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INVESTIGATION ON THE ADRIATIC ELASMOBRANCHIA LIVER OILS

by

S. ČMELIK

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S. ČMELIK

(Institut za oceanografiju i ribarstvo Split)

Introduction

In the preliminary report about the oils from the livers of the adriatic Elasmobronchias it was indicated to the possibility of their practical utilisation with regard to the high content of oil in the liver, as well as to the great percentile part of the liver in the weight of their bodies.

In the continuation of these works the physical and chemical characters of oils from the liver of different Selachias were examined, while the researches about the oils from the livers of other sorts of Elasmobranchias will be brought in the next demonstrations.

The greatest part of these sorts can also be found in other seas, thus their oils are examined in a great deal, especially by Tsujimoto, Schmidt-Nielsen and other authors. But as the results of the particular authors diverge, owing perhaps to the different methods of their works or even more perhaps to the ecological factors, the necessity appeared to carry out systematical researches on adriatic sorts by the application of a single method.

EXPERIMENTAL PART

Preparation of samples for analysis

For all the researches in the frame of this work the oils from the livers were obtained by filtering, except the oil of **Scyllium canicula**, which is gained by extraction with the help ot ethyl ether.

Grossly reducend to pieces the livers are kept in a termostate at a temperature of 70—80 degrees C for cca one hour. The separated oil is decanted and filtered over anhydrous natrium sulphate in a funel for the warm filtration. It can be observed that some livers separate the oil very easily, while some of them don't separate anything although they contain a great percentage of oil. From such livers the oil is being obtained by filtration through a tissue for collation. The oils are kept in brown bottles under inert gas (CO_2).

For the analysis native oils are used, without removing the glyceride which is being separated at lower temperatures.

A. PHYSICAL CHARACTERS OF OILS.

The specific gravity of the oil is being fixed by means of a pycnometer and calculated on $\frac{15}{4}$ C. The fixation of the refractive index has been carried out with the refractometer by Zeiss-Volny. The solidification point of the oil has been fixed according to the usual rules.

B. CHEMICAL CHARACTERS OF OILS.

1. The unsaponifiable matter.

The determination of the unsaponifiable part has been decided by Fahrion's method, which has proved to be a specially appropriate one for the examinations of fish oils. The shaking of the unsaponifiable part with the help of ethylether by this method has given much better results than the shaking with petrolether by other methods. The defectivness of Fahrion's method is the fact that in some cases there arise rather long lasting emulsions, which have to be removed by addition of small quantities of alcohol. The unsaponifiable rest is being dried at 100 degrees C to its constant gravity.

2. The free fatty acid value

The determination of the value of acidity has been decided in the usual way by the titration with n/10 KOH together with phenolphatalein as indisator. For the analysis the oilis being dissolved in a 50 ccm mash of equal parts of other and entirely neutral alcohol.

3. Saponification number

By this fixation it has been proceeded according to the rules, indicated in the **Jugoslaw pharmacopoeia.** About 2 gr. oil have been soaped with 25 ccm. of cca. 0,5n alcoholic KOH on a water bath, thus that it was being under a constant boiling for 30 minutes. The hot solution was being titrated with 0,5n HCl with phenolphtalein as indicator.

After that 25 ccm. of 0,5 n KOH was being warmed til its boiling for 30 minutes and titrated with 0,5 n HCl.

If we mark the weighed quantity of oil with A and the quantitö of the alcoholic KOH spent for soaping, the used quantity of oil with D, we get the saponification number calculating by the following formula:

$$\text{saponification number} = \frac{28{,}05 \,.\, D}{A}$$

4. Iodine number

A particular attention has been devoted to the determination of the iodine number. Because of the deficiency of the necessary chemicals the determination by Hanus or Wijs could not be made, therefore the soccalled bromometric method was used, which also gives very exact results. By the effect of kalium bromat on kalium bromide the free brome arises which binds itself on the oil. The superfluity of brome is being retitrated with the help of natrium arsenite.

0,15—0,2 gr. oil is being weighed in Erlenmayers' little flask with a glass stopper and dissolved in 10 ccm. of tetrachlormethane. 50 ccm. n/10 $KBr0_3$, 1 gr. KBr and 10 ccm. of diluted HCl (12,5%) is being added and sorrowly shaken. Before adding the acid the stopper is to be smeared with concentrated phosphor acid because of the isolation.

