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CHOLESTEROL CONTENT IN DIFFERENT PORK TISSUES

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SUMMARY

Results show high differences among tested tissues in lipid contents. Percentage of neutral lipids in total ones ranged 29.67 (in brain) to 99.64 (in fatty tissue);phospholipids were 0.27% (in fatty tissue) to 64.16% (in liver; close to it also in brain and spinal cord).Participation of glycolipids was low ranging 0.09-9.54%. There were high differences in quantities of neutral lipid fractions of different tissues.

Based on results of comparative study it is to be stated that there were high variations of cholesterol contents in different tissues of an animal. Cholesterol contents were: in muscle tissue - 239.7 mg/100 g, in liver - 338.9 mg/100 g, in fatty tissue - 122.9 mg/100 g, in brain - 1912.7 mg/100 g, in spinal cord - 2621.6 mg/100 g tissue.

More detailed data are expected through GC-MS-C combination of components of steroid type that are valuable for investigation of oxidative changes of cholesterol and other compounds in raw material processing and storage.

INTRODUCTION

The role of lipids in living organisms has been considerably investigated and there are numerous data on their importance in nutrition. In the past years special attention has been paid to the cholesterol content in food and the forms in which it can be found, as well as the undesired changes occurring during processing and storage.

Regarding the very limited data available in the literature on the cholesterol content in various animal tissues, except muscle tissue, (5,6,7,8,9,10,11,12) the purpose of this study was to investigate the cholesterol content in different hog tissues.

MATERIJALS AND METHODS

The muscle tissue (M.longissimus dorsi in the region of the 12^{th}-14^{th} vertebra), as well as liver, fatty tissue, brain and spinal cord of five Large White hogs, carcass mass about 81 kg, were taken from the slaughter line and immediately studied.

The total lipids were extracted by a procedure according to Folch et al (3) at about 15°C. The peroxide number in all extracted lipids was zero in all cases.

The lipid fraction was then fractionated by column chromatography - Silica Gel 60 (70-230 mesh) to neutral lipids, glycolipids and phospholipids according to the procedure described by Johnston (4). The composition of these fractions was determined gravimetrically upon evaporating the solvent in a stream of nitrogen and expressed as percent of total lipids.

By further fractionation of neutral lipids by column chromatography (Florisil,100-200 mesh) according to the procedure by Johnston et al (4), the following fractions were obtained: hydrocarbons, cholesterol esters, triglycerides, cholesterol, diglycerides, monoglycerides and free fatty acids. The content of each fraction was determined gravimetrically upon evaporating the solvent in a stream of nitrogen and expressed as percent of total lipids. The purity and identity of each fraction were determined by thin layer chromatography-Silica Gel G by comparing the obtained R_f values with standards developed under the same conditions.

About 1 g of total lipids was refluxed with 15 ml of 0.5 N methanolic KOH solution for about 20 minutes with a little distilled water. Upon triple extraction with diethyl ether and washing the ether extracts and drying wih Na_2SO_4, the eter was evaporated in a stream of N_2 to constant mass.

Separation of the fraction of cholesterol and unsaponifiable matter, as well as quantitative analysis were performed using a Varian 3400 capillary gas chromatograph on a SE 54 fused silica capillary stationary phase on deactivated siloxane, column length 25 m, inner diameter 0.25 mm with a FID detector. Carrier gas N_2, flow rate 1.18 ml/min. The injector and detector temperatures were 250° and 300°C, respectively. The analyses were performed at a programmed heating rate of

Table 1. Lipid content in tested tissues

Tissue	Total lipids %	Neutral lipids % of total lipids	Neutral lipids g/100 g tissue	Polar lipids Glycolipids % of total lipids	Polar lipids Glycolipids g/100 g tissue	Polar lipids Phospolipids % of total lipids	Polar lipids Phospolipids g/100 g tissue
Muscle tissue	2.47	74.89	1.85	0.74	0.02	24.37	0.60
Liver	3.49	31.03	1.08	4.80	0.17	64.16	2.24
Fatty tissue	87.48	99.64	87.16	0.09	0.08	0.27	0.24
Brain	8.65	29.67	2.56	9.54	0.82	60.79	5.26
Spinal cord	20.45	39.00	7.97	4.42	0.90	56.58	11.57

4 °/min.

