Fatty Acid Composition of Commercial Menhaden, *Brevoortia* spp., Oils, 1982 and 1983

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Introduction

Menhaden, *Brevoortia* spp., oil, the commercial fish oil produced in greatest volume in the United States, has been analyzed for its fatty acid composition by several investigators in recent years (Ackman et al., 1976, 1981; Ackman, 1980; Dubrow et al., 1976). In a summary of published information on fatty acid composition of menhaden oils, Ackman et al. (1981) showed that oils of this fish from colder waters of the Atlantic Ocean are somewhat more unsaturated than those of fish from warmer waters of the Gulf of Mexico. However, most of these data were obtained by chromatographic methods that have become outmoded for the analysis of marine fatty acids.

Ackman (1980) listed the fatty acids of Atlantic and Gulf coast menhaden oils, determined by modern high-resolution wall-coated open-tubular gas-liquid chromatography (GLC). There was, however, no indication of whether these oils were seasonal or annual composites. More recently, Stansby (1981) tabulated the percent ranges of 14 fatty acids of menhaden oils derived from both published and unpublished studies. Included in his report were narrower ranges of values in oils that had been composited annually to eliminate season as a variable. From this, he concluded that seasonal variation is greater than geographic variation in menhaden oils.

As none of these studies has clearly defined the extent of annual, seasonal, and geographic variations in fatty acid composition of commercial menhaden oils, this study was designed with that goal in mind. Compositional differences, if of sufficient magnitude, might suggest the feasibility of selective harvesting of menhaden, depending upon desired oil properties and intended markets for the oil.

Almost all fatty acids of marine plants and animals contain an even number of carbon atoms, generally from 12 to 24, in the molecule. If no double (olefinic) bonds are present, these fatty acids are known as saturates. Unsaturated fatty acids contain from one (monoenes) to a maximum of six (polyunsaturates) double bonds. The fatty acid shorthand notation used in this report has been suggested by the IUPAC-IUB Commission on Biochemical Nomenclature (1977) as a replacement for the "ω" (omega) notation, widely used for many years, but there is no basic difference in the two systems. Both specify, first, the number of carbon atoms and, second, the number of double bonds in the fatty acid molecule. This is followed by the position of the terminal olefinic bond relative to the hydrocarbon end of the molecule, i.e., the end-carbon chain, designated as "ω x" or "(n-x)". The symbols "ω" and "n-" are synonomous and "x" equals the end-carbon chain length. Thus 20:5ω3 and 20:5(n-3) both specify a fatty acid molecule that contains 20 carbons and five double bonds and is a member of the omega-3 family of fatty acids.

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Materials and Methods

Sample Preparation and Storage

During the 1982 fishing season, 12 commercial reduction plants partici-

ABSTRACT—Throughout the fishing seasons of 1982 and 1983, samples of commercially-rendered menhaden, Brevoortia *spp., oils from the coasts of the Atlantic Ocean and Gulf of Mexico were composited monthly and shipped to the Charleston Laboratory of the National Marine Fisheries Service for analysis. The fatty acid compositions of these oil samples, 65 in 1982 and 63 in 1983, were determined by GLC on flexible fused silica, high-resolution capillary columns. A microcomputer was used to assist in identification of 36 selected fatty acids and to provide descriptive statistics. Of these 36 fatty acids, the mean values of 10 fatty acids of nutritional or biochemical importance were statistically tested for annual, seasonal, and geographic differences by ANOVA on a mainframe computer. While there were few if any differences in annual or seasonal means of fatty acids of Atlantic oils, 9 of the 10 fatty acids in the Gulf oils had significantly different ($p < 0.001$) seasonal means and 4 had annual means that differed significantly. The geographic means of both 18:1ω9 and 22:6ω3 were highly different, statistically, in the Gulf oils.*

pated in the sampling program, three on the Atlantic coast and nine on the Gulf coast. Atlantic coast plants included those of two companies in Reedville, Va., and one in Southport, N.C. Gulf coast plants were located in Moss Point, Miss. (3), Empire, La. (1), Houma, La. (1), Intracoastal City, La. (1), and Cameron, La. (3). During the 1983 fishing season, there were 11 participating plants; only the two Reedville plants provided samples from the Atlantic coast. A total of 65 oil samples was received in 1982 and 63 in 1983.

Within each plant, an equal portion of each day's production was set aside to create monthly composite samples, beginning in mid- to late-April on the Gulf coast and in June on the Atlantic Coast and continuing through the month of October at all plants. At the end of each month, after thorough mixing of the composites, subsamples were transferred to 250 ml amber glass bottles with Teflon-lined[1] caps and shipped to the Charleston Laboratory of the NMFS Southeast Fisheries Center. After mixing again on a rotary-action mixer, a portion of each sample was used to completely fill a 15 ml glass culture tube with Teflon-lined cap for storage at −10°C until all monthly samples had been received.

