Lipids of marine origin: the rudderfish (*Centrolophus niger*)

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MUSCLE OF RUDDERFISH (*CENTROLOPHUS niger*), or black ruff, a rare mesopelagic fish caught in the South Atlantic, was found to contain 19.3% total lipids. The major part of the lipids (~70%) was unusual in not yielding glycerol but non-saponifiable glyceryl ether diols on alkaline hydrolysis. These ether diols included selachyl (C18:1), chimyl (C16:0) and batyl (C18:0) alcohols in amounts of 54.0, 21.6 and 8.8%, respectively. The remainder of the lipids was normal, comprising 24.2% triacylglycerols and small amounts of phospholipids, free fatty acids, cholesterol and squalene. The phospholipids were similar in composition to those of other fish species, consisting of 56% phosphatidylcholine, 20% phosphatidylethanolamine, 8% sphingomyelin, 7% phosphatidylinositol, 7% cardiolipins, 1% phosphatidylserine and 1% lyso phosphatidylcholine. The fatty acids of total lipids had oleic acid (C18:1, 37.7%) as the main component, whereas the phospholipids contained large amounts of dosahexaenoic acid (C22:6, 33.6%). Conflicting reports about the consequences for health of consuming rudderfish fillets are in circulation, but our work showed no adverse effects on consuming them.

Introduction

Lipids of the rudderfish (*Centrolophus niger*), also known as black ruff, have been investigated by Australian and Japanese workers.[1,2] They found that the lipid content of this fish, which frequents depths of 300–500 m, was in the range of 14–25% (wet basis). The lipids largely belong to the class of diacylglyceryl ethers, yielding glyceryl ether diols, such as batyl alcohol (3-octadecylglyceryl ether, C18:0), chimyl alcohol (3-hexadecylglyceryl ether, C16:0) and selachyl alcohol (3-octadec-9-enylglyceryl ether, C18:1), rather than glycerol, on hydrolysis. On saponification of the lipids, these glyceryl ether diols end up in the non-saponifiable fraction, which is therefore substantial. There are conflicting reports in the literature concerning the health consequences of consuming the fish: some claim a laxative and others a beneficial rather than a laxative effect.[1–3] Neither the Australian nor the Japanese workers made any attempt at isolating and analysing rudderfish phospholipids.

Almost 60 years ago, before the advent of chromatographic techniques, Karnovsky *et al.*[4] at the University of Cape Town developed methods for the analysis of the glyceryl ether diol content in the non-saponifiable fraction of the liver oil of several shark species. They frequently found some of them to be rich sources of these diols. Since only a cursory account of the fat content and the fatty acid composition of rudderfish lipis caught in the South Atlantic has been published,[5] I have investigated these lipids in more detail, especially concentrating on the composition of the diacylglyceryl ethers and phospholipids.

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Experimental

General. Deep-frozen, headed and gutted rudderfish caught by trawlers in the South Atlantic were supplied by the fish inspection team of the CSIR in Cape Town; muscle portions were excised and combined for analysis. The remainder of the fish was stored at –40°C until required for further analysis.

Extraction and purification of the lipids. In total, 203 g of the fish was extracted three times with 300 ml chloroform:methanol (2:1 volume ratio) in a Waring blender and purified by washing in a large volume of water according to the method of Folch *et al.*[6] The chloroform layer was dried over anhydrous sodium sulphate and the solvent removed on a rotary evaporator at a temperature below 40°C. The lipids were finally dried on a freeze drier, resulting in 39.2 g (19.3%) of viscous, straw-coloured oil with a phosphorus content of 0.051%, which solidified in the refrigerator to an off-white material.

Chemical analyses. Analysis of the lipids extracted from rudderfish followed procedures described before[7] except for methods of analysing diacylglyceryl ethers and squalene, which are described below.

Diacylglyceryl ethers and cholesterol. To a weighed portion (400–500 mg) of the lipids was added a small amount (10–20 mg) of stigmasterol (Merck, Darmstadt) as internal standard and the mixture saponified by refluxing with 10 ml 2 M ethanolic potassium hydroxide for 15 min, upon which 10 ml water was added through the top of the condenser and the refluxing continued for another 15 min. After cooling, it was washed with about 30 ml water into a separating funnel and extracted twice with 40 ml diethyl ether that was washed several times with water (until it tested neutral to litmus paper) and dried over anhydrous sodium sulphate.

