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CONSIDERATIONS OF FATTY ACIDS IN MENHADEN FROM THE NORTHERN LIMITS OF THE SPECIES

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The comparison of depot fat of menhaden *Brevoortia tyrannus* taken in Nova Scotian waters with other menhaden oils shows surprisingly little difference in qualitative and quantitative detail. It is concluded that the generally higher iodine value of North Atlantic samples compared with Gulf of Mexico samples reflects dietary factors rather than direct environmental effects. Copepods are plentiful in the same waters and modify the fatty acid composition of other fish, but the virtual absence of eicosenoic and docosenoic acids implies that wax esters are not absorbed with conversion of eicosenoic and docosenoic alcohols to the corresponding acids.

La comparaison entre les graisses de réserve de l'alose *Brevoortia tyrannus* capturé dans les eaux de la Nouvelle-Ecosse et celles d'autres aloses ne fait état que de différences étonnament petites tant au point qualificatif qu'au point de vue quantitatif. On conclut que les quantités d'iode en général plus élevées dans les échantillons venant d'Amérique du Nord que dans ceux venant du Golfe du Mexique résultent de facteurs alimentaires plutôt que d'effets environnementaux directs. Il y a une abondance de copépodes dans les mêmes eaux que l'alose et ils modifient la composition en acides gras des autres poissons alors que l'absence de fait des acides eicosénoîque et docosénoîque implique qu'il n'y a pas d'absorption des cires esters suivie d'une conversion des alcools eicosénoîque et docosénoîque en leurs acides correspondants.

Introduction

The Atlantic menhaden, *Brevoortia tyrannus* (Latrobe) 1802, is the principal source of fish meal and fish oil produced along the eastern seaboard of the United States (Tressler 1951; Butler 1967; Simmons 1958; Gruger 1963). Oil production was about 1 x 10^5 tons in 1974 (Anon 1975). The fatty acid composition of the oil was investigated in the late 1950's as part of the interest in relating dietary polyunsaturated fatty acids to serum cholesterol levels in humans (Gruger 1967; Peifer et al. 1962; Peifer 1967; Stansby 1969), and limited analytical compositions have been published for the oil in connection with flavor problems in poultry (e.g. Miller & Robisch 1969; Menge 1971) and swine (Kifer 1967). A comparative analysis of fish-muscle lipids, including Atlantic coast menhaden taken in November, has been published (Gruger et al. 1964). Despite the obvious importance of menhaden, the distribution of depot fatty acids in lots of particular fish has received little attention.

The occurrence of menhaden in Canadian waters may be sporadic (Leim & Scott 1966), although in recent years reports have been fairly numerous (Peppar 1974), including catches in gaspereau nets in January of 1975 in the Bay of Fundy near St. John, N.B. (R.F. Martin, *in verb.*). Changes, purportedly linked to water temperature, have been reported in iodine value of oils produced from fish taken respectively in the Gulf of Mexico or northwards along the Atlantic coast of the United States as far as New Jersey (Simmons 1958; Gruger 1963). A catch of menhaden in southwestern Nova Scotia offered us the opportunity of studying lipids and fatty acids in this species and of determining if any characteristics of the lipid or fatty acids could be ascribed to dietary changes or temperature effects from the colder waters north of Cape Cod.

Materials and Methods

Fish were caught in a seine off the southwest coast of Nova Scotia near Little Harbour, Yarmouth Co., in late June, 1972, and frozen by a commercial firm. Some were shipped to Halifax and in the fall 6 fish weighing about 1 kg each were thawed, weighed, measured, and eviscerated. The bodies were pooled for lipid extraction by the chloroform-methanol procedure of Bligh and Dyer (1959). Bodies were first processed in a Fisk mill and then mixed in a Hobart Silent Cutter. An aliquot was taken for lipid studies. Lipids were separated into polar and neutral fractions on gel columns (Drozdowski & Ackman 1969) with further purification of triglycerides on Adsorbosil-5 "Prekotes" (Applied Science Laboratories, Inc.) developed in hexane:diethyl ether:acetic acid::95:5:1. Methyl esters of triglycerides were prepared and analyzed in both natural and hydrogenated forms on open-tubular gas-liquid chromatography columns as described elsewhere (Ackman & Eaton 1971a; 1971b). A small scale reduction plant in Vancouver (Ackman et al. 1974) provided an oil from whole-fish bodies for comparison. Isolation and identification of unusual fatty acids (Ratnayake 1980) will be published elsewhere.