Chemistry and Chromatography

After warming to ambient temperature, each sample was transferred by a hexane rinse (about 20 ml) to a glass-stoppered 125 ml Erlenmeyer flask containing anhydrous crystalline Na_2SO_4. The air in the flask was displaced with N_2 and the contents shaken periodically for 1 hour to remove any contaminating water. The solution was then filtered through phase-separating filter paper into a 50 ml volumetric flask and made to volume with hexane. The concentration of oil in the solution was determined gravimetrically by transferring two 1.0 ml aliquots to tared aluminum weighing pans, evaporating the solvent in a 100°C oven for 30 minutes and reweighing the pans.

To prepare fatty acid methyl esters (FAME) for GLC, duplicate aliquots of the lipid solution, each containing about 35 mg oil, were transferred to two 15 ml conical centrifuge tubes and the solvent evaporated in a N_2 stream. Esters of the neat oil were prepared by the method of Christopherson and Glass (1969).

The esters were separated by GLC (Hewlett-Packard 5830A gas chromatograph) using a wall-coated open-tubular (capillary) flexible fused silica column, 50 m by 0.21 mm, coated with Silar 5-CP (Chrompack Inc., Bridgewater, N.J.). Helium was used as the carrier gas at 60 psig (4.5 kg/cm^2) and a column flow of 1 ml/minute. Nitrogen, the make-up gas, was provided at 40 psig and a flow of 30 ml/minute through the flame ionization detector. During analysis of the 1982 oils, initial analyses were carried out isothermally at 215°C, but as the column aged, resolution of early-eluting components decreased at this column temperature. This difficulty was overcome by carrying out later analyses using a two-step temperature program. The initial temperature of 200°C was held for 39 minutes, then increased to 215°C at 15°/minute to complete the analysis. For 1983 oils, a new column was installed just before beginning the analyses and all samples were analyzed isothermally at 205°C. The fatty acid composition of each sample was reported as area percent composition using a Hewlett-Packard 18850A GC terminal microprocessor.

Data Analysis

For analysis of 1982 data, retention times and percentages of the separated components of each sample were entered manually into a Radio-Shack Model III 48K microcomputer (Tandy Corp., Fort Worth, Tex.) and stored on floppy disks. The FAME were provisionally identified by means of a BASIC computer program that calculates equivalent chain length (ECL) values of the component FAME from their retention times (Jamieson, 1970), compares the ECL's with those of authentic primary and secondary standards, and reports probable identities. As Marmer et al. (1983) have noted, in studies involving GLC analysis of a large number of samples, complete computer automation is undesirable; human intervention is necessary to correct inevitable errors in peak identification or quantitation. Therefore, these tentative identifications were inspected and corrected as necessary with the Model III commercial word processor program, Superscripsit (Tandy Corp.), before any further data manipulation was attempted. Other BASIC programs calculated and tabulated mean percentages, standard deviations, and ranges of values of 36 fatty acids of particular interest in oils from the two regions. From these data, 10 fatty acids were selected for their nutritional or biochemical importance for more sophisticated statistical analysis.

Before the 1983 oils were analyzed, the chromatographic system was interfaced with dedicated microcomputers. An interface board (Hewlett-Packard 18833A digital communications interface) was installed in the gas chromatograph which, under software control, now sends all data (retention times, area counts, and percentages) through an RS-232C serial interface to an Apple IIe 64K microcomputer (Apple Inc., Sunnyvale, Calif.) where they are recorded on floppy disk. When convenient, the data are then transferred to the Radio-Shack microcomputer, using the commercial communications program, Videotex Plus (Tandy Corp.). These disk files provide the data for the identification and descriptive statistics programs.

The mean percentages of the 10 fatty acids of nutritional or biochemical importance were statistically tested for annual, seasonal, and geographic differences by analysis of variance (ANOVA) on a Burroughs B7800 mainframe computer using the program BMDP2V of the BMDP computerized statistical package. The 1982 and 1983 data were analyzed separately using a two-way ANOVA to identify significant differences in seasonal and geographic mean percentages. For the combined 1982-83

[1]Mention of trade names, commercial firms, or specific products or instrumentation is for identification purposes only and does not constitute endorsement by the National Marine Fisheries Service, NOAA.

data, three-way ANOVA was used to calculate significant differences in annual, seasonal, and geographic mean values.

Results

Before beginning analysis of the 1982 oils, a preliminary experiment was carried out to determine the precision of the planned analytic methodology. One of the oils was selected, dried, and transferred with hexane to a 50 ml volumetric flask as described in the previous section. Eight aliquots of the lipid solution, each containing about 35 mg oil, were transmethylated and analyzed by GLC. Mean percentage, the standard deviation, and the number of necessary analytic replications as a function of the relative standard error of the mean were calculated for each of the 10 fatty acids selected as being of particular interest.

These calculations showed that a relative standard error of ⩽4 percent could be expected for each of the 10 fatty acids from a single analysis of each oil (Table 1). With duplicate analyses, a relative standard error of ⩽2 percent could be achieved for all fatty acids except myristic acid (14:0) which would require three analyses to give this relative standard error. Since each GLC analysis required about 72 minutes, not including the time needed to prepare the sample for analysis, three analyses were judged impractical in terms of the total time required for analysis and later data manipulation. Therefore, a duplicated analysis of each oil was accepted as a satisfactory compromise and suitable procedure.

