH_3$; 13. Unident.; 14. C^{o}_{14}; 15. C^{o}_{15}; 16. $C^{1=}_{16}$; 17. C^{o}_{16}; 18. Unident.; 19. C^{o}_{17}; 20. $C^{2=}_{18}$; 21. C^{o}_{18}; 22. C^{o}_{18}; 23. $CH_3(CH_2)_7-C\equiv C-(CH_2)_7COOCH_3$ (Sterculic acid Methylester); 24.  $CH(CH_2)_{12}COOCH_3$ (Chaulmoogric acid Methylester) 25. Unident.; 26. Unident.; 27. $C^{1=}_{20}$; 28. From 28-31 Unident.; 32. 4,6,8(14)-cholestatrien; 33. 3,5-cholestadiene.

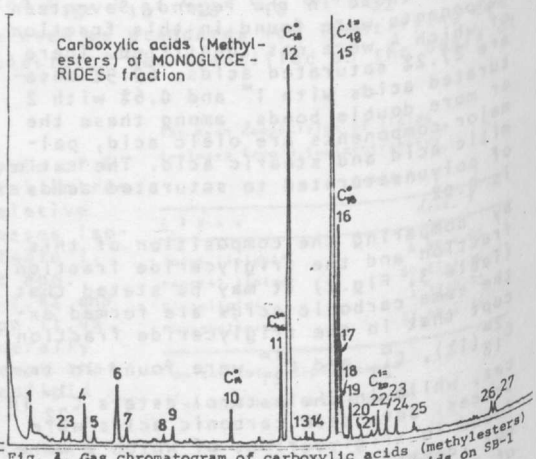


Fig. 4. Gas chromatogram of carboxylic acids (methylesters) from the monoglyceride fraction of neutral lipids on SB-1 (30 m glass capillary column) from 100°/6°/min.

1. Unident.; 2. Iso-Valeric acid Methylester; 3. $CH_3OOC-CH_2-CH_2-COOCH_3$; 4. $CH_3OOC-CH_2-CH(CH_3)COOCH_3$; 5. $CH_3OOC-(CH_2)_2CH(CH_3)COOCH_3$; 6. $CH_3OOC-C_6H_{12}-COOCH_3$; 7. $CH_3OOC-(CH_2)_4CH(CH_3)COOCH_3$; 8. Unident.; 9. $CH_3OOC(CH_2)_7-COOCH_3$; 10. C^{o}_{14}; 11. $C^{1=}_{16}$; 12. C^{o}_{16}; 13. Unident.; 14. Unident.; 15. $C^{1=}_{18}$; 16. C^{o}_{18}; 17.  $(CH_2)_{12}-COOCH_3$; 18. $CH_3-(CH_2)_7-C\equiv C-(CH_2)_7COOCH_3$ (Sterculic acid Methylester); 19.  $(CH_2)_{12}COOCH_3$ (Chaulmoogric acid Methylester); 20. Unident.; 21. Unident.; 22. $C^{1=}_{20}$; 23. From 23-25 Unident. 26. 4,6,8(14)-cholestatriene; 27. 3,5-cholestadiene.

acids, 0.8% sterculic acids and 3% chaulmoogric acid. As can be seen in Table 3 and Fig.2,3,4 and 5, the investigated fractions also contain acids with an odd number of C atoms. The obtained values for linear acids are in accordance with the available literature data, the references generally represent the lipid content of lean pork or the fatty acid content of glycerides mostly even acids /3,4,5,6,16,17/.

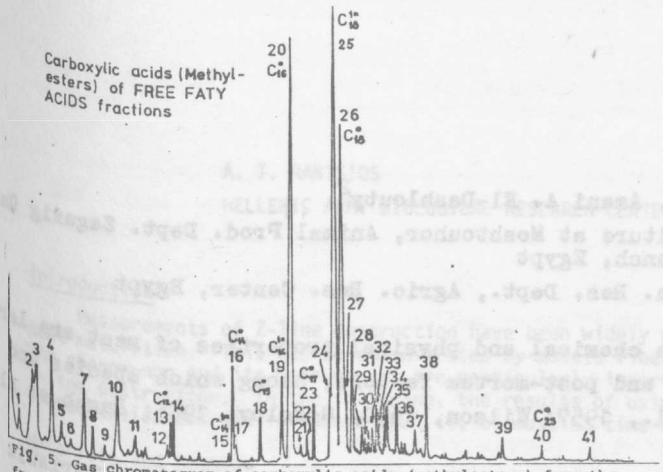


Fig. 5. Gas chromatogram of carboxylic acids (methylesters) from the free fatty acid fraction of neutral lipids on SB-1 (30 m fuseed silica capillary column) from 100°/6°/min.
1. From 1-3 Unident.; 4. $CH_3OOC-CH_2-CH_2-COOCH_3$; 5. $CH_3OOC-CH(CH_3)COOCH_3$; 6. Unident.; 7. $CH_3OOC-CH_2-CH(CH_3)COOCH_3$; 8. $CH_3OOC(CH_2)_2CH(CH_3)COOCH_3$; 9. C^{o}_{10}; 10. $CH_3OOC-C_6H_{12}-COOCH_3$; 11. $CH_3OOC(CH_2)_4CH(CH_3)COOCH_3$; 12. Unident.; 13. C^{o}_{12}; 14. $CH_3OOC(CH_2)_7COOCH_3$ (Azelainic acid methylester); 15. $C^{1=}_{14}$; 16. C^{o}_{14}; 17. $CH_3OOC(CH_2)_9COOCH_3$; 18. C^{o}_{15}; 19. $C^{1=}_{16}$; 20. C^{o}_{16}; 21. Unident; 22. $C^{1=}_{17}$; 23. C^{o}_{17}; 24. $C^{2=}_{18}$; 25. $C^{1=}_{18}$; 26. C^{o}_{18}; 27. $CH_3(CH_2)_7-C^{c=c}_{H}-(CH_2)_7COOCH_3$ (Sterculic acid methylester) 28. $CH(CH_2)_{12}COOCH_3$ (chalmoogric acid methylester); 29. $C^{3=}_{19}$; 30. $C^{4=}_{20}$; 31. $C^{3=}_{20}$; 32. $C^{2=}_{20}$; 33. $C^{1=}_{20}$; 34. C^{o}_{20}; 35. From 35-38 Unident.; 39. Cholesta -3,5-diene; 40. C^{o}_{25}; 41. Unident.

Beside cholesterol as the major component in the fraction of the same name, the presence of 4,6,8 (14) cholestatriene and 3,5 cholestadiene was noted.

In the hydrocarbon fraction, beside squalene as the major component and C_{13} to C_{31} saturated paraffins, the C_{19} n-olefin was also identified. There is also a three member homologous series which has not yet been identified in hydrocarbons of meat origin /18,19/. They are probably acetylenes or hydrocarbons with a cyclopropene, cyclopentene or cyclohexene ring. The structure of 20 components in this fraction was determined, i.e. assumed, while one compound remained unidentified.

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