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AN INITIAL INVESTIGATION OF LIPIDS AND FATTY ACIDS OF NOVA SCOTIAN "SOFT" COD

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"Soft" cod *Gadus morhua* are a regular economic problem in fisheries on the Scotian Shelf. A preliminary study has been conducted and a low fat content in viscera is the principal abnormality. The lipid content, lipid class profiles, and fatty acids of the fillets from two "soft oily" cod were also examined in this study and were relatively similar to those of one "control" fish as well as to "normal" Atlantic cod. It was however observed that the fatty acid details of the soft oily cod were slightly different from those in the literature for "normal" Atlantic cod. The aquarium-held control fish supplied by Fisheries and Oceans Canada also had a slightly different fatty acid composition. Lipids could not be directly associated with the soft cod condition.

La présence de cabillaud "mou" est un problème économique fréquent dans les pêcheries de la plate-forme "Scotian". Une étude préliminaire a montré que l'abnormalité principale est le teneur en lipides bas des viscères. Le teneur en lipides, le profile de classe de lipides, et des acides gras des filets de deux capabillauds "moux et gras" etaient examiné dans cette étude et on été relativement similaires a ceux d'un poisson de contrôle aussi bien qu'a ceux d'un cabillaud d'Atlantique "normale". Cependant, on a remarqué que les détails des acides gras de ces cabillauds étaient légèrement différentes de celles trouvées dans la littérature pour le cabillaud d'Atlantique "normale". La composition des acides gras du poisson fourni par la Département de Pêches et d'Océans du Canada et maintenu en aquarium était aussi légèrement différente. La condition des cabillauds "moux et gras" ne pouvait pas etre liés directement aux lipides.

Introduction

"Soft" cod is a seasonal and localized problem in the Nova Scotian fishing industry but of unknown etiology. In mid to late summer, and usually in specific areas, for example the Emerald Bank, cod *Gadus morhua* can be taken but are difficult to handle and/or process because of the "soft" musculature. This condition is sometimes apparent when the fish are caught, but it may also develop after a few days on ice, and can occur in haddock and other species. Fishermen avoid known soft cod areas and often have to go further afield to catch cod. It is therefore an economic problem of some significance.

Previous investigations by Fisheries and Oceans Canada showed that no known disease, protozoan, or bacterial infection could be associated with the soft cod problem. The observation that such fish were feeding heavily, often on sand launce

Ammodytes americanus, suggested a dietary factor. In an examination of sand launce oil (Ackman and Eaton 1971) a non-saponifable content of 2.4% was noted, but the material was not defined. This was higher by a factor of 2 to 4 than for herring oils, and agreed with a report of 3.56% of unsaponifiable material in North Sea sand launce (Holmer 1967). This suggested an investigation of lipids as a starting point for the soft cod problem.

Occasional reports of soft cod or haddock having a dimethyl sulfide odor (cf. Ackman et al. 1967) also suggested a dietary factor, but not necessarily lipid-oriented.

Materials and Methods

Fish samples

Fillets, stomachs and all other viscera from seven cod samples were supplied to the Canadian Institute of Fisheries Technology on 25th October, 1984 by Fisheries and Oceans Canada, for analyses for lipid content and lipid class and fatty acid profiles. Out of these seven fish, four were categorized by Fisheries and Oceans Canada as "soft oily" cod. The other three were control fish, from stocks maintained in the Fisheries and Oceans aquarium in Halifax. Fish described as dressed had been gutted prior to receipt and viscera were not available. The code names and the description of the samples are given in Table I.

Analyses

The lipids were extracted with chloroform: methyl alcohol: water according to the Bligh and Dyer (1959) procedure. The recovered lipids were analysed for lipid class composition by the Iatroscan (Technical Marketing Ass.) technique (Ackman 1981). Chromarods-S were developed in hexane: diethyl ether: formic acid 97:3:1 (v/v/v). Some selected total lipid samples were transesterified with boron trifluoride: methyl alcohol (Morrison and Smith 1964). The recovered fatty acid methyl esters were examined by Gas-liquid chromatography (*GLC*) on a Supelcowax-10 (Supelco Canada Ltd.) fused silica capillary column, 50 m x 0.24 mm, as described by Ackman (1986).