From about 60 component fatty acids in the menhanden oils, 36 were selected for calculation of annual and geographic means over the two 6-month fishing seasons. These 36 fatty acids comprised ⩾96 percent of the total fatty acids in the oils. Branched-chain fatty acids (iso-, anteiso-, and isoprenoid acids) were omitted, as were a few minor components of uncertain identity. Twenty-five fatty acids with an annual mean percentage of ⩾0.2 percent for the years 1982 and 1983 are listed in Table 2 for Atlantic oils and Table 3 for Gulf oils. The annual mean percentages of the major fatty acids were similar in Atlantic and Gulf coast oils and, within experimental error, all fell within the broader ranges reported by Stansby (1981), with the possible exception of 16:0 (palmitic acid) in 1982 Atlantic coast oils. These values also agree well with those reported by Ackman (1980) for Atlantic and Gulf coast oils.

A three-way ANOVA of percentages

Table 1.—Precision of analytic methodology. Replication required to give 1-5 percent relative standard error of the mean (RSEM).

	RSEM				
Fatty acid	1	2	3	4	5
	- - - - - - - Replication - - - - - - -				
14:0	11	3	2	1	1
16:0	2	1	1	1	1
16:1(n-7)	3	1	1	1	1
18:0	3	1	1	1	1
18:1(n-9)	3	1	1	1	1
18:1(n-7)	3	1	1	1	1
18:4(n-3)	2	1	1	1	1
20:5(n-3)	3	1	1	1	1
22:5(n-3)	4	1	1	1	1
22:6(n-3)	5	2	1	1	1

Table 2.—Weight percent composition of fatty acids from commercial Atlantic coast menhaden oils[1].

	1982 (N=13)			1983 (N=10)		
Fatty acid	Mean	±S.D.	Range	Mean	±S.D.	Range
14:0	9.2	1.72	6.6-12.3	8.4	1.00	6.6-10.5
15:0	0.7	0.14	0.5- 1.1	0.6	0.04	0.6- 0.7
16:0	17.6	1.83	14.3-20.4	19.2	1.59	16.3-20.8
17:0	0.8	0.24	0.6- 1.3	1.1	0.14	0.7- 1.3
18:0	3.2	0.39	2.5- 3.7	3.5	0.30	2.9- 4.0
14:1(n-5)	0.3	0.10	0.2- 0.4	0.3	0.05	0.3- 0.4
16:1(n-9)	0.2	0.10	0.2- 0.3	0.2	0.02	0.2- 0.2
16:1(n-7)	11.0	2.37	7.5-14.8	10.1	1.70	7.7-13.4
18:1(n-9)	6.6	1.08	3.9- 8.5	6.8	0.90	5.4- 8.1
18:1(n-7)	3.0	0.28	2.6- 3.4	3.0	0.24	2.6- 3.5
20:1(n-9)	0.9	0.20	0.5- 1.4	0.9	0.17	0.7- 1.2
16:2(n-4)	1.4	0.33	0.9- 2.0	1.4	0.24	1.2- 1.9
18:2(n-6)	1.3	0.20	1.0- 1.6	1.4	0.11	1.2- 1.6
16:3(n-4)	1.7	0.72	0.9- 3.0	1.5	0.20	1.3- 1.9
18:3(n-6)	0.4	0.20	0.2- 0.7	0.3	0.04	0.2- 0.4
18:3(n-3)	1.1	0.39	0.5- 1.7	1.2	0.24	0.8- 1.5
16:4(n-1)	1.2	0.47	0.5- 2.1	1.2	0.40	0.7- 1.9
18:4(n-3)	3.2	1.04	1.5- 4.6	3.3	0.35	2.9- 3.9
20:4(n-6)	1.0	0.41	0.6- 2.1	0.7	0.09	0.6- 0.9
20:4(n-3)	1.4	0.33	0.8- 2.2	1.4	0.10	1.3- 1.6
20:5(n-3)	14.5	1.59	12.3-17.1	14.8	1.68	12.9-18.1
21:5(n-3)	0.7	0.10	0.6- 0.8	0.6	0.04	0.5- 0.7
22:5(n-6)	0.4	0.10	0.3- 0.5	0.2	0.01	0.1- 0.2
22:5(n-3)	2.1	0.24	1.9- 2.7	2.1	0.08	2.0- 2.3
22:6(n-3)	9.5	3.21	4.5-14.5	10.6	1.83	7.3-13.1

[1]Fatty acids not listed but present at <0.2 percent include 20:0, 20:1(n-11), 20:1(n-7), 20:2(n-6), 20:3(n-6), 20:3(n-3), 22:0, 22:1(n-11), 22:1(n-9), and 22:4(n-6).

Table 3.—Weight percent composition of fatty acids from commercial Gulf coast menhaden oils[1].

