

**PHYSICO-CHEMICAL COMPARATIVE
CHARACTERIZATION OF *Squalus acanthias*,
Trygon pastinaca AND *Raja clavata* OILS
FROM THE BLACK SEA**

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ABSTRACT

There is already a tradition in using fish oils in pharmaceutical aim.

The necessities on the existing market of fish oils incorporated in pharmaceutical products are even more accentuated and the using of some native products could be more profitable.

The present paper makes a comparative presentation of the physico-chemical, biochemical and biologic active characteristics of the oils extracted from spiny dogfish (*Squalus acanthias*), sting ray (*Trygon pastinaca*) and thornback ray (*Raja clavata*).

The obtained data show a complex composition of the oils, which can be used in different extern and intern inflammatory scopes.

KEY WORDS : physico-chemical characteristics, fish oil, spiny dogfish, sting ray, thornback ray

INTRODUCTION

Elasmobranch is the scientific name for a very ancient group of fishes which includes the sharks, skates and rays. As many as 60 species of elasmobranches are native or more or less frequent visitors to waters adjacent to the Black Sea and many of these are caught in fisheries.

In general, elasmobranchs are more often taken as by catch. In fisheries for other species rather than as target species in their own right and most fisheries around the Black Sea take some elasmobranch by catch.

Relative to other animals that are widely held in captivity, research on elasmobranch species is extremely limited. The small amount of research available indicates that this group may be primarily nocturnal, with endogenous and exogenous rhythms specific to each species. However, the vast majority of these studies focus on sharks, with very little reference to ray species (DEMIRHAN *et al.*, 2005).

Spiny dogfish and rays are the most abundant and subject to more research than any other elasmobranch species in the southern Black Sea (MATER *et al.*, 2005), because this species are commonly used as laboratory subjects and aquarium attraction, it is essential that the comparatively characteristics of oils from this group be well understood.

DEMIRHAN (2004) also produced a detailed account for biology and ecology of the spiny dogfish, sting ray and thornback ray. A recent study carried out in the area revealed that the ratios of this elasmobranch species in the total benthic biomass were 16% (GENC, 2002). CILOGLU *et al.* (2002) stated that the ratio of spiny dogfish, sting ray and thornback ray in the total benthic biomass was 24.21%, 2.17% and 1.87%. Spiny dogfish is a piscivorous and opportunist feeder (Ellis *et al.*, 1996). The sting and thornback rays are known to feed predominantly on invertebrate species, namely crustaceans (QUINIOU & ANDRIAMIRADO, 1979; CUNHA *et al.*, 1986; EBERT *et al.*, 1991; SMALE & COWLEY, 1992) although they also feed on fish (ERDEM *et al.*, 2001; MORATO *et al.*, 2003). Due to the narrow continental shelves and geo-ecologic characteristics of the Black Sea (BREWER & MURRAY, 1973), all species occur at depths from 25 to 180 m. Despite the overlap in their geographic and bathymetric distributions, little is known about comparative oils between this elasmobranch.

The present study was, therefore, undertaken to study the physico-chemical characteristics of the oils from this species in the southeastern Black Sea.

MATERIAL AND METHODS

Specimens of the three species were collected in the southeastern Black Sea by commercial purse seines. Sampling was conducted seasonally and 3 sting rays, 3 thornback rays and 2 spiny dogfishes were caught. The minimum and maximum lengths for spiny dogfishes observed was 43.3 and 114.8 cm for females and 38.1 and 123.0 for males, respectively. Sting rays ranged between 34.3 to 88.2 cm for females and 48.0 to 95.0 cm for males. Thornback rays

also ranged between 30.2 to 85.3 cm for females and 40.5 to 90.0 cm for males.

Livers with contents were placed in numbered plastic bags and frozen for subsequent analysis. The liver contents were weighed and the volumes of those were measured to the nearest g and ml, respectively.

The oils from all species was melted and then filtered to eliminate the proteic extracts. Then, for cleaning, the oils were washed with distilled water (BANU, 1977).

