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Fish processing

INVESTIGATING CHOLESTEROL CONTENT IN THE TISSUES OF SELECTED MARINE FISH

BADANIA NAD ZAWARTOŚCIĄ CHOLESTEROLU W TKANCE MIĘŚNIOWEJ NIEKTÓRYCH RYB MORSKICH

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Muscles of the sixteen species of fishes originating from fishing grounds of Middle and North West Atlantic and Baltic Sea were tested for total, free and connected cholesterol contents and for unsaponifying substances content.

INTRODUCTION

Cholesterol is created in animal tissues as metabolic product, namely in the process of carbohydrates protein and fat oxygenation (**Supniewski**, 1960). The simpliest compound of biosynthesis in acetic acid which is created in the tissues, mainly through oxygenation of hexose, higher fatty acids, ketoacids and aminoacids. In pathological cases cholesterol is stored on the walls of veins (in arteriosclerosis), in the gull channels. At present, the most popular theory for pathogenesis of arteriosclerosis is the metabolic-filtration theory. It is based on the assumption, that arteriosclerosis is caused by cholesterol and some fats being stored on the artery wall.

In particular, significant role in lowering the high cholesterol level in the human and animal blood, is played by unsaturated fatty acids, apperaing in large quantities i.a. in fish and marine mammal oils (**Wood**, **Topliff**, 1961, **Braekkan**, et.al., 1962, **Reed**, 1964). Deficiency of those acids is the reason of disturbance in the production of phospholipides and indirectly of ATPase, this causing in turn the lipids Transportation of the body

changing to worse. Thus, the cholesterol level in the blood is rised, as well as storing of cholesterol in the arteries (**Hill** et.al., 1965).

As proved in several investigations (Wood, 1960, **Załęski**, 1962) cholesterol level is of total unsaturation of the fats consumed than on the presence of any particular unsaturated fatty acids. Fish fats, being very rich in polyunsaturated fatty acids, contain also some quantity of cholesterol.

The cholesterol content in fish, as compared with other food stuffs, is relatively low amounts to 35–60 mg/100 g of tissue, while for instance in eggs the amount is 680 mg/100 g, ox's liver 320 mg/100 g, calf's liver 350 mg/100 g and so on.

Shimma and **Taguchi** (1964) report, that in fish the cholesterol content oscillates from 16 to 160 mg/100 g of tissue. In fresh water fish, such as smelt, shanny and trout, the cholesterol content is – according to the above authors – 25 to 72 mg/100 g, 71 mg/100 g and 70 mg/100 g, respectively. **Dąbrowski** and others (1968), investigating cholesterol content in the tissues of selected fresh water fish from Polish waters, report the content to be from 34 mg/100 g in the tissues of burbot to 107,5 mg/100 g of eel tissue.

Similar investigations with crustaceas and molluscs have been carried out by **Thomson** (1964), who reports the cholesterol content in those organisms to be 37 to 157 mg/100 g of tissue.

Numerous investigations have been carried out on cholesterol content, except in fish. Only few works on cholesterol in fish and in other marine organisms have been found in the available foreign sources, as well as among local references. Thus, the investigations on cholesterol content in marine fish caught in bulk by Polish deep sea fleet, appears to be in good time.

The paper is an attemp to examine the cholesterol content in several marine fish species.

MATERIAL AND METHOD

Samples necessary for the work have been taken from 16 species of marine fish. Fish selected for the investigations, have been caught in the middle and north-west Atlantic waters and in the Baltic. I cod (*Gadus morrhua L.*), the tissue, the roe and liver were analysed.

The selected fish were filleted, skinned and its muscles minced. The material thus prepared was used as samples to examine: water content, denoted by means of drying at 105°C; total nitrogen – by means of Kjeldahl method; the fat, using the extraction method in the Soxhled device; ash, by durning in the muffle furnace at the temperat, of 660°C. Fat extractions necessary to denote the unsaponifying substances and cholesterol (**Sperry, Webb**, 1950, **Tretiakova, Grodenski**, 1959) have been prepared in the following way: the flesh muscles were dehydrated using anhydrous sodium sulphate, then the fat extracted by means of chloroform, repeating the process three times using each time new portion of dissolvent. Denotation of total cholesterol: having denoted the quantity of unsaponifying substances, total cholesterol has been denoted in the following way: the