	1982 (N=52)			1983 (N=53)		
Fatty acid	Mean	±S.D.	Range	Mean	±S.D.	Range
14:0	9.2	0.57	7.9-11.1	8.9	0.43	7.8-10.0
15:0	0.6	0.10	0.4- 0.8	0.6	0.08	0.4- 0.8
16:0	19.8	1.17	16.9-22.8	20.3	1.06	17.7-22.4
17:0	0.8	0.20	0.3- 1.1	0.9	0.12	0.5- 1.0
18:0	3.4	0.33	2.7- 4.3	3.5	0.22	2.9- 3.9
14:1(n-5)	0.2	0.10	0.1- 0.4	0.2	0.04	0.1- 0.3
16:1(n-9)	0.2	0.10	0.2- 0.3	0.2	0.02	0.2- 0.3
16:1(n-7)	11.7	0.87	10.3-14.5	12.0	0.50	11.0-13.9
18:1(n-9)	8.2	1.62	3.9-11.3	8.7	1.31	6.5-12.3
18:1(n-7)	3.0	0.17	2.6- 3.2	3.1	0.09	2.8- 3.3
20:1(n-9)	1.2	0.36	0.5- 1.8	1.3	0.22	0.9- 1.9
16:2(n-4)	1.7	0.20	1.3- 2.2	2.0	0.20	1.7- 2.6
18:2(n-6)	1.1	0.26	0.7- 1.7	0.9	0.15	0.6- 1.2
16:3(n-4)	2.1	0.26	1.5- 2.8	2.5	0.20	2.2- 3.1
18:3(n-6)	0.6	0.10	0.3- 0.8	0.3	0.02	0.2- 0.3
18:3(n-3)	0.8	0.20	0.4- 1.2	0.9	0.20	0.4- 1.2
16:4(n-1)	1.1	0.44	0.4- 2.1	1.1	0.48	0.4- 2.1
18:4(n-3)	2.1	0.26	1.5- 2.8	2.1	0.20	1.6- 2.6
20:4(n-6)	1.0	0.26	0.5- 2.1	1.1	0.22	0.5- 1.4
20:4(n-3)	1.2	0.10	0.8- 2.0	1.1	0.08	0.9- 1.3
20:5(n-3)	13.5	1.26	11.4-17.7	13.3	0.86	11.7-15.8
21:5(n-3)	0.7	0.10	0.5- 0.9	0.6	0.01	0.5- 0.7
22:5(n-6)	0.3	0.10	0.1- 0.5	0.4	0.15	0.2- 0.7
22:5(n-3)	2.3	0.32	1.7- 3.0	2.2	0.33	1.5- 2.9
22:6(n-3)	7.0	1.38	4.2-10.6	6.6	1.32	4.2- 8.2

[1]Fatty acids not listed but present at <0.2 percent include 20:0, 20:1(n-11), 20:1(n-7), 20:2(n-6), 20:3(n-6), 20:3(n-3), 22:0, 22:1(n-11), 22:1(n-9), and 22:4(n-6).

of 10 selected fatty acids in 1982 and 1983 Gulf coast oils showed highly significant differences ($P \leq 0.001$) in the annual means of 16:0, 16:1(n-7)(palmitoleic acid), 18:1(n-7) (cis-vaccenic acid), and 22:5(n-3) (docosapentaenoic acid) (Table 4). No significant differences could be detected in the 1982 and 1983 annual mean percentages of 18:0 (stearic acid), 18:4(n-3) (octadecatetraenoic acid), or 20:5(n-3) (eicosapentaenoic acid). In the Atlantic oils, significant differences in annual mean percentages of the 10 fatty acids were slight or none at all.

Listed in Tables 5 and 6 are the 1982 and 1983 seasonal mean percentages of the 10 fatty acids in Atlantic and Gulf coast oils, respectively. On the Atlantic coast, the menhaden fishery begins in early June, whereas on the Gulf coast it begins in mid- to late-April. As a result, the April-May Gulf coast data were not included in ANOVA calculations since a matching data set was not available from the Atlantic coast plants. Highly significant differences ($P \leq 0.001$) were found in the seasonal mean percentages of all Gulf oil fatty acids except 14:0 ($P \leq 0.01$) (Table 4). In addition, there were significant differences ($P \leq 0.001$) in mean percentage of 18:1(n-9) (oleic acid), 18:1(n-7), and 22:6(n-3) (docosahexaenoic acid) in oils from the different plants. No differences were found in the mean percentages of 20:5(n-3) in oils produced by the nine Gulf plants. In 1982 and 1983 Atlantic oils, little or no significant differences were detected in seasonal means of the 10 fatty acids, and there were no significant differences in mean values of fatty acids in oils from the different plants. Seasonal changes in percentage of six fatty acids from 1982 and 1983 Gulf and Atlantic oils are compared in Figures 1-3.

Although the Gulf coast was not partitioned into eastern, central, and western regions for ANOVA calculations, seasonal percentages of three fatty acids in 1982 and 1983 oils, produced by the three Moss Point plants and the three Cameron plants, are illustrated in Figure 4. As indicated in Table 4, the probability of significant differences in mean annual percentages of 18:1(n-9) in the Gulf oils was low ($P \leq 0.05$), but both seasonal and geographic mean values differed very significantly ($P \leq 0.001$).