Several physico-chemical characteristics were analysed from oils with regards to the importance of those utility in pharmaceutical domains, including:

- dry substance (%), relative density (d_{20}^{20}), refraction index, acidity index, iodine index, saponification index (XFR, 1993) and mineral residue (%), (POPESCU *et al.*, 1986).

The dry substance was realized using 2-3 g of sample and then was dried at $105 \pm 2^{\circ}\text{C}$ into etuve device, for 3-4 hours. If the results obtained from two consecutive dries are not been different from 0.1%, the process is considered over.

For mineralization was used azotes and perchloric acid. The glass was then warmed until complete discoloration.

The relative density of the sample was determined using a picnometer filled with 1 ml oil.

The refraction index was obtained using an ABBE Refractometer (Carl Zeiss, Jena, 389820 type) from Physico-optics laboratory (Ovidius University of Constantza). The oil was solubilised in ethanol (0.25 mg/25 ml ethanol) and then the refraction index was measured.

The acidity index was measured by titration of the oil with KOH, after that solubilisation into ethanol/ether mixture (1:2 v/v) was realized.

The iodine index was determined as follows: 0.11 g sample were dissolved in 10 ml CHCl_3 and subsequently stirred in 25 ml of Hannus solution (keeping the mixture in a dark place for 1 h at room temperature). Then, 20 ml of a KI solution in 100 ml water was added and 6 ml $\text{Na}_2\text{S}_2\text{O}_3$ in the presence of 1 ml amidon solution until disappearance of the blue colour. Blank sample (no oil) was analysed in parallel using the same procedure and quantity.

The saponification index was also determined. 2 g sample were stirred in 25 ml KOH and extracted in a sohxlet apparatus for 1 hour, until complete saponification. The final mixture was then titrated with a HCl 0.5N solution using phenolphthalein as indicator.

All data were statistical analysed by Student (t Test) two-tails distribution. The level of signification was accepted at $p < 0,05$. Data are

presented as mean values $\pm$ S.D. (standard deviation) (CONOVER & IMAN, 1981).

UV spectra were recorded on a GBC CINTRA 5 Spectrophotometer in 10 mm quartz cells. 1 g oil was dissolved in isopropanol (1:10, v/v) and then the absorption measurements were collected in the 210-390 nm range (POGANY & BANCUIU, 1983).

RESULTS AND DISCUSSION

The physico-chemical characteristics of the three oils are presented in Table 1.

Table 1

Physico-chemical characteristics of the *Squalus acanthias*,
Trygon pastinaca and *Raja clavata* oils

Entry	Physico-chemical characteristics	<i>Squalus acanthias</i>	<i>Trygon pastinaca</i>	<i>Raja clavata</i>
1.	Dry substance %	97.5 $\pm$ 0.1	96.0 $\pm$ 0.1	98.5 $\pm$ 0.1
2.	Mineral residue %	10.0 $\pm$ 0.1	9.0 $\pm$ 0.1	10.5 $\pm$ 0.1
	Total phosphorus %	2.3 $\pm$ 0.1	2.0 $\pm$ 0.1	3.1 $\pm$ 0.1
	Fe salts % mg/g	0.018 $\pm$ 0.1	0.019 $\pm$ 0.1	0.250 $\pm$ 0.1
	Zn salts mg/g	0.03 $\pm$ 0.1	0.05 $\pm$ 0.1	0.15 $\pm$ 0.1
	Co salts mg/g	0.003 $\pm$ 0.1	0.003 $\pm$ 0.1	0.0075 $\pm$ 0.1
3.	Relative density (d_{20}^{20} , g/cm ³)	0.920 $\pm$ 0.100	0.922 $\pm$ 0.110	0.934 $\pm$ 0.099
4.	Refraction index (n_D^{20})	1.4770 $\pm$ 0.1	1.5140 $\pm$ 0.1	1.7230 $\pm$ 0.1
5.	Acidity index (ml KOH/g)	0.6 $\pm$ 0.5	0.7 $\pm$ 0.5	0.9 $\pm$ 0.5
6.	Iodine index (ml Na ₂ S ₂ O ₃ /g)	150 $\pm$ 0.54	160 $\pm$ 0.55	200 $\pm$ 0.56
7.	Saponification index (ml HCl/g)	160 $\pm$ 0.1	190 $\pm$ 0.1	200 $\pm$ 0.1

Several interesting differences could be found between the liver oils. In terms of dry substance and mineral residue oils from *Squalus acanthias* and *Trygon pastinaca* had similar values compared to oil from *Raja clavata*, which has a much more high concentration of Fe, Zn and Co salts.

