Fatty Acid Composition of Oils From 21 Species of Marine Fish, Freshwater Fish and Shellfish[1]

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Abstract

The fatty acid composition of body lipids was determined by GLC for 14 species of saltwater fish, three species of freshwater fish and four species of shellfish. In addition, liver lipids of two species and egg lipids of one species were analyzed for comparison with the fish body lipids. The various species ranged from lean to fatty and contained from 0.7–15.5% oil in the tissues. Certain major fatty acids were found to vary widely among the species, as follows: 1.6–8.0% myristic, 9.5–33.4% palmitic, 2.0–11.2% palmitoleic, 5.2–29.1% oleic, 0.7–10.5% eicosenoic, 5.0–21.5% eicosapentaenoic, 0.2–11.6% docosenoic and 5.9–26.2% docosahexaenoic acids. Analyses of two separate mullet-oil samples illustrated the wide differences that are possible for a single species caught during different seasons. Significant differences in the amt of particular fatty acids were found in comparing freshwater-fish analyses with analyses for marine fish. Oysters and scallops showed large amt of pentaenoic and hexaenoic acids in their oils.

Introduction

THIS INVESTIGATION was undertaken to learn what fatty acids are found in various oils from different commercially important species of fish and to determine if there are any distinct differences among the oils from these species. The species were chosen to include various fish having a wide range of oil contents, specifically from a low of 0.7% to a high of 15.9% oil. Determinations were made of the fatty-acid composition of body lipids from 14 saltwater fishes, three freshwater fishes, and four marine shellfishes. In addition, two species of liver lipids and one species of egg lipids were analyzed for comparison with the body lipids. Our results from GLC show the complexities of fish oil fatty acids in relation to the numerous chain lengths and degrees of unsaturation and to the variable composition of fish species. This is the first time that fatty acid analyses are reported for many of these fish oils.

Fish oils are recognized by researchers as being the best natural source of highly unsaturated fatty acids, notably those acids with more than three ethylenic groups or double bonds. Scientists in the biochemical and nutrition fields now show a growing awareness of the usefulness and importance of fish oils as sources of highly unsaturated fatty acids. Investigations of fish oils, for example, have been reported in relation to the biogenesis of polyunsaturated fatty acids (1,2), biological potency and essential fatty acid activity (3) and hypercholesteremia and heart disease (4–9).

Although there is considerable information about fatty acids from fish oils (10,11), only recently it has been possible to greatly expand our knowledge of the complexities of these fatty acid mixtures with the use of GLC. The complexities of fish lipids have been further demonstrated by reports of fatty acids with branched chains and with odd-numbered carbon atoms (8,12,13). Results from GLC have shown that fish oil fatty acids are far more complex than they were thought to be in the past.

Experimental

The experimental portion of this investigation comprised four stages: the collection of necessary samples of fish, the extraction of lipids from fish tissues, the preparation of fatty acid methyl esters from the lipids and the analyses of the methyl esters by GLC.

Sampling of Fish and Shellfish. The fish collected included five saltwater species from the Atlantic coast, nine saltwater species from the Pacific coast and three freshwater species. In addition, four species of marine shellfish were collected. All except two samples were obtained fresh and were immediately frozen and held frozen until time for extraction of the lipids. The exceptions were crab meat and salmon eggs, as noted later. The number of fish used in each sampling, the dates of catch, and the wt of fish are listed in Table I. Where the wt was unknown, the estimated average wt is given.

Particular sections of fish and whole fish were used to obtain representative samples of the species, as follows: the entire edible portions of ocean perch, sablefish and sea scallop; the entire bodies (including entrials) of menhaden, striped mullet, littleneck clam and Pacific oyster; and the whole livers from Atlantic cod and spiny dogfish. Whole fillets were used from the Atlantic cod, Pacific herring, lake herring mackerel, lake whitefish and rainbow trout, whereas only the dorsal portion of rockfish fillets were used. Steak samples, taken ahead of the dorsal fin, were used in the cases of spiny dogfish, Pacific halibut and four species of salmon. Blue crab meat was obtained from a 1-lb can of commercial pasteurized product. Pink salmon eggs were obtained from freshly frozen skeins from Alaska.