Table 4.—Statistical significance of differences in mean percentages of 1982 and 1983 menhaden oils[1].

	Fatty acid									
Year, oil source	14:0	16:0	18:0	16:1(n-7)	18:1(n-9)	18:1(n-7)	18:4	20:5	22:5	22:6
1982-1983, All oils										
Annual	***	***	**	N.S.	N.S.	*	N.S.	N.S.	*	N.S.
Seasonal	***	***	***	***	N.S.	***	N.S.	***	***	***
Geographic	N.S.	***	N.S.	***	***	*	***	***	***	***
1982-1983, Atlantic oils										
Annual	N.S.	*	*	N.S.	N.S.	N.S.	**	*	*	N.S.
Seasonal	N.S.	**	*	*	N.S.	*	N.S.	**	*	*
Plant location	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
1982-1983, Gulf oils										
Annual	**	***	N.S.	***	*	***	N.S.	N.S.	***	**
Seasonal	**	***	***	***	***	***	***	***	***	***
Plant location	N.S.	*	**	N.S.	***	***	*	N.S.	**	***
1982, All oils										
Seasonal	***	***	***	***	N.S.	***	N.S.	***	***	**
Geographic	N.S.	***	*	*	***	N.S.	***	***	***	***
1983, All oils										
Seasonal	***	***	***	***	N.S.	***	***	***	**	***
Geographic	***	***	N.S.	***	***	***	***	***	N.S.	***

[1]*** = $P \leq 0.001$, ** = $P \leq 0.01$, * = $P \leq 0.05$, N.S. = Not significant.

Discussion

One of the more important characteristics of a triacylglycerol (triglyceride) oil is its fatty acid composition, since this often determines feasible uses for the oil. Chemical modification of fatty

Table 5.—Seasonal differences in mean percentages of biochemically important fatty acids in Atlantic coast menhaden oils.

	June				July				August				September				October			
	1982 (3 samples)		1983 (2 samples)		1982 (3 samples)		1983 (2 samples)		1982 (2 samples)		1983 (2 samples)		1982 (2 samples)		1983 (2 samples)		1982 (3 samples)		1983 (3 samples)	
Fatty acid	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.
14:0	10.8	1.59	10.0	0.78	10.0	0.72	8.0	0.49	8.3	0.42	7.5	1.06	9.0	3.04	8.3	0.28	7.6	0.86	8.3	0.42
16:0	15.2	1.12	16.4	0.14	16.9	1.26	18.9	0.49	18.1	0.28	20.5	0.21	19.8	0.85	20.4	0.49	18.9	0.92	19.9	0.28
18:0	2.8	0.46	3.1	0.07	3.1	0.21	3.6	0.21	3.7	0.07	3.9	0.07	3.5	0.35	3.7	0.04	3.5	0.12	3.7	0.07
16:1(n-7)	13.2	0.65	13.1	0.28	12.5	2.21	10.0	1.06	9.2	0.14	8.3	0.85	10.2	3.61	9.5	0.07	9.1	1.76	9.7	0.07
18:1(n-9)	6.6	0.47	6.1	0.28	7.0	0.91	6.5	0.35	7.1	0.14	6.7	1.63	5.1	1.63	6.9	0.63	7.2	1.25	8.1	0.07
18:1(n-7)	3.3	0.06	3.4	0.14	3.2	0.10	3.0	0.14	3.0	0.07	2.8	0.07	2.7	0.14	2.9	0.07	2.8	0.29	2.9	0.01
18:4(n-3)	3.3	1.39	3.2	0.07	2.9	0.92	3.8	0.21	3.3	0.00	3.8	0.02	3.1	2.19	3.2	0.07	3.4	1.22	3.0	0.07
20:5(n-3)	16.1	0.56	17.8	0.34	15.1	1.01	14.7	0.85	13.0	0.28	13.3	0.42	14.7	3.25	13.9	0.78	13.4	0.76	14.6	0.49
22:5(n-3)	1.9	0.06	2.1	0.04	2.0	0.06	2.1	0.05	2.2	0.00	2.2	0.07	2.4	0.49	2.3	0.07	2.4	0.21	2.2	0.07
22:6(n-3)	6.9	1.15	7.8	0.28	7.7	2.82	11.4	1.84	12.2	0.42	12.8	0.35	10.3	5.94	11.3	0.07	11.7	2.75	9.7	0.14

Table 6.—Seasonal differences in mean percentages of biochemically important fatty acids in Gulf coast menhaden oils.