unsaponifying substances were dissolved in a mixture of 25 ml of absolute etyl alcohol with acetone (proportion 1:1). This mixture was put, in 1 ml portions, into the centrifugal tube. To such a 1 ml portion, 1 ml of 0.5% digitonine solution fresh prepared was added. The test tubes were placed in tight glasse container and kept for 18 hours at ambient temperature. Then, for 10 min. the test tubes were whirled at 4000 rpm. The liquid over the sediment was removed and the white cholesterol digitonid was rinsed using 5 ml of petroleum ether and acetone mixture (1:2), and again whirled, then rinsed twice in pure ether. The dried sediment was dissolved in 1 ml of ethyl acetate, adding 8 ml of colour producing reagent (mixture of: 92% H_2SO_4, glacial acetic acid, 1% $FeCl_3$ in 95% acetic acid – proportions 1,1:1:0,1 (**Badzio** 1964). Then the content of the test tubes was heated for 15 minutes at 50°C, and the test tubes cooled to reach the ambient temperature, and the extinction measured on the photocolorimeter using a filter of 530 nm wave lenght. By means of the base curve previously prepared, the total cholesterol content was read in the sample and reported in mg/ml.

Denotation of free cholesterol: for this purpose, 25 ml of the chloroformed extract was taken and decoloured using plant carbo. The mixture was heated to the boiling point and drained. Then the chloroform was removed by distillation, and the remainder dissoled in a mixture of abolute ethyl alcohol with acetone, transported quantitively to the measuring glask of 25 ml. From this flask, 1 ml of mixture was taken and put into the centrifugal tube and precipitated using alcoholic solution of digitonine of 0.5% concentration. The succeeding procedure was the same as with denoting total cholesterol.

The connected cholesterol content was calculated as difference between total and free cholesterol.

RESULTS OF INVESTIGATIONS AND THEIR ANALYSIS

Chemical analysis of flesh tissue of the marine fish under review reveals significant differences not only as regards the content of water, protein, fat and ash, but also as regards cholesterol content (Table 1 and 2).

The percent content of total cholesterol in the fat of the fish under review oscillates from 0.58% in redfish to 9.26% in Spanish meckerel. The fat of the analysed fish contains 4.53% of total cholesterol, on an average (Fig. 1).

Cod liver shows low cholesterol content in its fat, amounting to 0.44%. Similar is the cholesterol content per 1 gram of fat. The investigations show, that total cholesterol in the flesh tissue of the analysed marine fish oscillates from 24 to 44 mg/100 g of tissue. These results are close to the results reported by the Japanese scientists (**Shimma, Taguchi,** 1964). **Dąbrowski** et.al. (1968) report, that 100 g flesh tissue of fresh water fish contains 50.7 mg of total cholesterol, on an average.

Idler and **Bitners** (1959) denoted cholesterol content in the flesh tissue of *Oncorhyncus nerca,* between 29 to 37 mg/100 g.

The results of the investigations show, that the lowest quantity of cholesterol is found to be in the tissue of *Epinephelus gigas* (24.1 mg/100 g) and in the tissue of *Sargus sargus*

Table 1

Chemical composition of selected marine fish

Fish species	Water	Protein	Fat	Ash
	in percent			
Redfish (Sebastes marinus (L.))	72.1	20.0	6.2	1.0
Tuna (Thunnus thynnus L.)	66.1	21.5	10.3	1.4
Hairtail (Trichiurus lepturus L.)	70.2	16.5	12.1	1.2
Sprat (Sprattus sprattus (L.))	75.6	16.2	6.6	1.1
Baltic herring (Clupea harengus L.)	77.0	19.1	2.5	1.2
Horse mackerel (Trachurus trachurus (L.))	77.0	20.4	1.2	1.2
Halibut (Hippoglossus hippoglossus (L.))	78.2	18.2	2.0	1.0
Grouper (Epinephalus gigas, (Brünnich))	77.1	20.7	0.3	1.2
John Dory (Zeus faber L.)	78.4	19.7	0.3	1.5
Pampano (Lichia vadigo, (Risso))	75.7	20.9	1.8	1.3
White bream (Sargus sargus L.)	77.5	20.3	0.4	1.5
Barracuda (Sphyraena barracuda, Walbaum)	78.0	20.0	0.3	1.2
Bream (Brama raii, Bloch)	75.1	23.2	0.4	1.2
Baltic cod (Gadus morrhua L.)	81.1	17.4	0.3	1.1
Cod liver	29.0	11.2	58.6	0.9
Cod roe	82.2	15.3	0.8	1.0
Tasergal (Temnodon saltator L.)	78.7	19.1	0.2	1.2
Spanish mackerel (Scomber colias, Gmelin)	75.3	22.1	0.9	1.5

(26.4 mg/100 g), while the highest content is found in the tissue of *Scomber colias* (43.5 mg/100 g) and tuna (44.2 mg/100 g).