After 20 hours standing in darkness it is being well shaken again, adding 10 ccm. n/2 Natrium arsenite to it and shaken for so long till the solution gets entirely discoloured. Thereafter 20 ccm. of concentrated HCl is being added and titrated with n/10 $KBr0_3$ with an addition of some drops of indigocarmine till a faint yellow colour.

The blind eäperiment has been made in that way that 10 ccm. tetrachlormetane with 25 ccm. n/10 $KBr0_3$ and 10 ccm. diluted HCl

(12,5%) has been left in the dark for 20 hours, thereafter, after adding 10 ccm. of Natrium arsenite and 20 ccm. of concentrated HCl, titrated with some drops of indigocarmine till a faint yellow colour.

If we mark the number of cubics of n/10 $KBrO_3$ spent on the titration of the oil (50 measured — consumption on the titration) with a, the cubics spent on the titration of the blind experiment (25 measured — consumption on the titration) with b and the weighed quantity of oil with g, then the iodine number is to be calculated by the following formula:

$$\text{iodine number: } = \frac{(a-b) \cdot 1{,}2692}{g}$$

5. Reichert-Meissl' s number.

With the water vapour the fatty acids, which are marked by Reichert-Meissl' s number, are being determined by means of an apparatus of strictly prescribed dimensions. For the analysis 5 g of oil soaped with 2 ccm. 50% NaOH and the addition of 5 ccm. waterless glycerin is being used. After 5 minutes soaping, 90 ccm. hot distilled water, 50 ccm. diluted H_2SO_4 (20 ccm. of concentrated acid on 1 liter of water) are being added.

The distillation is being regulated in that way that 110 ccm. of the distillment is to be put down in 20 minutes. Of the filtrated distillment 100 ccm. with n/10 KOH and an addition of phenolphtalein as indicator are being titrated.

The blind test is performed in the same way, but without the use of oil.

The difference of the consumption of kalium hydrate by the titration at the main test multiplicated with 1,1 gives the Reichert-Meissl's number.

6. The determination of Hehner's number.

By this number the quantity of in water insoluble fatty acides, included the unsaponifiable matter, is being expressed in percentages.

2—3 g of oil is being soaped on a water bath with 50 ccm. alcohol and 1 g. kalium hydrate. After the alcohol was disvapoured, the rest is being dissolved in 50 ccm. warm water and acidulated with 20% HCl till the strong sour reaction. After the

separation of the fatty acids a small weighed quantity of paraffin is being added, less the acides might not pass through the filter. The solution has to be left on a hot water bath, till the fatty acids are separated on the surface and the fluid below them gets clear. When the acids have got solid, the solution is being filtered through a weighed filter and washed for so long till the reaction on chlorids disappears. The filter with the fatty acids is being dried in a small tared bowl for weighing at 95 degress C till its constant weight. Because of the prevention of the oxidation of fatty acids it is being dried in an atmosphere of inert gas.

C. THE PHYSICAL AND CHEMICAL PROPERTIES OF FATTY ACIDS.

The fixation of physical and chemical constants shown on the Table 3 is made according to the former indicated prescriptions. The quantity of insoluble hexabromides is being fixed in the following way:

From the water solution of kalium salts of fatty acids, remained at the determination of the unsaponifiable rest, the fatty acids are precipitated with a 20 percentile HCl acid and shaken with ether. The ether solution is being washed with water till the disappearence of the reaction on chlorides and dried with the help of waterless natrium sulphate. The larger part of ether is being foredistilled on a water bath and the rest removed by drying in the drying place at 60—70 degress C in the current of inert gas.

About 1 g. of the fatty acids is being dissolved in 10 ccm. of ether and cooled in the refrigerator through 10 minutes at -10 degrees C. Into this cooled up solution 0,4 ccm. of bromine are being added, drop by drop from the microbirette at constant stirring. After 2 hours of cooling at -5 degrees C the bromed mass is being moved into the little tube of the centrifuge and centrifuged cca 5 minutes. The etheral solution is being thrown away, while hexabromides are being shaken with 5 ccm. of ether (-10 C) and centrifugated anew. This is being repeated for so long till the hexabromides get entirely white. After drying at 100 degrees C till the constant gravity the weight is being calculated on the used quantity of fatty acids.