The ether extract was filtered and evaporated on the rotary evaporator; the residue dried overnight in a desiccator over silica gel, weighed and the non-saponifiable content calculated.

This non-saponifiable fraction was silylated by treatment with 0.5 ml chlorotrimethylsilane (Merck) and 10 drops of hexamethyldisilazane (Merck) in 3 ml anhydrous pyridine. The flask was gently heated while swirling in a small flame for about 1 min and the excess reagent removed in a stream of nitrogen. The sample was dissolved in 2–3 ml hexane and injected in a 6890 Hewlett-Packard gas chromatograph using an HP-5 30-m capillary column with an i.d. of 0.32 mm, with 5% cross-linked PH ME Siloxane as stationary phase and nitrogen as carrier gas. The column temperature was programmed to rise from 150 to 300°C at 4°C per min with the injection port at 250°C and the detector at 300°C. Under these conditions, the internal standard stigmasterol eluted after 38.77 min, cholesterol after 36.28 min and the various glyceryl ether diols between 20.31 and 28.18 min. Chimyl and batyl alcohols, from Sigma (St Louis), eluted after 24.45 and 28.18 min, respectively. P. Nichols (CSIRO Marine Research, Hobart, Tasmania) also analysed the glyceryl ether diols. His analysis agreed with ours.

Squalene. The hydrocarbon squalene was determined in the total lipids by gas chromatography on the 6890 Hewlett-Packard gas chromatograph using the same column as described above; the column temperature was programmed to rise from 200 to 300°C, at 30°C per min. Stigmasterol acetate was used as internal standard; it was prepared (89% yield) by acetylating stigmasterol with acetic anhydride in pyridine, it melted at 142–144°C (lit.[8] 144°) and eluted after 12.96 min. Sigma (St Louis) supplied pure squalene, which eluted after 6.53 min.

Results and discussion

Lipid yield. The total lipid content of rudderfish muscle was 19.3%, which falls within the range of 14–25% quoted by Nichols *et al.*[1] for Australian rudderfish, is similar to the 21.0% found by Mori *et al.*[2] for the fish caught in Japanese waters, and the 22.7% of Davis *et al.*[5] for rudderfish from the South Atlantic. The non-saponifiable content of the lipids was 30.4%, similar to the value of 30.5% found for the Japanese sample.[2]

Glyceryl ether diols. The composition of the glyceryl ether diols is shown in Table 1. It ranged from 3-tetradecylglyceryl ether (C14:0), with retention time of 20.31 min, to 3-eicosaenylglyceryl ether (C20:1), with retention time of 31.18 min; silylated stigmasterol had a retention time of 38.77 min. The major component (54.0%) of the ethers was mono-unsaturated selachyl alcohol, the next in abundance (21.6%) was chimyl alcohol, whereas

Table 1. Glyceryl ether diol composition of rudderfish (*Centrolophus niger*) lipids (as percentages of the corresponding di-*O*-trimethylsilyl ether).

Glyceryl ether diol	%
14:0	3.1
15:0	1.5
16:1	3.6
16:0 (chimyl)	21.6
17:0	3.0
18:1 (selachyl)	54.0
18:0 (batyl)	8.8
20:1	4.4

batyl alcohol the third most abundant (8.8%). These values are somewhat different from those of the fish from Australia and Japan, for which Nichols *et al.*[1] found 65% selachyl, 15% chimyl and 11% batyl alcohol, while Mori *et al.*[2] quote values of 62.5%, 16% and 6.7%, respectively.

Cholesterol. The cholesterol content of the lipids, determined as its *O*-trimethylsilyl ether, was 0.3%, which calculated on the wet muscle is 0.058%, similar to the Patagonian toothfish (*Patagonotothus guntheri*)[9] but much less than the average of 0.13% found for anchovy (*Engraulis capensis*), red eye (*Etrumeus whiteheadii*), maasbanker (*Trachurus trachurus*) and pilchard (*Sardina ocellata,* Jenyns), all pelagic fish caught in South African waters.[10]

Triacylglycerol. The glycerol content of the total lipids was 2.52%, yielding a triacylglycerol concentration of 24.2%, higher than the average of 7% quoted by Nichols *et al.*[1] for lipids of Australian rudder fish. Unfortunately, Mori *et al.*[2] give no quantitative figure for the triacylglycerol content of the Japanese rudderfish, but inspection of their thin-layer chromatogram — that provides only a qualitative assessment — indicates that it was close to our value. It should be mentioned here that a considerable time was expended on trying to convert glyceryl ether diols into glycerol by rupturing the ether linkage without affecting the two free hydroxyl groups. If successful, a glycerol determination[9] would provide a convenient and accurate method for estimating the amount of glyceryl ether diols. So far this has not proved possible.