Cod liver, due to the high content of fat (58.6%), has relatively high total cholesterol value expressed per 100 g of liver (141.7 mg), although the percent content of cholesterol in the fat is very low. Even more total cholesterol is contained in cod's roe (172.5 mgr/100 g), although the roe is rather poor in fat.

Table 2

Total, free and connected cholesterol and unsaponifying substances content in fat and in muscles of selected marine fish

Fish species	Fat				Total cholesterol		
	unsaponifying substances	Cholesterol			in unsaponifying substances	in fat	in flesh muscles
		free	connected	total			
	%	%	%	%	%	mg/g	mg/100g
Redfish	1.4	0.30	0.28	0.58	42.5	5.8	42.5
Tuna	2.2	0.37	0.30	0.67	30.1	6.7	44.2
Hairtail	3.3	0.57	0.58	1.15	34.4	11.5	38.5
Sprat	2.7	0.56	0.75	1.31	48.1	13.1	38.5
Baltic herring	2.3	0.44	0.96	1.40	60.8	14.0	36.5
Horse mackerel	14.5	1.98	2.28	4.26	29.3	42.6	41.0
Halibut	5.7	1.60	2.94	4.54	78.8	45.3	41.0
Grouper	8.0	2.00	2.83	4.83	60.2	48.4	24.1
John Dory	14.9	1.08	3.81	4.89	32.7	48.8	34.0
Pampano	6.1	1.44	3.88	5.32	87.5	53.2	31.5
White bream	15.6	2.34	3.32	5.66	36.2	56.6	26.4
Barracuda	12.9	2.58	3.31	5.89	45.6	58.9	36.5
Bream	18.0	2.25	4.58	6.83	38.0	68.3	41.0
Baltic cod	16.5	3.28	4.05	7.33	44.3	73.3	38.6
Cod liver	1.3	0.30	0.14	0.44	33.2	4.4	141.7
Cod roe	11.5	1.80	5.75	7.55	65.3	75.5	172.5
Tasergal	18.0	3.38	5.12	8.50	47.2	85.0	34.0
Spanish mackerel	21.3	5.11	4.15	9.26	43.5	92.5	43.5

The percent content of total cholesterol in unsaponifying substances is rather differentiated with particular fish species. The lowest level of cholesterol was found in the unsaponifying substances of horse mackerel, amounting to 29.3%, the highest – with *Lichia vadigo:* 87.5%. The vaerage content of total cholesterol in the unsaponifying substances of the analysed fish was found to be 47.5%.

Cod liver contains 33.2% of total cholesterol in the unsaponifying substances, while cod roe – 65.3%.

In the fat of the analysed fish, the content of free cholesterol oscillates between 0.3% in redfish, this being 51,7% of total cholesterol, to 5.1% in Spanish mackerel – 55.2% of total cholesterol, the mean value being 1.8% (40.4% of total cholesterol).

