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INVESTIGACION

Preparation of polyunsaturated fatty acid concentrates from the liver oil of dogfish *(Squalus acanthias)* from the Black Sea

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RESUMEN

Preparación de concentrados de ácidos grasos poliinsaturados de aceite de hígado de cazón *(Squalus acanthias)* del Mar Negro.

Se ha determinado la composición en ácidos grasos del aceite de hígado de cazón *(Squalus acanthias)* del Mar Negro, así como sus variaciones estacionales.

Se propone un esquema para la concentración de ácidos grasos poliinsaturados de aceite de hígado de cazón mediante complexión de urea. A partir de 360 g de ácidos grasos libres se obtuvo un concentrado de 48g, que contenía 7,8% $C_{20:4}$, 16,4% $C_{20:5}$, 9,2% $C_{22:5}$ y 49,7% $C_{22:6}$.

PALABRAS-CLAVE: Aceite de hígado de cazón – Ácido graso poliinsaturado (concentrado) – Mar Negro.

SUMMARY

Preparation of polyunsaturated fatty acid concentrates from the liver oil of dogfish *(Squalus acanthias)* from the Black Sea.

The fatty acid composition of the liver oil from the Black Sea dogfish *Squalus acanthias*, as well as its seasonal variations were determined.

A scheme for concentration of polyunsaturated fatty acids from the dogfish liver oil by urea complexation was proposed. From 360g of free fatty acids a 48g concentrate was obtained, containing 7,8% $C_{20:4}$, 16,4% $C_{20:5}$, 9,2% $C_{22:5}$ and 49,7% $C_{22:6}$.

KEY-WORDS: Black sea – Liver oil of dogfish – Polyunsaturated fatty acid (concentrate).

1. INTRODUCTION

The increasing interest in the nutritional and pharmacological properties of the n-3 polyunsaturated fatty acids (PUFA) has led to efforts to develop procedures for the preparation of highly concentrated fractions of some n-3 fatty acids (FA).

Despite the high glyceryl ether content the dogfish liver oil seems to be a good source of PUFA (Ackman et al., 1988; Ratnayake et al., 1988). The fractionation of PUFA from shark liver oil by urea inclusion compounds was performed earlier by Abu-Nasr et al., (1954) which find that free PUFA form urea adducts more difficult than their esters. The isolation of PUFA can therefore be accomplished most easily by fractionation of the free FA. The proposed by Abu-Nasr ten-step fractionation of PUFA appears to be very labourconsuming. An urea complexing of the free FA from the liver oil of *S. acanthias*, followed by esterification and low-pressure distillation of the FA ethyl esters produced a concentrate containing 65.5% PUFA (Ratnayake et al., 1988).

Although in the last years the population of S. *acanthias* in Black Sea increased, until now there are no practical utilization of their liver oil. In this paper we present a suitable method for the preparation of PUFA-concentrates from dogfish liver oil.

2. EXPERIMENTAL

The samples of *S. acanthias* were collected in different regions of Bulgarian Black Sea coast at two seasons (spring and summer). At least four fishes for every experiment were caught.

The livers were separated from the body and placed immediately in ice and transported to the laboratory. The lipids were extracted three times with a mixture of chloroform and methanol (2:1 v/v) in a Waring blender and the combined extracts were purified according to the method of Bligh and Dyer (1959). The further procedures are shown in figure 1.

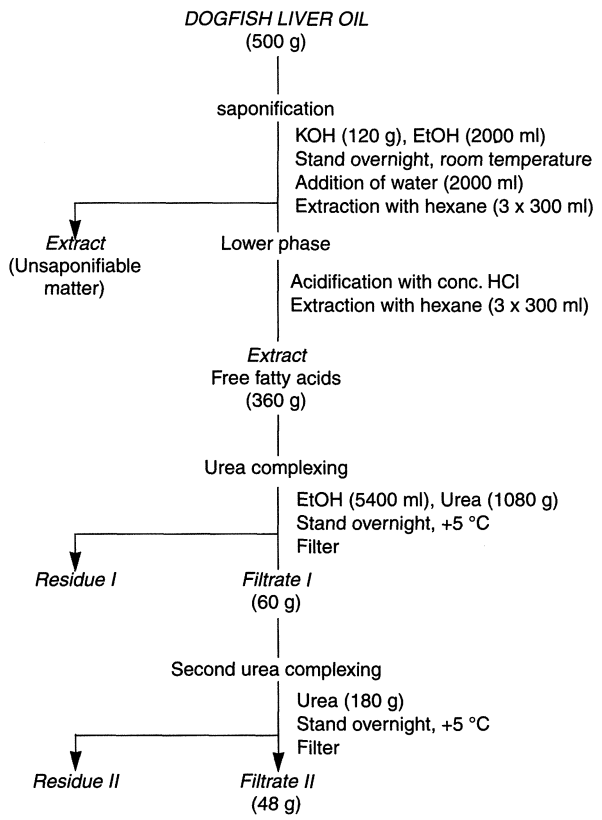

Figure 1

Scheme for concentration of the total fatty acids in Black Sea dogfish *S. acanthias* by urea complexation