The percents of total surface area in quantitative analysis, obtained by a Spectra Physics System I Computing Integrator, were converted to percent mass by comparing with mixtures of known composition. Cholesterol (Fluka AG, Buch SG) was used as the reference sample.

RESULTS AND DISCUSSION

The results presented in Table 1 indicate the considerable differences in the content of total, neutral and polar lipids in various tissues. In the investigated tissues the total lipid content varied from 2.47 to 87.48%, the fraction of neutral in total lipids from 29.67% in the brain to 99.64% in fatty tissue, phospholipids from 0.27% in fatty tissue to 64.16% in the liver and very similar in the brain and spinal cord. There are considerably less glycolipids as compared to neutral lipids and phospholipids, the most 9.54% in the brain and the least in fatty tissue 0.09%

By separating neutral lipids by column chromatography data were obtained concerning various fractions, i.e. hydrocarbons, cholesterol esters, triglycerides, cholesterol, diglicerides, monoglycerides and free fatty acids (Table 2). Significant differences in the amounts present in various fractions were noted. Triglycerides, the major component in muscle tissue neutral lipids with 66.10%, are present in the spinal cord with 23.27%. So the participation of cholesterol in neutral lipids of fatty tissue is 0.15%, while in the brain it is 78.68%.

The gas chromatograms of the cholesterol fraction (Fig. 1) prove the good separation of cholesterol by column chromatography. On the basis of weight measurements of all fractions and gas chromatographic data of the purity of the cholesterol fraction, it was determined that the amount of cholesterol, (Table 3) i.e. Δ^5-cholesten-3β-ol in muscle tissue is 239.69 g/100 g, in the liver 338.87, fatty tissue 122.97, brain 1921.69 and spinal cord 2621.57 g/100 g tissue.

Regarding the data concerning cholesterol esters fraction, significant differences are also noted in the investigated tissues. The data indicate that in the brain and fatty tissue cholesterol is mostly present in the free form, while in muscle tissue, especially in the liver and spinal cord there is also a considerable amount in the ester form. The fraction of cholesterol esters in the neutral lipid fraction in the muscle tissue is 8.50, in the liver 6.55, in fatty tissue 0.09, in the brain 0.17 and 26.99% in the spinal cord.

Besides cholesterol, which was identified in an amount of more than 94% in the cholesterol lipid fraction in all tissues, except in spinal cord - 81.6%, the presence of other compounds (Fig. 1) was also determined by gas chromatographic analysis. In the spinal cord lipid fraction, a homologous series of unknown structure was found in front of cholesterol. The four peaks appearing after cholesterol are, judging by the retention times, probably compounds of steriod structure; for the three

Table 2. Composition of neutral lipid fractions of tested tissues

Fraction	Muscle tissue			Liver			Fatty tissue			Brain			Spinal cord		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Hydrocarbon	2.01	1.50	0.04	7.08	2.20	0.08	0.45	0.45	0.39	1.46	0.43	0.04	2.69	1.05	0.21
Cholesterol ester	8.50	6.36	0.16	6.55	2.03	0.07	0.09	0.09	0.08	0.17	0.05	0.004	26.99	10.53	2.15
Triglycerides	66.10	49.50	1.22	39.29	12.19	0.42	65.53	65.29	57.11	10.56	3.13	0.27	23.27	9.80	1.85
Cholesterol	13.20	9.88	0.24	32.08	9.95	0.35	0.15	0.15	0.13	78.68	23.34	2.01	40.31	15.72	2.21
Diglycerides	5.37	4.02	0.10	6.49	2.01	0.07	14.47	14.42	12.61	1.96	0.58	0.06	0.39	0.15	0.03
Monoglycerides	0.89	0.66	0.02	3.49	1.08	0.04	15.78	15.72	13.75	0.57	0.17	0.01	0.34	0.13	0.03
Free fatty acids	3.50	2.62	0.06	5.01	1.55	0.05	2.63	2.62	2.29	6.39	1.89	0.16	4.92	1.92	0.39