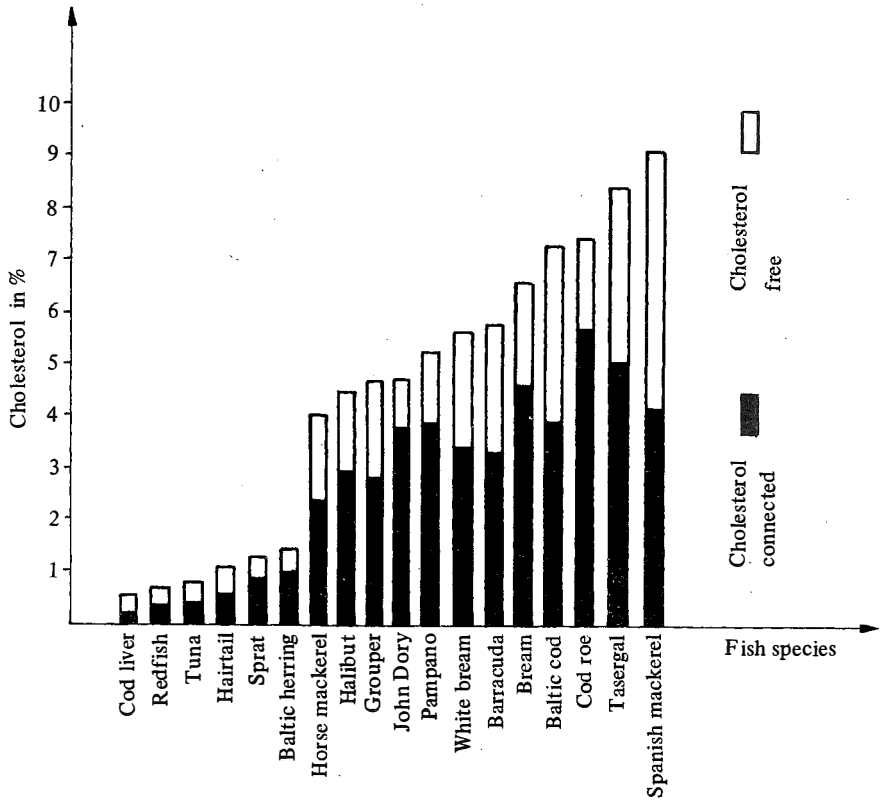

Fig. 1. Free and connected cholesterol content in the fat of marine fish

The fat of cod liver contains small, quantities of free cholesterol: 0.3% – as compared with the fat of the analysed fishthis being 68.2% of total cholesterol.

Mean content of connected cholesterol in the fat of the analysed fish is higher than of the free cholesterol, and amounts to 2.7%, this meaning 59.6% of total cholesterol. Here again, the lowest quantity of connected cholesterol is found in the fat of redfish: 0.28% (this being 48.3% of total cholesterol), while in the Spanish mackerel the value is 4.15% (44.8% of total cholesterol), in tasergal 5.12% (60.2% of total cholesterol).

Analogous to free cholesterol, the lowest quality of conected cholesterol is contained in the fat of cod liver 0.14%, this meaning 31.8% of total cholesterol. The fat of cod roe contains the highest level of connected cholesterol: 5.75% (76.2% of total cholesterol).

The content of unsaponifying substances in the fat of the analysed fish osicllates between 1.4% and 21.3% and amounts to 10.2%, as an average.

Correlation has been found between the quantity of unsaponifying substances and cholesterol content. And so: the lowest cholesterol content in fat has been found in those fish, that have the lowest quantity of unsaponifying substances. For instance: the lowest quantity of in fat unsaponifying substances are found in: redfish (1.4%), tuna (2.2%) and

herring (2.3%), while the highest content have the following fish species: bream (18.0%), tasergal (18.0%) and Spanish mackerel (21.3%). Also the lowest content of cholesterol is found to be in the fat of redfish, tuna and herring, while the highest – in the fat of bream, tasergal and Spanish mackerel.