Fatty acid	April-May			June			July			August			September			October		
	1982 (9 samples)		1983 (9 samples)	1982 (9 samples)		1983 (9 samples)	1982 (9 samples)		1983 (9 samples)	1982 (9 samples)		1983 (9 samples)	1982 (8 samples)		1983 (9 samples)	1982 (7 samples)		1983 (8 samples)
	Mean	±S.D.	Mean	Mean	±S.D.	Mean	Mean	±S.D.	Mean	Mean	±S.D.	Mean	Mean	±S.D.	Mean	Mean	±S.D.	Mean
14:0	9.4	0.59	9.0	9.4	0.27	9.0	9.3	0.39	8.6	9.3	0.51	8.8	8.8	0.21	8.9	8.6	0.40	9.2
16:0	18.6	1.07	19.3	19.1	0.65	19.6	19.7	0.73	20.0	20.6	0.86	20.9	20.8	0.99	21.1	20.6	0.75	21.2
18:0	3.2	0.26	3.3	3.2	0.19	3.3	3.4	0.36	3.4	3.6	0.29	3.6	3.7	0.31	3.7	3.7	0.20	3.6
16:1(n-7)	12.7	1.07	12.2	12.2	0.58	12.2	11.7	0.47	11.9	11.5	0.40	11.8	11.0	0.37	11.8	10.7	0.26	12.0
18:1(n-9)	9.2	2.27	10.1	8.0	2.05	9.4	8.0	1.41	8.3	8.5	0.79	8.3	8.0	0.92	8.1	8.2	0.37	8.1
18:1(n-7)	3.1	0.07	3.2	3.1	0.05	3.2	3.0	0.17	3.2	3.0	0.20	3.1	2.9	0.16	3.1	2.9	0.11	3.1
18:4(n-3)	2.3	0.38	2.2	2.4	0.14	2.3	2.2	0.15	2.3	2.0	0.14	2.1	2.1	0.19	2.0	2.0	0.13	2.0
20:5(n-3)	14.7	1.28	14.4	14.1	0.65	14.0	13.7	0.67	13.3	12.7	0.75	12.5	12.7	0.97	12.7	12.9	0.62	12.8
22:5(n-3)	1.7	0.44	1.8	2.2	0.11	1.9	2.4	0.24	2.1	2.4	0.14	2.3	2.5	0.07	2.4	2.7	0.13	2.5
22:6(n-3)	5.7	1.04	5.4	7.0	1.41	6.2	7.1	1.70	7.3	7.0	1.28	7.2	7.7	1.05	7.3	7.7	1.05	6.8
14:0	9.4	0.59	9.0	9.4	0.27	9.0	9.3	0.39	8.6	9.3	0.51	8.8	8.8	0.21	8.9	8.6	0.40	9.2
16:0	18.6	1.07	19.3	19.1	0.65	19.6	19.7	0.73	20.0	20.6	0.86	20.9	20.8	0.99	21.1	20.6	0.75	21.2
18:0	3.2	0.26	3.3	3.2	0.19	3.3	3.4	0.36	3.4	3.6	0.29	3.6	3.7	0.31	3.7	3.7	0.20	3.6
16:1(n-7)	12.7	1.07	12.2	12.2	0.58	12.2	11.7	0.47	11.9	11.5	0.40	11.8	11.0	0.37	11.8	10.7	0.26	12.0
18:1(n-9)	9.2	2.27	10.1	8.0	2.05	9.4	8.0	1.41	8.3	8.5	0.79	8.3	8.0	0.92	8.1	8.2	0.37	8.1
18:1(n-7)	3.1	0.07	3.2	3.1	0.05	3.2	3.0	0.17	3.2	3.0	0.20	3.1	2.9	0.16	3.1	2.9	0.11	3.1
18:4(n-3)	2.3	0.38	2.2	2.4	0.14	2.3	2.2	0.15	2.3	2.0	0.14	2.1	2.1	0.19	2.0	2.0	0.13	2.0
20:5(n-3)	14.7	1.28	14.4	14.1	0.65	14.0	13.7	0.67	13.3	12.7	0.75	12.5	12.7	0.97	12.7	12.9	0.62	12.8
22:5(n-3)	1.7	0.44	1.8	2.2	0.11	1.9	2.4	0.24	2.1	2.4	0.14	2.3	2.5	0.07	2.4	2.7	0.13	2.5
22:6(n-3)	5.7	1.04	5.4	7.0	1.41	6.2	7.1	1.70	7.3	7.0	1.28	7.2	7.7	1.05	7.3	7.7	1.05	6.8

Figure 1.—Seasonal and annual percentages of 14:0 (A) and 16:0 (B) in menhaden oils from Atlantic (circles) and Gulf (dots) coast rendering plants.

Figure 2.—Seasonal and annual percentages of 16:1(n-7) (A) and 18:1(n-9) (B) in menhaden oils from Atlantic (circles) and Gulf (dots) coast rendering plants.

Figure 3.—Seasonal and annual percentages of 20:5(n-3) (A) and 22:6 (n-3) (B) in menhaden oils from Atlantic (circles) and Gulf (dots) coast rendering plants.