A - % of neutral lipids B - % of total lipids C - g/100 g tissue

Table 3. Δ^5 - cholesten - 3β - ol content in tested tissues

	Muscle tissue	Liver	Fatty tissue	Brain	Spinal cord
mg/100 g tissue	239.69	338.97	122.97	1921.69	2621.57

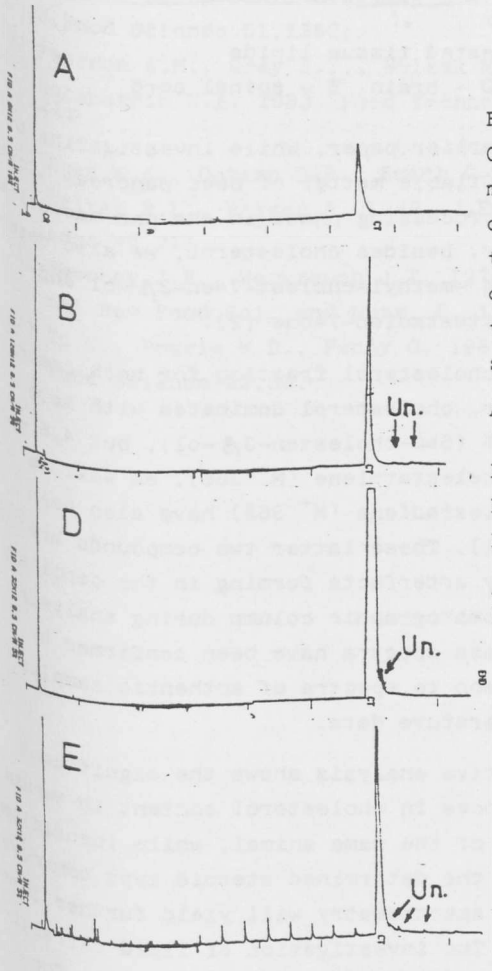

Fig. 1. Gas chromatograms of cholesterol fraction of tested tissue lipids

A - muscle tissue
B - liver
D - brain
E - spinal cord
Un- unidentified compounds

Table 4. Unsaponifiable matter content in tested tissues

Tissue	% of total lipids
Muscle tissue	27.95
Liver	30.94
Fatty tissue	35.43
Brain	38.89
Spinal cord	39.02