Squalene. The squalene content was 0.2%, which agrees reasonably well with the value of 0.9% of Mori *et al.*[2] Nichols *et al.*[1] found no squalene in their *Centrolophus niger,* but found large quantities (>80%) of it in the lipids of *Tubbia* species, most likely flabby driftfish (*Tubbia tasmanica*),[11] which confusingly they also refer to as rudderfish.

Free fatty acids. Free fatty acids (FFA), which are an enzymatic spoilage product of fish, were present in an amount of 1.4%. One anomalous sample of rudderfish from Australia contained as much as 21.6% FFA, indicating extensive spoilage, whereas two other samples had FFA contents of 0.6 and 1.0%.[1]

Phospholipids. The total lipids contained 0.051% P, which means 1.46% phospholipids or, based on wet muscle, 0.29%. This value is, like cholesterol, identical to that of the Patagonian toothfish[9] but less than the 0.9% of pilchard and 1.5% of hake (*Merluccius capensis* Castelnau).[12,13]

The phospholipid composition, calculated using an average molecular weight of the fatty acids of 298 (see below), is shown in Table 2. The composition holds no surprises and is similar to that of other fish analysed in our laboratory,[14] with the major component of phosphatidylcholine (56%) followed by phosphatidylethanolamine (20%) and minor quantities of sphingomyelin (8%), phosphatidylinositol (7%), phosphatidylserine (1%), lysophosphatidylcholine (1%) and cardiolipins (7%).

Table 2. Main constituents of rudderfish (*Centrolophus niger*) phospholipids and their calculated composition.

Constituent	Percentage of phospholipids
P	3.6
Total choline	9.66
Lecithin-choline	8.16
Sphingomyelin choline	1.20
Lysolecithin-choline	0.3
Ethanolamine	1.60
Serine	0.13
Myo-inositol	1.44
Calculated composition[a]	
Phosphatidylcholine (lecithin)	56
Phosphatidylethanolamine	20
Phosphatidylserine	1
Phosphatidylinositol	7
Lyso-phosphatidylcholine (lysolecithin)	1
Sphingomyelin	8
Cardiolipins[b]	7

[a]Quoted as whole numbers.
[b]Obtained by difference.

The phospholipids contained no unsaponifiable glyceryl ether diols such as chimyl, batyl or selachyl alcohols: in other words, although the total lipids were abnormal in containing large amounts of these non-saponifiable alcohols, the phospholipids were normal.

Wax esters. Thin-layer chromatography showed there were no wax esters in the lipids of the rudderfish, in agreement with the Australian and Japanese rudderfish,[1,2] although Sargent[15] records that *Centrolophus* sp. (called erroneously *Centropholus*) contains both wax esters and diacylglycerol ethers.

Fatty acid composition. The fatty acid composition of total lipids and phospholipids are recorded in Table 3. The table shows that their compositions are markedly different: total lipids comprise large amounts of oleic (C18:1, 37.7%) and palmitic (C16:0, 20.6%) acids, whereas of the phospholipids the docosahexaenoic acid was the most abundant polyunsaturated fatty acid (C22:6, 33.6%).

Health effects. Conflicting reports about the laxative effects of eating rudderfish prompted us to test this by asking 5 persons in the laboratory to consume portions (about 200 g) of the cooked fish. No adverse effects (such as diarrhoea) were noticed.

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Table 3. Fatty acid composition of rudderfish (*Centrolophus niger*) total lipids and phospholipids (as percentages of the corresponding methyl esters).

Fatty acid	Total lipids	Phospholipids
14:0	3.3	0.5
15:0	0.7	
16:0	20.6	18.9
17:0	2.0	1.1
18:0	5.7	7.0
19:0		1.4
20:0	0.4	
22:0	0.2	
16:1	5.2	1.1
18:1	37.7	15.6
20:1	7.2	0.8
22:1	5.4	
24:1	2.9	0.9
18:2	0.7	
20:4 $n = 6$	1.1	6.1
20:5 $n = 3$	1.9	5.3
20:4 $n = 3$	0.4	
21:5	0.2	0.6
22:6 $n = 3$	3.8	33.6
22:5 $n = 3$	0.4	2.0
Unidentified	0.2	5.1
Average molecular mass	290	298

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