Figure 4.—Seasonal and annual percentages of 18:1(n-9) (A), 20:5(n-3) (B) and 22:6(n-3) (C) in menhaden oils from three east Gulf (circles) and three west Gulf (dots) rendering plants.

acid composition, winterization, or hydrogenation, for example, yields fats and oils with different physical properties and potential uses. Partial hydrogenation of menhaden oils and other fish oils is necessary for their incorporation into margarines or shortenings, common utilization products of fish oils in Europe and Canada for many years, although not in the United States since the early 1950's (Stansby, 1973; 1978). Oils such as herring oil that contain relatively greater percentages of saturated and monoenoic fatty acids are somewhat more suitable for partial hydrogenation than more unsaturated oils such as menhaden oil, since they require less hydrogen and catalyst for reaction. However, recent research by biochemists and physicians in a number of countries suggests that highly unsaturated marine oils, particularly those rich in 20:5(n-3), may have great potential in nutritional or therapeutic treatment of certain cardiovascular diseases (Dyerberg et al., 1978; Sanders et al., 1981; Saynor and Verel, 1980; von Lossonczy et al., 1978). Thus, since 20:5(n-3) is one of the principal fatty acids in menhaden flesh and oils, menhaden might be an excellent raw product from which pharmaceuticals or food supplements could be prepared.

The analytic methodology adopted for this study differs from that usually applied in fatty acid analyses of commercial triacylglycerol oils, such as vegetable oils which are generally homogeneous and usually not contaminated with water. Most, if not all, of the oils analyzed in this study were not homogeneous at ambient temperature, but were cloudy or contained particulate material that disappeared upon warming, suggesting the presence of stearines, a mixture of precipitated, saturated fatty acids. To eliminate the possibility of reprecipitation of stearines during drying of the warmed oils, they were not warmed but, instead, were solubilized in hexane before treatment with anhydrous Na_2SO_4 to remove water.

Ideally, GLC analyses of samples in a series should be carried out under the same instrumental methods. However, when a series is as large as that encountered in this study which required 3 months during each of 2 years to complete, modifications in chromatographic conditions may become necessary. In 1982, our isothermal analysis initially permitted baseline separation of 16:4 (n-1) and 18:0, two fatty acids of considerable interest. However, as the column aged, separation of these two components declined and it was necessary to reduce the column temperature to maintain adequate resolution. Analysis of one sample in both isothermal and temperature-programmed modes demonstrated no quantitative differences in composition of the sample.

Stansby has stressed orally (1980) and in print (1981) the importance of adequate sample size in attempting to define the fatty acid composition of oils or lipids from any species of fish. In 1982, about 800,000 metric tons (t) of menhaden were processed by Gulf coast plants; somewhat more, about 850,000 t, were processed in 1983. Assuming the catch were equally divided among the 11 plants operating on the Gulf coast, the oils from the nine participating plants would represent about 650,000 t of menhaden in 1982 and 700,000 t in 1983. Even if this assumption of equality is very inaccurate, the oils from the Gulf plants still represent a very large number of menhaden taken from Gulf waters. A similar estimate for menhaden harvested by the participating Atlantic coast plants is more difficult to derive since 6 of the 13 rendering plants on this coast are multispecies plants. However, menhaden landings for 1982 and 1983 were about 390,000 and 375,000 t, respectively, and only three plants (two in 1983) participated in the sampling program. Thus the Atlantic oils represent a far smaller sampling of the resident menhaden population than the Gulf oils.

Illustrated in Figures 1-3 are seasonal and geographic mean percentages of the six fatty acids present in greatest amount in menhaden oils. None of these fatty acids had significantly different annual mean values in 1982 and 1983 Atlantic oils, and only 16:0 and 16:1(n-7) annual means differed significantly in the Gulf oils (Table 4). However, seasonal and geographic differences were prominent. In both 1982 and 1983, the mean percentages of 16:0 (Fig. 1B) and 18:1(n-9) (Fig. 2B) were significantly greater while those of 20:5(n-3) (Fig. 3A) and 22:6(n-3) (Fig. 3B) were significantly lower in Gulf oils than in Atlantic oils. In addition, in 1983, 14:0 (Fig. 1A) and 16:1(n-7) (Fig. 2A) percentages were also significantly higher in the Gulf oils. Seasonal differences were equally prominent for these six fatty acids, and their seasonal variations were similar in 1982 and 1983 oils from the two regions. Even though menhaden oils from the Gulf contain significantly less 20:5(n-3) and 22:6(n-3) than those of the Atlantic coast, the Gulf oils yield far larger quantities of these polyunsaturates which have great potential as nutritional supplements or therapeutic agents, due to the larger menhaden catch on the Gulf coast.

In general, mean percentages of fatty acids from 1982 and 1983 Atlantic oils agreed well with data reported by Ackman (1980) on menhaden oils from the Chesapeake Bay and mid-Atlantic coastal waters. In contrast, rendered menhaden oils from Nova Scotian-caught fish contained almost twice as much 18:1 (sum of all isomers) (Ackman et al., 1981) as 1982 and 1983 Atlantic oils. Ackman et al. (1981) noted that 18:1 > 16:1 is characteristic of menhaden oils from colder Atlantic waters, whereas 16:1 > 18:1 characterizes menhaden oils from warmer waters of the Gulf (Ackman, 1980). In this study, however, the annual mean percentage of 16:1 exceeded that of 18:1 in both 1982 and 1983 Atlantic oils (Table 2).