Table I Description of the samples and their lipid percentages

Sample*	% Lipid (w/w) Fillet	Viscera**	Stomach and Contents
Dressed SO-1	0.77	—	—
Round SO-2	0.71	13.4	0.98
Dressed SO-3	0.61	—	—
Round SO-4	0.63	16.2	2.04
Round CO-1	0.68	55.3	4.82
Round CO-2	0.64	36.6	1.85
Dressed CO-3	0.67	—	—

* SO = "soft, oily" fish
CO = control fish
** including liver
— Not determined

Results and Discussion

The lipid percentages, lipid compositions and the fatty acid compositions of the various cod samples are given in Tables I, II and III respectively.

The Iatroscan flame ionization detector area responses for fillets (Table II) were heavily biased by the dominance of the phospholipids. Conversely, lipids of the viscera and stomachs were dominated by triglycerides. They are therefore reported simply as area % since normal correction procedures (Parrish and Ackman 1985) would have been very difficult to apply.

An unexpected result was the very low lipid content of the viscera of two soft cod (Table I). It should be noted that the lipid content in the viscera is variable, depending mainly on the nutritional and reproductive status of the fish. The viscera did not include ripe or ripening gonads and therefore the liver was quantitatively the most important component. The liver is normally 30-50% fat and plays an important role in the "condition" of the fish (Jangaard et. al. 1967a). As expected the visceral lipids from the two control samples were mostly triglycerides, whereas those of the soft oily cod showed only about 90% triglycerides, the rest being mostly phospholipids (Table II). In a study of Nova Scotian cod livers (O'Keefe, 1984) only 3 of 71 livers had less than 25% lipids.

The variations in the stomach lipid compositions (Table II) are apparently due to the different types of food in the stomachs. The lipid content in the stomachs were low and varied from 0.98 to 4.82%. Visual examination of the soft oily cod (SO-2 and SO-4) showed some undigested clams and fish bones in their stomachs. The stomach contents of the control fish could not be identified by visual examinations. They are normally fed chopped herring, supplemented with beef liver.

Table II Lipid composition of the cod samples as determined by the Iatroscan TLC/FID

Sample	Iatroscan Area % *					
Fillets	SE	FFA**	FFA†	TG	CHL	PL
Dressed SO-1	—	TR	TR	TR	1.4	98.6
Round SO-2	TR	TR	TR	TR	5.4	94.6
Dressed SO-3	TR	TR	TR	TR	4.3	95.7
Round SO-4	—	—	—	—	TR	100
Round CO-1	—	TR	TR	TR	4.7	95.3
Round CO-2	—	TR	—	TR	3.5	96.5
Dressed CO-3	—	—	—	—	1.7	98.3
Viscera						
Round SO-2	—	—	—	90.8	TR	9.2
Round SO-4	—	—	—	90.4	TR	9.6
Round CO-1	—	—	—	100	—	—
Round CO-2	—	—	—	100	TR	—
Stomach						
Round SO-2	—	1.7	2.6	1.2	15.5	78.9
Round SO-4	—	TR	TR	83.4	2.7	13.9
Round CO-1	—	—	TR	95.1	TR	4.9
Round CO-2	—	3.3	4.5	56.7	4.1	29.5

* The Iatroscan TLC/FID responses were not corrected to w/w%. TR, trace; SE, steroid esters; FFA, free fatty acids; TG, triglyceridecs; CHL, cholesterol; PL, phospholipid.

** Primarily saturated acids (e.g. 16:0).

† Primarily polyunsaturated acids (e.g. 22:6n3).

Table III Fatty acid composition of total lipids of selected cod samples in w/w %.