Results and Discussion

The advanced gonad development (unpublished data) and time of year indicate that our fish should correspond approximately to those found in the usual Atlantic commercial fishery. The moderate fat content of 8.2% for the body suggests however that they had been feeding somewhat less than to the limit of the food supply (Dubrow et al. 1976). This is only one of the difficulties in making comparisons of this type (Stansby 1981). Gruger et al. (1964) recovered 15.5% fat from whole fish by wet rendering. Triglyceride was 85% of the lipid recovered from the bodies of Nova Scotian fish, and the polar lipid, essentially phospholipid, was 15%. These figures are not very dissimilar from those reported for autumn-caught mackerel, *Scomber scombrus* (L.), a typical northwest Atlantic fatty fish from an area overlapping somewhat with the menhaden (Ackman & Eaton 1971b; see also Hardy & Keay 1972, for other lipid details in North Atlantic mackerel). The proportions of the 2 lipid types are similar to those determined by Ueda (1972) for jack mackerel, *Trachurus japonicus* (Temminck & Schlegel). The distribution of lipid among organs and tissues is not exactly the same in all fish with fatty bodies (Ackman 1980), and triglycerides can have varying compositions in different deposits of some species (Ackman & Eaton, 1971b), but not of others (Ackman et al. 1975).

The adult menhaden is a planktivorous fish, remarkable for the efficiency of its filtration process. The commercially produced crude oil has a distinct greenish cast, presumably because of chlorophyll from the phytoplanktic contents of the digestive system. Leim and Scott (1966) reported that copepods and euphausiids are included in the diet. If the menhaden deposits fat in a species-oriented composition, then the triglycerides should reflect the basic plant diet, and the presence of 18:3ω3 at 2.2%, and of a more noticeable amount of 18:4ω3 at 3.8% shows a residual influence of fatty acids from phytoplankters (Ackman et al. 1968; Eaton et al. 1975; see also average composition of phytoplankter fatty acids and discussion in Ackman et al. 1970). Plant fatty acids, such as 16:4ω1 and 18:4ω3, have recently been shown to be high in oils from high-fat feeding capelin and depleted in low-fat spawning capelin (Eaton et al. 1975).

A variety of C_{16} and some C_{18} acids with ω1, ω4, and ω7 unsaturation have been identified in menhaden oil (Ratnayake 1980). The major 16:4ω1 acid was erroneously listed as 16:4ω3 in Ackman et al. (1976). Some of the C_{16} acids were chain extended to 18:2ω4, 18:3ω1, and 18:4ω1, all fatty acids not commonly observed in oils from fish such as herring, mackerel, etc. Other specific analyses of menhaden oils for fatty acids include oils of iodine value 145-147 for 1 year from the Gulf of Mexico, and of iodine value 175 from the Atlantic (Ackman 1980). The median iodine values given by Simmons (1958) or Gruger (1963) are: — gulf (west of Mississippi delta) 152.7; gulf (east of Mississippi delta) 152.5; east coast of Florida, 157.5; Carolinas, 162.8; Chesapeake Bay, 169.2, South Jersey and Delaware, 182.6; and northern New Jersey and Long Island, 176.6. Superficially this indicates that iodine values increase as the production site moves north and the common view (Storch 1966; Stickney 1971; Linden et al. 1973; Fuller et al. 1974; Yavin & Menkes 1974a, 1974b; de Torrengo & Brenner 1976; Wodtke 1978; Hazel 1979; Vigh et al, 1979) would be that this is a reflection of a decrease in water temperature affecting the metabolic needs of the fish. However, it is equally probable that the fatty acids of the food are only partially modified or selected, and deposition in depot fat therefore reflects a dietary factor.

Other analyses not included in Table I (e.g. Hallgren & Stenhagen 1960; Gruger et al. 1964; Peifer et al. 1962; Gauglitz 1967) report quite remarkable variations in individual components. It is worthy of note that neutral lipids from juvenile menhaden (78-83 mm fork length) taken in Virginian waters, and presumably in the carnivorous stage, resemble our adults in fatty acid composition, being slightly higher in 16:0 and lower in 20:5ω3 (Weaver 1974). Dietary fatty acids vary in proportion with food (Jeffries 1975).