Table I.

Physical properties of oils from adriatic Elasmobranchia livers

Species	Specific gravity $d\ \frac{15}{4}$	Refractive index $n\ \frac{20}{D}$	Solidification point	Colour
Acanthias vulgaris Risso	0,9203	1,4769 1,4786	2	light yellow-nearly colourless
Scyllium stellaris L.	0,9215	1,4787	−2	gold coloured
Scyllium canicula L.	—	1,4816	—	light brown
Mustellus vulgaris (Müll. et Henle)	0,9291	1,4805	7	light yellow
Mustellus laevis. Risso	0,9247	1'4749 1,4798	8	orange coloured
Galeus canis Bp.	0,9394	1,4805	6−7	light yellow
Lamna cornubica (Gmel.)	0,9311	1,4836	5 − 6	light yellow-nearly colourless
Squatina angelus Bp.	0,9323	1,4846 1,4875	4	gold coloured

Table II.

Chemical properties of oils from adriatic Elasmobranchia livers

Species	Unsaponifiable matter	Free fatty acid	Saponification No.	Jodine No.	Reichert Meissl No.	Hehrer No.
Acanthias vulgaris Risso	6,28	0,7	176—192,3	156	0,2	98,?
Scyllium stellaris L.	6,32	0,3	195	175—175,7	0,3	94,6
Scyllium canicula L.	—	4,3	201,3	150—150,9	0,9	87,8
Mustellus valgaris (Müll et Henle)	2,56	2	180,9	167	0,8—1	96,4
Mustellus laevis Risso	1,95	0,3	195,3	146	0,1	93,2
Galeus canis Bp.	0.71	0,5	186,7	159,8	0,16	88,4
Lamna cornubica (Gmel)	3,25	1,1—1,3	195,6	176	0,27	92,7
Squatina angelus Bp.	1.05	0,4	191	201,3—206	0,1	95,6

Table III.

Physical and chemical properties of fatty acids from adriatic Elasmobranchia liver oils

Species	So idification point	Melting point	Colour	Neutralization No.	Jodine No.	Unsoluble hexabromides
Acanthias vulgaris Risso	30	32	light brown	178,5	133—139	28,5
Scyllium stellaris L.	28	34	yellow	197,6	113,9 - 120,3	20,9
Mustellus vulgaris (Müll. et Henle)	31	34	light brown	196,1	165,1—168,5	45,9
Mustellus laevis Risso	32	35	orange coloured	193,2	168—170	51,7
Galeus canis Bp.	32	36	light brown	195,2	165,4—167,5	19,7
Lamna cornubica (Gmel.)	32	35	light brown	186,8	185,5	15,5
Squatina angelus Bp.	32	34	brown	190,8	170,5	19,9

Table IV.

Qualitative reactions of adriatic Elasmobranchia liver oils

Species	H_2SO_4 conc	HNO_3 fumans	$CS_2 + H_2SO_4$	$CHCl_3 + H_2SO_4$	Tortelli Jaffe	Phosphomolyb. daenic acid	HCl conc
Acanthias vulgaris Risso	violet	violet	violet	dark violet	after 1 min. intensively green	dark green	—
Scyllium stellaris L.	yellow	feebly violet	brown	yellow	after 5 min feebly green	green	—
Scyllium canicula L.	red brown	brown	red brown	brown	—	light green	—
Mustellus vulgaris (Müll et Henle)	dark violet	violet	dark violet	dark violet	yellow	green	—
Mustellus laevis Risso	red brown	brown	light violet	yellow	after 5 min. olive-green	dark green	—
Galeus canis Bp.	violet	violet	dark violet	dark violet	after 5 min. olive-green	light green	—
Lamna cornubica (Gmel.)	brown	feebly violet	brown	brown	after 5 min. green	light green	—
Squatina angelus B.	red brown	red brown	violet red	brown	after 5 min. green	light green	—

DISCUSSION

In regard of the specific gravity and the refractometrical index it doesen't almost exist any difference between one's own researches and datas, which have been found for the same sorts in other seas. In some cases there have been achieved smaller and in others greater results. For the solidification point of oil there almost have not been found any datas, which could be compared with one's owns, except for the picked dogfish, for which Berlingozzi (3) alleges the solidification point of -3 till — 5 C.