Fatty* Acid	Fillet SO-2	Fillet SO-4	Fillet CO-2	Viscera SO-2	Stomach SO-2
14:0	2.6	2.9	6.0	7.9	6.0
Iso 15:0	—	—	—	0.7	—
15:0	0.7	0.6	0.7	0.9	1.2
Iso 16:0	—	—	—	0.3	—
Anteiso 16:0	—	—	—	0.3	—
16:0	29.2	32.0	24.1	17.9	21.6
17:0	0.3	0.2	0.3	0.3	0.8
Iso 18:0	0.2	0.1	0.2	0.2	0.3
18:0	3.8	3.9	2.6	2.0	5.4
19:0	0.2	0.2	0.1	—	0.2
20:0	—	—	<0.1	<0.1	0.1
Total saturates	37.0	39.9	34.0	30.5	35.6
16:1n7	2.4	2.4	5.2	12.3	5.8
16:1n5	0.5	0.4	0.6	0.4	0.4
18:1n11	—	—	—	—	0.3
18:1n9	6.5	5.7	12.4	12.6	8.3
18:1n7	4.0	2.8	3.0	5.1	3.2
18:1n5	0.3	0.3	0.5	0.4	0.3
20:1n11	0.3	0.2	0.7	1.3	3.2
20:1n9	1.4	1.4	4.0	5.9	2.6
20:1n7	0.1	0.1	0.1	0.5	0.3
22:1n5	—	—	—	0.1	0.1
22:1n11 + 13	0.2	0.6	1.0	0.5	0.3
22:1n9	<0.1	—	0.1	0.5	0.3
22:1n7	—	—	—	0.1	0.1
24:1n9	—	0.1	0.3	0.2	0.2
Total monoenes	15.7	14.0	27.9	42.8	27.2
16:2n6	—	—	—	—	0.5
16:2n4	0.1	0.2	0.4	0.7	1.1
18:2n6	1.1	0.7	2.0	1.3	0.9
18:2n4	0.1	0.1	0.1	0.2	0.2
20:2NMID	—	—	—	—	0.8
20:2n6	0.1	0.1	0.1	0.2	0.2
Total dienes	1.4	1.1	2.6	2.4	3.7
16:3n4	0.3	0.1	0.2	0.4	—
18:3n6	0.1	0.1	0.1	0.1	0.2
18:3n4	0.1	0.1	0.1	0.2	0.2
18:3n3	0.3	0.2	0.3	0.7	0.2

The level of fillet lipids in the present study fell within the range of values expected (0.5-0.7%) for Atlantic cod, *Gadus morhua* (Jangaard et. al. 1967a; Addison et. al. 1968). There was no apparent difference in the fillet lipid levels between the control and the soft oil samples or in relation to visceral lipid.

The lipid class compositions for the fillets (Table II) are all generally similar, except for one soft oily cod (SO-4) which showed even more phospholipid than in the other samples. Probably this finding was correlated with a low visceral lipid. Phospholipid

Table III continued

Fatty Acid	Fillet SO-2	Fillet SO-4	Fillet CO-2	Viscera SO-2	Stomach SO-2
20:3n6	—	—	0.1	<0.1	0.1
20:3n3	0.1	0.1	<0.1	0.1	0.1
Total trienes	—	—	—	0.1	—
16:4n3	—	—	0.2	0.5	0.2
16:4n1	—	—	0.2	0.5	0.2
18:4n3	0.4	0.3	0.4	1.5	0.6
18:4n1	0.1	0.1	0.1	0.2	0.1
20:4n6	1.7	1.4	2.5	0.4	2.2
20:4n3	0.3	0.3	0.3	0.4	0.3
22:4n6	—	—	0.4	—	0.2
Total tetraenes	2.5	2.1	4.0	3.1	3.6
18:5n3	—	—	—	0.1	2.5
20:5n3	17.1	16.9	12.3	8.1	12.1
21:5n3	0.3	0.5	0.3	0.3	0.6
22:5n6	0.6	0.4	0.3	0.2	0.4
22:5n3	0.8	1.4	1.3	1.0	1.6
Total pentaenes	18.8	19.2	14.2	9.7	17.2
22:6n3	24.0	23.6	16.5	10.3	12.5
Calc. iodine value	224.8	220.9	188.1	149.9	179.6