Changes in gas-liquid chromatographic detectors and other technological considerations make examination of some details and comparisons of small changes in some fatty acids difficult to interpret unless they are done as a sample group or series in one laboratory. One set of published data (Gruger 1963) shows that the percentage of 22:6ω3 in commercial menhaden oil produced at definite locations in 1960 varied as follows: — Empire, La. (Gulf of Mexico) 3.3; Fernandina Beach, Fla. (Atlantic Coast) 5.0; Beaufort, N.C. 5.0; Port Monmouth, N.J. 5.1 (May); 6.5 (July). Samples specified only as "south" and "north" had 4.8% and 5.6% respectively. It is not always known if samples such as that marked "Reedville" are winterized (cold-cleared, which increases iodine value because the oil then includes more 20:5ω3 and 22:6ω3), but it is clear that the oil from Nova Scotian menhaden has more 22:6ω3 and less 20:5ω3 than oil from Gulf of Mexico fish. It should be specifically noted that the "European" menhaden sample is a blend of several shipments of unwinterized oil.

Analyses of fatty acids of the oil from reduction of whole fish taken off Nova Scotia, and of fatty acids of triglycerides from the body are in reasonable agreement, and show less 16:1 and more 18:1 than the other oils on Table I. This point is of interest because we have found 16:1>18:1 in Nova Scotian sand launce *Ammodytes americanus* DeKay 1842, whereas in European waters this species has

18:1 16:1 (Ackman & Eaton 1971a). This type of systematic difference, also noted in some oils from capelin, *Mallotus villosus* (Müller) 1777, where 16:1>18:1 is observed in Canadian oils, (Eaton et al, 1975). The observation that 18:1 exceeds 16:1 in menhaden from off Nova Scotia, a proportion quite different from the Gulf samples, is supported by 3 other Gulf samples (Ackman 1980), where the ratio of 18:1 to 16:1 was about 2:3. This is probably important, as in another set of analyses (Gruger 1963), 18:1>16:1 is definitely associated with a colder time of year (May) for a Fort Monmouth, N.J. sample, or the sample of unspecified origin marked "north". As noted earlier, the possibility of technical problems affecting results must always be kept in mind, but it seems reasonably established that in addition to a higher iodine value for oils of more northerly origin, they are marked by 18:1>16:1. Adult menhaden basically sweep up a considerable proportion of phytoplankters (Reintjes 1969). Probably they also ingest some lipids from copepods containing wax esters (Ratnayake & Ackman 1979a; 1979b) and possibly from common euphausids (Ackman et al. 1970). Plants essentially lack 20:1 and 22:1 (Ackman et al. 1970). The menhaden oils differ from the oils of other North Atlantic fatty fish, such as sand launce (Ackman & Eaton 1971a), capelin (Eaton et al. 1975), and herring (Addison et al. 1969), in having 16:1 and 18:1 as almost the only monoethylenic fatty acids, whereas the oils of sand launce, capelin, herring, and mackerel all have at least 10% (total) 20:1 + 22:1. These 2 longer-chain fatty acids are replaced in menhaden oil by higher (25-30 w/w%) total proportions of 14:0, 16:0 and even 18:0, which seldom total more than 20 to 25 w/w% in the other fish oils from Nova Scotian waters.

One monoethylenic fatty acid isomer detail for Nova Scotian fish (Ackman et al. 1976) suggests that when fatty acids are biosynthesized in the menhaden, the route 16:0→18:0→18:1ω9 is preferred over 16:0→16.1ω7→18:1ω7. In considering a large number of samples of monoethylenic fatty acids of marine origin, we have concluded that the former route is taken when food supply is adequate and additional unsaturated acids are not required. The latter route seems to be taken when there is a requirement for unsaturated acids, but is also postulated to take place in capelin in the autumn, when they are feeding heavily, to modify excess 16:0 originating in the C_{16} wax ester alcohol and acid from copepods (Eaton et al. 1975). The low proportion of 18:1ω7 to 18:1ω9 in the Nova Scotian menhaden, compared with Gulf of Mexico fish (Ackman 1980), suggests that zooplankters the wax-rich copepod type are not important in the diet of this particular lot of menhaden. This view is reinforced by the virtual absence of the 22:1ω11 (and accompanying 20:1ω9) fatty acids of copepod fatty alcohol origin (Ackman et al. 1980; Ratnayake & Ackman 1979a; 1979b). It is remotely possible that menhaden lack the digestive system enzyme which must be present in most pelagic fish (Patton et al. 1975; Sargent et al. 1979) to promote absorption of the 22:1 alcohol. This process leads to the 22:1 fatty acid in the depot fats of herring, mackerel etc. taken in the same area as the Nova Scotian menhaden (Ackman et al. 1980).