Interestingly, there were differences in density between the three oils, being the *Raja clavata* oil more dense compared with *Squalus acanthias* and *Trygon pastinaca* oils. This fact led us to think that there will be significant differences between the oils from spiny dogfish, thornback ray and oil from

sting ray, in terms of compounds present in the mixture. Refraction index of the three oils were also in the XFR limits established for food and pharmaceutical oils.

Nevertheless, a remarkable difference was found in acidity index. The spiny dogfish and sting ray oils were close to 0.2 times little in acidity than oil from thornback ray. This suggests the presence of important quantities in free fatty acids in those mixtures. Moreover, the 0.1 ml KOH/g difference between the *Squalus acanthias* and *Trygon pastinaca* oils suggested that there may not be major differences between the two species in terms of quantity of free fatty acids present in the oils.

The iodine index is characteristic of the content of (poly)unsaturated fatty acids and it is also an indicative of the physiological condition of the marine organisms. The value of the iodine index is also greatly influenced by the position of the double bond(s) with respect to the carboxylic group. Interesting differences were also found for the two oils from *Squalus acanthias* and *Trygon pastinaca* compared to *Raja clavata* oil. The higher iodine index in the *Raja clavata* oil (200 ml Na₂S₂O₃/g) compared to *Squalus acanthias* and *Trygon pastinaca* oils (150 and 160 ml Na₂S₂O₃/g, respectively) suggested a higher content of (poly)unsaturated fatty acids, which is extremely important in terms of nutritive value. The presence of these unsaturated compounds is even more noticeable in *Trygon pastinaca* oil compared to *Squalus acanthias* oil.

The saponification index indicates the average molecular weight of the fatty acids in fats/lipids. The measurements provided extremely interesting results in the particular case of the *Raja clavata* oil, which showed an extremely higher saponification index (200 ml HCl/g) compared to *Squalus acanthias* and *Trygon pastinaca* oils (160 ml HCl/g and 190 ml HCl/g, respectively). This suggest the presence of a higher content of low molecular weight fatty acids in *Raja clavata* oil compared to *Squalus acanthias* and *Trygon pastinaca* oils, in which a higher content of high molecular weight fatty acids was found.

All values obtained for investigated oils were in the range of the minimum-maximum allowed as established in the XFR and CEE regulations (CEE/2472/97 and CEE 796/02) of the European Union Commission.

UV spectra of the *Squalus acanthias*, *Trygon pastinaca* and *Raja clavata* oils, were run after dilution of the oils in isopropanol. The most relevant data which can be extracted from the UV spectra is summarized in table 2.

Table 2

Relevant UV data of the three diluted oils from *Squalus acanthias*,
Trygon pastinaca and *Raja clavata*.