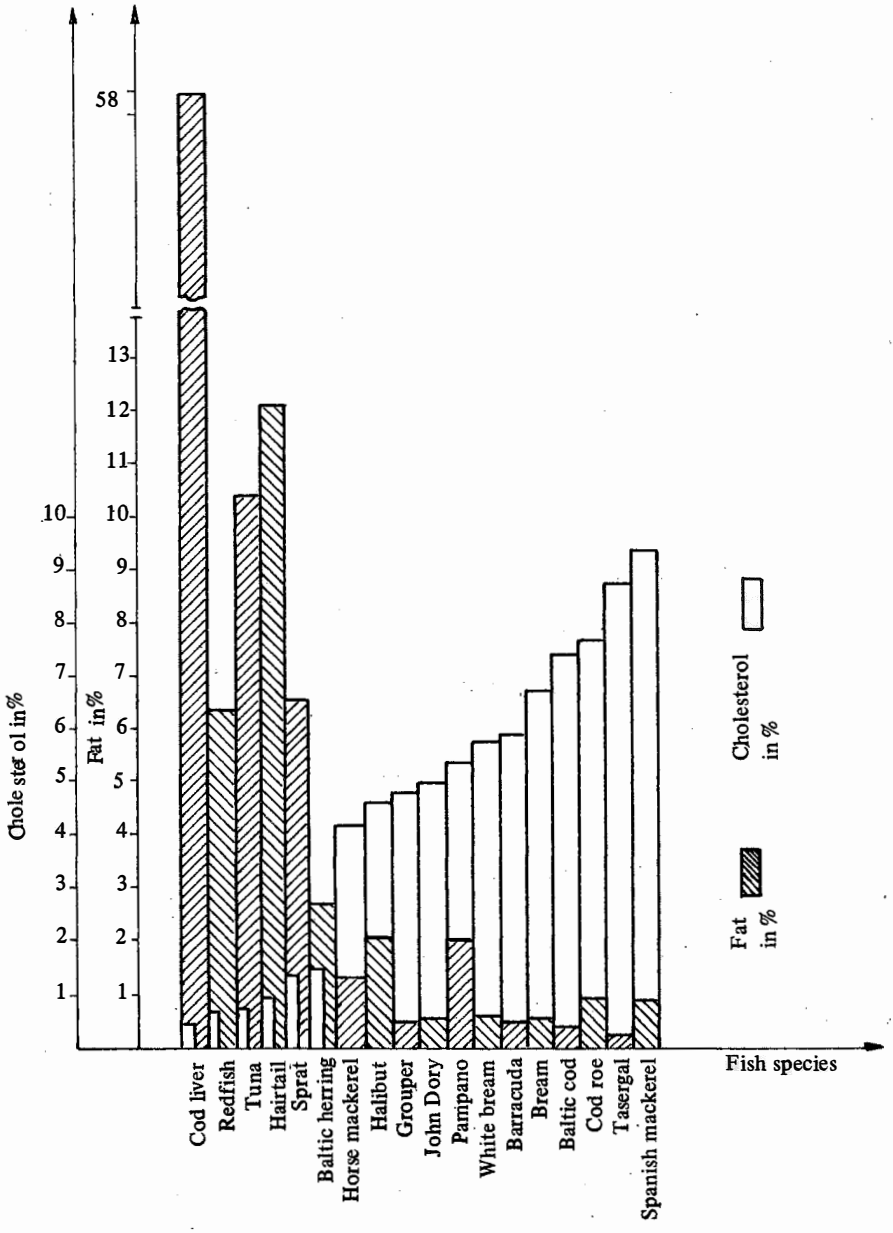

Fig. 2. Cholesterol (in the fat) and fat content in the flesh tissue of marine fish

The results of the investigations lead to the conclusion, that the content of cholesterol an unsaponifying substances in fat, is reciprocal to the quantity of fat in the flesh tissue of the fish (Fig. 2).

And so: fatty fish (as herring, redfish, sprat, tuna hairtail) show lower content of unsaponifying substances than lean fish (cod, bream, tasergal and other species). The content of those substances in the fat of fatty fish scillates 1.4% to 3.3%, while in the fat of lean fish, from 5.7% to 21.3%.

CONCLUSIONS

Basing on the above investigations, the following conclusions may be drawn:

1. Marine fish contain insignificant quantity of cholesterol.
2. The analysed fish species contain in their fat the following mean quantities of cholesterol: 4.53% of total chořesterol, free choresterol being 40.4% of total cholesterol, and the connected cholesterol – 59.6%.
3. Fatty fish, when compared with lean ones, contain lower quantities of cholesterol and of unsaponifying subastances.

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BADANIA NAD ZAWARTOŚCIĄ CHOLESTEROLU W TKANCE MIĘŚNIOWEJ NIEKTÓRYCH RYB MORSKICH

Streszczenie

Przeprowadzono badania nad zawartością cholesterolu ogólnego, wolnego i związanego oraz substancji nie zmydlających się w tkance mięśniowej 16 gatunków ryb pochodzących z łowisk środkowego i północno-zachodniego Atlantyku oraz Morza Bałtyckiego.

Stwierdzono, że zawartość cholesterolu w analizowanych rybach waha się w granicach od 24 mg/100 g do 44 mg/100 g w zależności od gatunku. Średnio – zawartość cholesterolu związanego jest wyższa niż cholesterolu wolnego i wynosi 60% cholesterolu ogólnego. Zawartość sunstancji nie zmydlających się w tłuszczu badanych ryb waha się w granicach od 1,4% do 21,3%.

Na podstawie uzyskanych wyników stwierdzono, że ryby tłuste w porównaniu z rybami chudymi zawierają mniejsze ilości choresterolu i substancji nie zmydlających się.

ИССЛЕДОВАНИЯ НАД СОДЕРЖАНИЕМ ХОЛЕСТЕРИНА В МЫШЕЧНОЙ ТКАНИ НЕКОТОРЫХ МОРСКИХ РЫБ

Р е з ю м е

Проведены исследования над содержанием общего, свободного и связанного холестерина, а также неомыляющихся веществ в мышечной ткани 16 видов рыб, выловленных в районах промысла центральной и северо-западной Атлантики и Балтийского моря.

Установлено, что содержание холестерина в анализируемой рыбе колеблется в пределах от 24 мг/100 г до 44 мг/100 г в зависимости от вида. В среднем содержание связанного холестерина является более высоким, чем содержание свободного холестерина, и составляет 60% общего холестерина. Содержание неомыляющихся веществ в жире исследуемой рыбы колеблятся в пределах от 1,4% до 21,3%.

На основании полученных результатов установлено, что жирная рыба по сравнению с тощей содержит меньшее количество холестерина и неомыляющихся веществ.

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