The preponderence of analyses showing 20:5ω3>22:6ω3 strongly suggests the close link between menhaden and phytoplankters. Our details on the inclusion of 16:2ω4, 16:3ω4 and 16:4ω1 (Ratnayake 1980), and the 18:3ω3 and 18:4ω3, also of plant origin, support this view. The 20:5ω3 is almost certainly accumulated in large part directly from its plant origin (see Ackman et al. 1968; 1970), and can be converted to 22:6ω3. We suggest that chain extensions and other complex fatty acid transformation in menhaden fatty acid biochemistry emphasize disposal of the 18:2ω6 and 18:3ω3 plentiful in the phytoplankters respectively by catabolism of almost all ingested 18:2ω6, and excess 18:3ω3, and by conversion of some 18:3ω3 to 20:5ω3 and/or 22:6ω3. Notwithstanding differences among menhaden

Table I. Proportions of selected fatty acids (w/w%) of menhaden triglycerides and commercial oils.

	Nova Scotian Fish Triglycerides							
	Laboratory Extract of Body Only	Pilot Plant Reduction of Whole Fish	Other Reduction Oils					
	Halifax Extract	Vancouver Reduction	Reedville[a] (Virginia)	Atlantic[b] (New Jersey)	Gulf of Mexico		Average USA[a]	As Exported to Europe[c]
14:0	8.1	7.9	10.2	10.6	11.8[a]	13.5[b]	12.0	7.3
16:0	25.4	21.5	22.2	23.6	21.8	22.4	22.0	19.0
18:0	4.3	3.1	2.7	3.0	2.7	2.6	2.9	4.2
16:1	8.8	10.4	12.3	14.8	16.2	17.3	14.9	9.0
18:1	17.2	18.8	10.7	12.6	11.9	14.1	17.6	13.2
20:1	1.8	1.4	3.2*	2.7	2.1*	1.6	2.2*	2.0
22:1	0.1	0.5	1.3	1.0	1.4	0.9	1.0	0.6
18:2ω6	2.7	1.4	3.5	1.2	2.3	0.9	1.2	1.3
18:3ω3	2.3	1.4	-	1.0	-	0.4	-	1.3
18:4ω3	3.8	2.9	3.8	0.9	2.6	1.1	1.2	2.8
20:4ω6	0.3	0.1	1.1	0.8	1.6	0.8	0.9	0.2
20:5ω3	10.1	11.6	13.8	13.1	13.2	13.5	11.5	11.0
22:5ω3	0.4	0.3	2.2	1.4	1.4	1.5	1.4	1.9
22:6ω3	8.3	9.2	9.9	6.5	6.0	3.3	5.5	9.1
Iodine Value	138	148	184	-	158	-	-	162

*includes 18:3ω3

[a] Unpublished data courtesy of E.H. Gruger

[b] Gruger (1963), also published in Stansby (1969).

[c] Unilever Research, Vlaardingen, *in litt.*, reproduced by permission of W. Schokker and H. Boerma.

oils, the fatty acid patterns in the triglycerides fit quite well into general marine oil patterns (Harlow et al. 1966; Litchfield et al. 1967; Litchfield 1973).

In cultured rat-brain cells the conversion 18:3ω3→20:5ω3→22:6ω3 may be a 2-step and temperature-dependent process (Yavin & Menkes 1974a), but it is not known if fish require 1 or 2 steps. The basic problem is that studies of this type in fish usually apply to functional membrane *lipids* (de Torrengo & Brenner 1976; Wodtke 1978; Hazel 1979). Very little has been done on the influence of temperature on the fatty acids of triglycerides (Leger et al. 1977). Menhaden have adapted to an environment niche by eating organisms relatively low in fat, by depositing some fatty acids (e.g., 20:5ω3) relatively unchanged, and by converting other food material to the readily biosynthesized saturated acids and their desaturated monoethylenic analogues. It does not seem necessary to involve lower water temperature in northerly latitudes and functional fish biochemistry to account for the higher iodine value of menhaden *oils*. Rather this seems to be a case of a species-peculiar composition dictated by both a minimum energy expenditure on conversion of ingested food and the absence of low iodine value 20:1 and 22:1. We postulate that it is the higher degree of unsaturation of the plant food organisms *in toto* (cf. Vigh et al. 1979), rather than geographic and environmental influence acting directly on the metabolism of the menhaden, which modifies the iodine value of the depot fats and resulting commercial oils.

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