The colour of the oil is characteristical for each sort and only in rare- probably pathological — cases a difference in the colour can be observed. Till, the temperature of 20 degrees C they are clear and transparent. Below this temperature they begin to get troubled and below 15 degrees C the larger part of them separates solid glycerides.

This group of fish oils distinguishes it self generally by a high content of unsaponifiable substances, which reaches by some sorts also 80%. These unsaponifiable substances consist in the main point of unsatured hydrocarbon squalen, further batyl and selachyl alcohol and, cholesterin. All these substances diminish the practical value of oils. In all sorts of adriatic Elasmobranchis, researched up to now, the unsaponifiable rest is a very small one, in some cases even smaller than the value found for the same sorts by other autors. Thus Schmidt-Nielsen and A. Flood (1) have found by the picked dogfish 7,7-12,7%, and Lexov (2) 12,31%. According to the work of Berlingozzi (3) the quantity of the unsoapy rest amounts at this sort to 1,53% thus less than it has been found at one's own researches. Also by some other sorts the own results are greater ones than the value found for the same sorts in other seas. Marcelet (4) has found only 0,9% by the sort **Scyllium stellaris** and Tsujimoto (5) 2,51% of unsoapy part by the mackerel shark (**Lamma cornubica**). The compound of the unsoapy part is not comprehended in these researches.

The free acid value by this oils is a very small one as rule and only in rare cases is it greater than 2. In this point the literary datas are conform with the own results.

The saponification number generally differs between 180 and 195. The same values have been found at the same or similar sorts by other researchers. Solely Schmidt-Nielsen (1) has found a smaller number of saponification (153—166) by picked dogfish (**Acanthias vulgaris**).

At the determination of the iodine number there have been found rather different values. By the picked dogfish the iodine number is a much greater one than the values found by Schmidt-Nielsen (1) 103—128, Lexov (2) 110,1 and Berlingozzi (3) 140. Also by **Squatina angelus** a greater iodine number was found than it is alleged by Lewkowitch (7) 157,3. In the other cases the iodine number conforms with the values found by other authors as far as the question is of oils gained in the same way, e. i. by filtering. According to the quantity of unsaturated acids. these oils can be comprised among druying oils. An exceptive case of the oil from the livers of the Elasmobranchis with a low iodine number (56,5) has been found by Tsujimoto (6) e. i. at a sort of shark (mejiro-zame) from the Pacific.

About the vapourable and in water dissoluble fatty acids there cannot be found any datas in the disposable literature. Solely Berlingozzi alleges the Reichert Meissl's number 0,6 for the **Acanthias vulgaris**. In one's own work the values found are very small ones and correspond to the values of the cod liver oil.

For Hehner s number e. j. the quantity oil in water insoluble fatty acids, the unsaponifiable rest included, there has also been found the data at Berlingozzi (3) e. i. for **Acanthias vulgaris** (94). Of the examined sorts two distinguish themselves by the relatively low Hehner's s number, e. i. **Syllium yanicula** and **Galeus canis,** while at the other sorts it is between 93—98.

In regard of some characters, especialy physical ones, the fatty acids show a great conformation, while in chemical constants, as the iodine number and the insoluble hexabromides they differ considerably. Verifying the datas from the literature it was not possible to confirm Marcelet's (6) statement according to which the acids from the liver oil of **Scyllium stellaris** contains 46% insoluble hexabromides.

Also in regard of the qualitative reactions between these oils there exist certain differences, that is very interesting just with

regard to the fact that the question is of very nearly related sorts. There are in general two tökes, e. i. one, which gives with concentrated sulphur acid and fumans nitric acid a dark violet colouring, and the other, which gives with the same reagents a brown or red-brown colour. Acording to Tortelli-Jaffe the reaction for fish oils is also not equal everywhere. By the picked dogfish it is intense green, by the sort **Mustellus vulgaris** yellow and by the sort **Geleus canis** olive green. It is interesting that the reaction with phosphomolydaenic acid either doesn't always give the same colour, but changes all possible nuances between dark green and light green.