* Fatty acid notation indicates: Number on left of colon is the number of carbon atoms in the fatty acid chain; on the right of the colon, the number of ethylenic bonds; the number to the right of "n" is the position of the ethylenic bond nearest to the terminal methyl group.

was the major lipid component in all the fillet samples, and sterol was the only other quantitatively important lipid component. Triglyceride was found in small amounts in all samples and was found to be accompanied by an equal amount of diacyl glyceryl ethers (Ratnayake et al., 1986), hitherto not reported for cod flesh lipids. It is concluded that there was possibly more lipid in the fillet from the soft oily cod than the controls, but the small number of samples precluded a definitive comparison.

The fatty acid profiles of five cod lipid samples are given in Table III. The fatty acid compositions of the fillets from the two soft oily cod samples (SO-2 and SO-4) were basically similar to those published by Jangaard et. al. 1967b and by Addison et. al. 1968, in studies on the flesh lipids of Atlantic cod, but were different in important details. Palmitic acid (16:0) at 29.2 and 32.0% was much higher than literature values (ca. 20%). Conversely octadecenoic acids (18:1) at 10.8 and 8.8% respectively were less than previous totals of 12-13%. Of the two major polyunsaturated acids, 20:5n3 at about 17% was almost exactly the same as in previous reports, whereas 22:6n3 at about 24% was less than previous reports of 30-33% from the two independent studies.

Unexpectedly the fatty acid composition of the lipids from the control fish differed from previous reports and was also different from the two soft oily cod. According to the Addison et. al. (1968) results, the major fatty acids of male cod flesh are 16:0, 18:1, 20:5n3 and 22:6n3 with weight percentages of 19.6, 13.8, 17.0 and 29.8 respectively. From the Table III results, it is clear that the percentages of the above four major fatty acids from the lipids of the control cod are distinctly different. Especially, the control

cod flesh lipid showed relatively low proportions of 20:5n3 and 22:6n3 and correspondingly was enriched with monoethylenic fatty acids. Unfortunately a sample of only one fish is always suspect (Takahashi et. al. 1985) and a more thorough investigation is indicated. It should be noted that Jangaard et. al. (1967b) averaged 12 seasonal samples and Addison et. al. published averages from four fish of each sex. Whether aquarium-held cod are reliable comparison samples for lipid and fatty acid studies is an open question.

The fatty acid composition of the viscera of SO-2 was similar to that of normal Atlantic cod livers (Jangaard et. al. 1967b; Addison et. al. 1968) with 16:0, 16:1, 18:1, 20:1, 22:1, 20:5n3, and 22:6n3 as major fatty acids. A novel fatty acid, 20:2 Δ 5, Δ 13 was tentatively identified from the stomach of a soft oily cod (SO-2). This and other non-methylene-interrupted fatty acids are minor fatty acids among those of Atlantic molluscs and other invertebrates (Paradis and Ackman 1977). In this connection *Limacina helicina*, a mollusc, is a known factor in the food web transfer of *dimethyl-2-carboxyethyl sulfonium chloride* and its breakdown product, dimethylsulfide, to economically valuable fish such as cod (Ackman et. al. 1967; Botta et. al. 1985) or mackerel (Ackman et. al. 1972). The acrylic acid accompanying the dimethylsulfide could lead to protein breakdown post-mortem but this line of investigation was not pursued. Protease activity (Ando et. al., 1986) seems more probable. These enzymes are known to produce a "jellied" condition in American plaice *Hippoglossoides platessoides* (Templeman and Andrews 1956), or in other species (Konagaya 1984).

These preliminary results suggest that although the lipid content in fillets of "soft cod" is similar to that reported in the literature for normal fish, the visceral lipid is abnormally low. There also appears to be abnormalities in the fatty acid composition of fillets of "soft cod", but substantiation of these inferences requires detailed studies on large samples of control fish and of "soft cod".

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