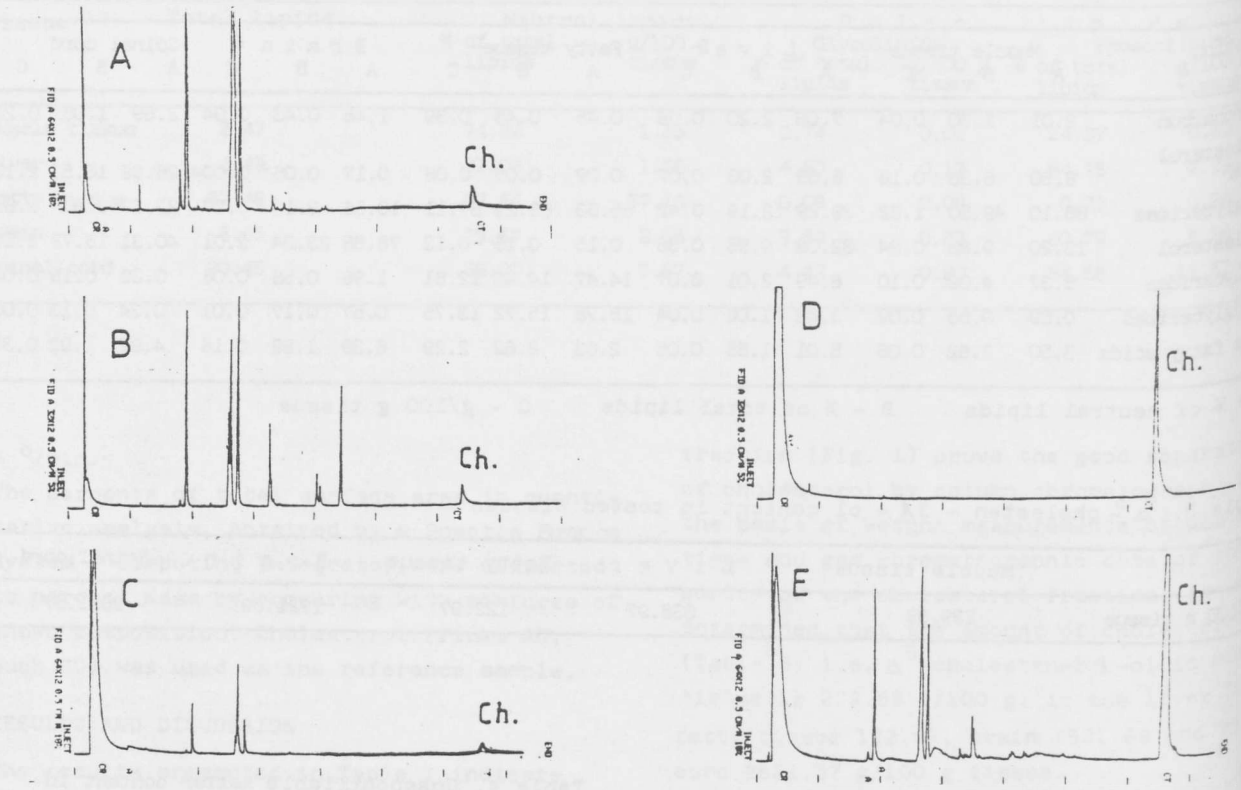

Fig. 2. Gas chromatograms of unsaponifiable matter of tiested tissue lipids
A - muscle tissue, B - liver, C - fatty tissue, D - brain, E - spinal cord

larger peaks identification by GC-MS-C is in progress. In the cholesterol fraction of brain lipids the presence of a steroid type compound in an amount of 2.6% was determined. In the liver fraction two compounds, the identification of which is in progress,were found.

The data on unsaponifiable matter of tested tissue lipids are presented in Table 4.The gas chromatograms of the unsaponifiable part (Fig.2) also indicate the differences in the composition of the lipid components of the various investigated tissues. The amount of cholesterol in total lipids cannot be observed on the basis of chromatograms of the unsaponifiable part only the ratio of cholesterol to other components of unsaponifiable matter. In the unsaponifiable matter of the brain and spinal cord the cholesterol fraction is considerable. In the unsaponifiable matter of the spinal cord, as opposed to the brain, many other components with similar retention times as in the case of unsaponifiable matter of lipids originating from muscle tissue or the liver. These components also appear in the unsaponifiable matter of fatty tissue, although there are less of them.

In an earlier paper, while investigating the unsaponifiable matter of beef pancreas lipids in the processing phase in the pharmaceutical industry, besides cholesterol, we also determined 24 -methyl-cholest-7-en-3β -ol and $\Delta^{3,5}$-sitostadien-7-one (2).

In the cholesterol fraction for both castrate and boar, cholesterol dominates with more that 95% (5α-cholesten-3β-ol), but 4,6 - 8(14)-cholestatriene (M^+ 366), as well as 3,5-cholestadiene (M^+ 368) have also been found (1). These latter two compounds are possibly arterfacts forming in the capillary gas chromatographic column during analysis. Their mass spectra have been confirmed by comparison to spectra of authentic samples and literature data.

Comparative analysis shows the significant differences in cholesterol content in various tissues of the same animal, while identification of the determined steroid type components by mass spectrometry will yield further information. The investigation of lipid extractions of fresh tissues is also valuable for studing oxidative changes of cholesterol and

other compounds during the storage of raw materials in the frozen state and their processing in the meat industry as well as,in the case of spinal cord, it is important for the pharmaceutical industry.

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