<i>Squalus acanthias</i> 1:10 dilution			<i>Trygon pastinaca</i> 1:10 dilution			<i>Raja clavata</i> 1:10 dilution		
λ (nm)	A (absorb-tion)	Chromo-phore	λ (nm)	A (absorb-tion)	Chromo-phore	λ (nm)	A (absorb-tion)	Chromo-phore
200	0.51	$\nu_{\text{CH}_2=\text{CH}-\text{COOH}}$	-	-	$\nu_{\text{CH}_2=\text{CH}-\text{COOH}}$	200	8.12	$\nu_{\text{CH}_2=\text{CH}-\text{COOH}}$
205	3.12	$\nu_{\text{CH}_3-(\text{CH}=\text{CH})_4-\text{CH}_3}$	-	-	$\nu_{\text{CH}_3-(\text{CH}=\text{CH})_4-\text{CH}_3}$	205	1.13	$\nu_{\text{CH}_3-(\text{CH}=\text{CH})_4-\text{CH}_3}$
-	-	$\nu_{\text{R}-\text{COOR}'}$	210	2.14	$\nu_{\text{R}-\text{COOR}'}$	210	2.52	$\nu_{\text{R}-\text{COOR}'}$
225	3.43	$\nu_{\text{C}_4\text{H}_8-\text{CH}=\text{CH}-\text{COOH}}$	225	3.37	$\nu_{\text{C}_4\text{H}_8-\text{CH}=\text{CH}-\text{COOH}}$	225	4.27	$\nu_{\text{C}_4\text{H}_8-\text{CH}=\text{CH}-\text{COOH}}$
227	3.49	$\nu_{\text{CH}_3-\text{CH}=\text{CH}-\text{CH}=\text{CH}_2}$	227	3.45	$\nu_{\text{CH}_3-\text{CH}=\text{CH}-\text{CH}=\text{CH}_2}$	227	4.75	$\nu_{\text{CH}_3-\text{CH}=\text{CH}-\text{CH}=\text{CH}_2}$
255	3.53	$\nu_{\text{CH}_3-(\text{CH}=\text{CH})_6-\text{CH}_3}$	255	3.52	$\nu_{\text{CH}_3-(\text{CH}=\text{CH})_6-\text{CH}_3}$	225	3.53	$\nu_{\text{CH}_3-(\text{CH}=\text{CH})_6-\text{CH}_3}$
-	-	$\nu_{\text{CH}_3-(\text{CH}=\text{CH})_2-\text{CH}=\text{O}}$	270	1.48	$\nu_{\text{CH}_3-(\text{CH}=\text{CH})_2-\text{CH}=\text{O}}$	-	-	$\nu_{\text{CH}_3-(\text{CH}=\text{CH})_2-\text{CH}=\text{O}}$
-	-	$\nu_{\text{CH}_3-(\text{CH}=\text{CH})_6-\text{CH}_3}$, E vitamin	285	1.25	$\nu_{\text{CH}_3-(\text{CH}=\text{CH})_6-\text{CH}_3}$, E vitamin	-	-	$\nu_{\text{CH}_3-(\text{CH}=\text{CH})_6-\text{CH}_3}$, E vitamin
326	1.65	$\nu_{\text{CH}_2=\text{CH}-\text{CH}=\text{CH}_2}$, A vitamin	-	-	$\nu_{\text{CH}_2=\text{CH}-\text{CH}=\text{CH}_2}$, A vitamin	-	-	$\nu_{\text{CH}_2=\text{CH}-\text{CH}=\text{CH}_2}$, A vitamin

The *Squalus acanthias* oil showed 5 significant bands at 200, 205, 225, 227 and 255 nm. The bands were attributed to different compounds present in the oil as detailed in Table 2. The most interesting chromophore in the mixture

was the A vitamin (retinol) that was identified by its characteristic absorption bands at 326 nm.

Trygon pastinaca oil present much more weak wavelengths of absorption comparatively with *Squalus acanthias* and *Raja clavata* oils. The UV spectra of the diluted oil also revealed the presence of an absorption band at 285 nm, characteristic to E vitamin (α -tocopherol).

The UV spectra of the *Raja clavata* diluted oil exhibited a similar profile that of *Squalus acanthias* and *Trygon pastinaca* oils, but the bands present in the spectra were much diverse. The 200-250 nm range was pretty similar to all three species and may be due to the absorption of the various unsaturated fatty acids in the oils.

In any case, the UV results provided enough data to demonstrate the presence of different characteristic chromophores that were in good agreement with preview physico-chemical analysis.

CONCLUSIONS

We have investigated three oils from different fish species (*Squalus acanthias*, *Trygon pastinaca* and *Raja clavata*) from the southeastern Black Sea. The two oils studied from *Squalus acanthias* and *Trygon pastinaca* were found to be different from *Raja clavata* oil, in terms of physico-chemical proprieties.

The analysed mixtures were found to be very complex, which reinforced the potential utilisation of the oils as nutritional supplement.

All examined species represent an interesting opportunity for further research and investment due to necessity to develop and establish a brand new market of “greener” products from natural resources with specific and tolerant activities to be used in different extern and intern inflammatory scopes.

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