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ISTRAŽIVANJA O ULJIMA IZ JETARA ELASMOBRANCHIA

II. saopćenje

Zaključak:

Ulja iz jetara različitih Elasmobranchia već su više puta bila predmetom istraživanja. Između rezultata pojedinih autora postoje dosta velika razmimoilaženja, što je vjerojatno posljedica različitih metoda rada, a još više možda ekoloških faktora. Da bi se dobio pregled o svojstvima ulja iz jadranskih Elasmobranchia, te ista moglo usporediti sa svojstvima istih vrsta u drugim morima, podvrgnute su sve jadranske vrste sistematskom ispitivanju i to sa istom metodikom. Pri tome su određene i razne druge konstante kojih dosadašnja literatura ne spominje.

U priloženim tabelama izneseni su rezultati izvršenih istraživanja. Za ispitivanje upotrebljena su ulja dobivena iz jetara cijedjenjem i to bez prethodnog uklanjanja čvrstih glicerida koji se počinju izlučivati kod temperature ispod 20 C.

Primjećuje se, da je količina neosapunjivih tvari razmjerno vrlo mala, pogotovo ako uzmemo u obzir da kod ulja iz jetara nekih Elasmobranchia može doseći i do 80%. U pogledu nekih konstanata postoje vrlo male razlike između pojedinih vrsta. Jedino kod jodnih brojeva, te kod netopivih heksabromida postoje znatne razlike između pojedinih vrsta.

Kod kvalitativnih reakcija postoje između ovih ulja stanovite razlike, što je zanimljivo s obzirom na činjenicu da se radi o vrlo blizu srodnim vrstama.

Uspoređujući dobivene rezultate sa svojstvima ulja iz jetara bakalara (**Oleum jecoris aselli**) primjećujemo da između njih postoje samo vrlo male razlike. Prema tome postoji mogućnost praktične upotrebe ovih ulja kao zamjene za bakalarevo ulje, tim više što je na temelju preliminarnih ispitivanja zaključeno da sadrže znatne količine vitamina. Istraživanja o vitaminima bit će objavljena u jednom od slijedećih saopćenja.

ИССЛЕДОВАНИЯ ЖИРОВ ИЗ ПЕЧЕНИ ELASMOBRANCHIA

II. СООБЩЕНИЕ

Вывод

Жиры из печени разных Elasmobranchia были уже несколько раз предметом исследования. Между результатами отдельных исследователей существуют большие расхождения что, вероятно, является последствием различных методов работы, а возможно еще в большей мере последством влия-и ния среды на условия жизни организмов. Чтобы получить картину о качествах жиров от Elasmobranchia Адриатического моря, и что бы их можно было сравнить с качествами жиров тех же сортов из других морей, подвергнуты все сорта жиров Адриатического моря систематическому исследованию по тем же методам. При этом были определены и разные другие постоянные о которых существующая литература не упоминает.

В приложенных таблицах показаны результаты сделанных исследований. Для исследования были взяты жиры, полученные из печени цеженьем без предварительного отстранения твердых глицеридов, которые начинают выделяться при температуре 20^0 С.

Замечается что количество неомыляемых веществ сравнительно очень мало; особенно, если принять во вниманье, что у жиров из печени некоторых Elasmobranchia оно может достигнуть и 80%. Что касается некоторых постоянных существует очень маленькая разница между отдельными сортами. Единственно у иодистых чисел и у нерастворяемых гексабромидов существует значительная разница между отдельными сортами.

В качественных реакциях существует между этими жирами определённая разница, и это очень интересно ввиду того, что это относится на очень похожие сорта.

Сравнивая полученные результаты с качествами жира из печени трески (oleum jecoris aselli), замечаем, что между ними существует очень маленькая разница. Поэтому возможно практическое употребление этих жиров вместо жира трески, тем более, что на основании предварительных опытов заключено, что они содержат значительное количество витаминов. Исследование витаминов будет показано в одном из следующих сообщений.

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