3. RESULTS AND DISCUSSION

Table I represents the data on the fatty acid (FA) composition of the liver oil from *S. acanthias* from different regions of the world and from Black Sea. It is evident that the total content of PUFA is almost the same in all samples investigated. The Black Sea shark seems to content twice more docosahexaenoic (22:6) acid than the ocean samples. The high content of docosapentaenoic (22:5) acid in the sample of Nikolaeva et al. (1991) can be due to the seasonal variations in the sharks of Black Sea. The amount of the PUFA is about 20% of the total FA in dogfish oil. It is evident that this mixture cannot be used for pharmacological purposes without further fractionation.

Figure 1 shows a two-step fractionation by urea adducts of the free FA from Black Sea dogfish. The shark oil contains a large amount of ether lipids, so the removal of the unsaponifiable matterial as a first step of fractionation procedure is obligatory. We have studied the influence of the urea: fatty acids: ethanol ratio in the PUFA in the Filtrate I. The best results were obtained when the ratio was 3:1:15. It seems that the same urea/fatty acid ratio is most convenient for preparation of PUFA concentrates from cod liver oil, but with methanol (Haagsma et al., 1982).

The FA composition of Filtrate I and Residue I are shown on Table II. It is evident that Filtrate I contains more than 55% PUFA, while the content of these acids in Residue I is less than 3%. In order to enhance the PUFA concentration, a new portion of urea was added

Table I

Fatty acid composition (wt % of total) of the liver oil of the dogfish Squalus acanthias from different regions

Fatty acid	Sea of Japan* (Hayashi, 1983)	Puget Sound, USA* (Malins et al., 1965)	Atlantic coast, USA* (Gruger et al., 1964)	Pacific coast, USA (Ratnayake et al., 1988)	Black Sea coast (Kozyubra, and Khristofersen, 1984)	Russia (Nikolaeva et al., 1991)**	Black Sea coast, Bulgaria Our data***
14:0	3.0	2.7	1.6	1.7		4.2-4.8	3.3-2.6
15:0	0.4	0.3	0.3	–		0.9-0.8	0.3-0.7
16:0	16.7	23.2	13.2	12.7	20.8	23.3-22.6	15.3-16.8
18:0	3.1	4.0	4.3	4.6		3.3-2.3	3.1-2.2
16:1	6.7	6.8	5.7	5.8	–	6.0-3.4	5.9-6.7
18:1	26.7	35.7	28.5	24.1	42.5	28.9-19.0	25.6-22.8
20:1	10.9	7.0	10.5	5.3	–	4.5-2.7	6.1-6.2
18:2	1.5	0.7	0.7	2.7	4.8	2.1-2.1	2.0-2.5
18:3	1.1	0.5	0.6	1.7	2.4	0.7-0.5	0.2-0.5
18:4	1.3	–	0.8	0.4	–	–	–
20:4	2.0	0.6	0.8	0.3	3.6	0.6-0.4	1.6-1.9
20.5	3.8	3.7	3.7	3.6	6.5	–	3.9-3.3
22:5	1.3	1.5	3.1	2.8		7.4-18.3	1.6-2.5
22:6	5.9	5.1	6.5	6.6	15.0	1.5-3.4	12.1-12.9

* Mean values for the triacylglycerols from male and female speciments
** Seasonal variations winter/autumn
*** Seasonal variations spring/summer

to filtrate I at 60-65 °C. After cooling to +5 °C overnight, a second filtrate was obtained, containing more than 83% PUFA. This composition is very close to the data for PUFA-concentrates from other fish oils (Haagsma et al., 1982; Ackman et al., 1988). It is evident that the proposed scheme is promising for preparation of PUFA-concentrates from the liver oil of dogfish from the Black Sea.

Table II

Fatty acids composition of different fractions after urea complexation of Black Sea dogfish liver oil (see also fig. 1)

Fatty acid	Residue I	Residue II	Filtrate I	Filtrate II
14:0	5.1	4.7	0.5	0.1
15:0	0.8	0.6	traces	traces
16:0	22.2	22.1	14.5	1.4
16.1	9.0	8.4	2.5	0.5
18:0	5.2	5.2	0.3	0.2
18:1	34.7	35.1	11.5	1.4
18:2	2.0	2.2	4.1	4.3
18:3	0.2	0.2	2.0	2.2
20:1	8.5	8.1	0.2	0.2
20:4	0.2	0.6	5.8	7.8
20:5	0.4	1.4	14.0	16.4
22:5	0.5	0.9	5.0	9.2
22:6	1.2	4.6	33.9	49.7
others	10.0	5.9	5.7	6.6

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