Ackman (1980) has described menhaden oils obtained from plants located in eastern, western, and Mississippi Delta regions of the Gulf coast, although the sampling procedures were not detailed. In this comparison, no difference was found in the percentage of 18:1(n-9) in oils from the three areas (mean, 6.2; range, 6.0-6.4) (Ackman, 1980). However, during both 1982 and 1983, substantial differences in the percentage of this fatty acid were found in oils from the three Moss Point plants in the east Gulf and the three Cameron plants in the west Gulf (Fig. 4A). A three-way ANOVA of 1982 and 1983 Gulf oils (Table 4) indicated highly significant differences ($P \leq 0.001$) in both seasonal

and geographic means of 18:1(n-9).

The seasonal percentages of 18:1(n-9) found in each of the Gulf oils for the years 1982 and 1983 are illustrated in Figure 5. Plants located east of the Mississippi River include only the three plants in Moss Point, Miss.; those west of the River include plants in Intracoastal City and Houma, La., and three plants in Cameron, La. Only one of the plants included in this survey is located on the Mississippi Delta, at Empire, La. While the oils may be characterized as originating in east, west, or Delta region plants, the fish processed by any of the plants may have been caught in a different region of the Gulf, although economics would surely encourage harvesting in waters as near the plant as possible. Information kindly provided by W. Borden Wallace[2] supports the conclusion that there are significant differences in the composition of eastern and western Gulf menhaden oils, exemplified by 18:1(n-9) percentages, at least during the first half of the fishing season. In 1982, boats from the Empire plant fished in the western Gulf, along with boats from the Cameron plants during the April-May period. In June, however, they fished the eastern Gulf, along with boats from the Moss Point plants. In 1983, boats from the Empire plant fished exclusively in western waters during April-May and largely in the same region during June. During July and August, fishing occurred in both eastern and western waters, but during September and October, fishing was, again, in the western Gulf. As shown in Figure 5, the 18:1(n-9) content in oils from the Empire plant reflects that in oils from eastern or western plants in accordance with Mr. Wallace's description of fishing areas for boats of the Empire plant.

These geographic and seasonal differences in 18:1(n-9) percentage suggest the possibility that menhaden harvested in the Gulf may represent two biochemically different populations. However, as the menhaden is a filter-feeder whose depot fat is derived from its phytoplankton diet, it could be that the differences

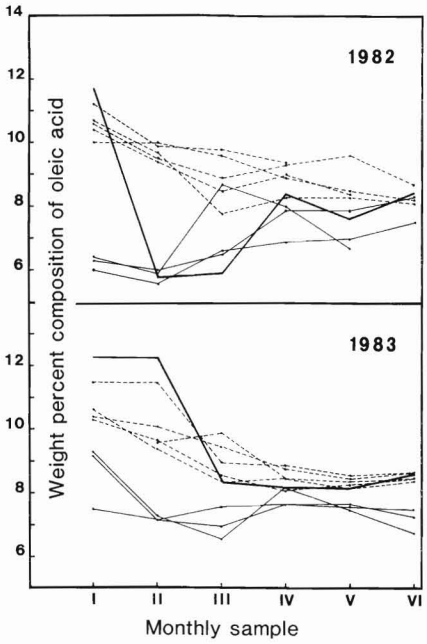

Figure 5.—A comparison of 18:1(n-9) percentages in menhaden oils produced by the plant in Empire, La. (heavy line) with those in oils from five west Gulf (dashed lines) and three east Gulf (light lines) plants.

in fatty acid composition of the menhaden oils are due to differences in phytoplankton populations of the eastern and western Gulf during the early months of the fishing season. Possibly, colder freshwater from the Mississippi River outflow during the late-spring and early-summer months creates an ecologic barrier to mixing of phytoplankton populations. On the other hand, Pristas et al. (1976) have shown that tagged menhaden, returning to inshore waters after winter offshore migration, return generally to the region from which they were originally released, indicating little mixing of fish populations during winter migration. Thus, it may be more likely that differences in phytoplankton populations in waters of their winter residence explain the observed differences in composition of east and west, Gulf-produced, early-season menhaden oils.

Conclusions

The results of this 2-year study of Atlantic- and Gulf-produced menhaden oils do not show differences of sufficient magnitude to support the concept of selective harvesting for specific utilization of the oils. While annual mean percentages of 14 component fatty acids of 1982 and 1983 Atlantic and Gulf coast menhaden oils fell within the broader ranges listed by Stansby (1981), the fact that statistically significant differences were found in the annual mean percentages of some of the fatty acids indicates that no absolute composition for menhaden oil can be predicted from year to year, over and above ranges of values. However, additional data may reveal the existence of annual cycles in fatty acid composition.

Differences in seasonal and geographic means of some fatty acids of Gulf oils, 18:1(n-9) in particular, suggest that fatty acid composition might be used as a biological tag in Gulf menhaden population studies. This application can be realized, however, only if in future studies the compositional data on the oils can be related to the specific waters from which the fish were taken. Even with cooperation of the fishing industry in providing this essential information, a multiyear study will probably be required before reliable conclusions can be drawn. Therefore, composite oil samples collected during the 1984 menhaden fishing season will again be monitored for fatty acid composition by staff of the Charleston Laboratory.

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