Extraction of Lipids. Prior to extraction of lipids, the samples of fish, fish livers, Pacific oysters and littleneck clams were minced in a mechanical food chopper for two min, which resulted in thorough mixing and permitted the selection of representative homogeneous samples of tissues. By use of the procedure of Bligh and Dyer (14), lipids were extracted from 100 g samples of minced tissues by homogenizing the tissues in a mechanical blender with a mixture of chloroform and methanol. The blue crab meat and sea scallops were extracted without preliminary mincing. The extracted lipids were protected from oxygen by pure dry nitrogen and were stored at −18C until ready for the preparation of methyl esters for analysis. The oil content by this procedure (14) is reported in Table I for the various samples.

Oil from pink salmon eggs was extracted by the technique of Sinnhuber (15). This involved passing 13.6 kg salmon-egg skeins through a coarse meat grinder into 27.3 kg 4% aqueous sodium chloride solution preheated to 55C. The grinder was operated,

[1] Presented in part at the AOCS Meeting, New York, 1960.

TABLE I

Oils from Various Species of Fish and Shellfish

Source of oil			Date of catch	Quantity of fish	Sample weight[b]	Oil in flesh[d]
Classification	Common name	Scientific name[a]				
				No.	Kg	%
Saltwater fishes, Atlantic coast	Atlantic cod	Gadus morrhua	Nov. 1959	10	0.36	**0.7**
	Atlantic cod liver	Gadus morrhua	Nov. 1959	8	0.45[c]	52.6
	Mackerel	Scomber scombrus	Nov. 1959	10	3.18[c]	12.9
	Menhaden	Brevoortia tyrannus	Dec. 1959	8	0.91[c]	15.5
	Ocean perch	Sebastes marinus	Dec. 1959		0.91[c]	2
	Striped mullet	Mugil cephalus	Dec. 1959	5	0.91[c]	2.8
Saltwater fishes, Pacific coast	Spiny dogfish	Squalus acanthias	Dec. 1959	4		14.1
	Spiny dogfish liver	Squalus acanthias	Dec. 1959	4		62.7
	Pacific halibut	Hippoglossus stenolepsis	1959	5	36.0	1.6
	Pacific herring	Clupea harengus pallasi	Dec. 1959	8	0.12	12.8
	Rockfish	Sebastodes pinniger	Nov. 1959	5	1.98	3.1
	Sablefish	Anoplopoma fimbria	Dec. 1959	1	1.36	6.4
	Chinook salmon	Oncorhynchus tshawytscha	Oct. 1959	5	3.91	13.2
	Chum salmon	Oncorhynchus keta	Nov. 1959	5	2.09	3.3
	Coho salmon	Oncorhynchus kisutch	Nov. 1959	5		7.5
	Pink salmon	Oncorhynchus gorbuscha	Oct. 1959	5	2.72	9.2
	Pink salmon egg	Oncorhynchus gorbuscha	Aug. 1960	..	13.62[c]	3.7[e]
Freshwater fishes	Lake herring	Coregonus artedii	Nov. 1959	5	0.18	2.5
	Rainbow trout	Salmo gairdneri	Dec. 1959	5	0.14	2.5
	Lake whitefish	Coregonus clupeaformis	Nov. 1959	1	.....	2.2
Shellfishes	Blue crab	Callinectes sapidus	Nov. 1959		0.45[c]	2.1
	Littleneck clam	Prototheca staminea	Aug. 1960	45	0.018	0.5
	Pacific oyster	Crassostrea gigas	Aug. 1960	12		2.5
	Sea scallop	Placopecten magellanicus	Dec. 1960		0.2[c]	<1

[a] American Fisheries Soc., Special Publication No. 2, 2nd Ed., Ann Arbor, Michigan, 1960.
[b] Average wt of whole fish or sampled portion, e.g. fillets and steaks (cf. Experimental).
[c] Total wt of sampled fish or fish portions.
[d] Oil determined as the extracted lipids based on method by Bligh and Dyer (14).
[e] Mainly free triglycerides from wet rendering process; not solvent extracted.

insofar as possible, in a manner that allowed the eggs to just be broken and not to be ground to an emulsion. To minimize emulsions, we poured some of the warm 4% salt solution through the grinder with the eggs. The egg mash was gently stirred by hand for five min, then covered and allowed to stand undisturbed under nitrogen for four hr at 38–48C. After four hr, the oil was skimmed from the top of the mixture, placed in 500 ml centrifuge bottles and centrifuged at 2100 rpm for 15 min. Finally, the oil was siphoned into containers, bubbled with nitrogen and stored at −18C. The yield of oil by this method was about 4% of the wt of the eggs.

Preparation of Methyl Esters. Following the extraction of lipids from the various fish tissues, methyl esters of the constituent fatty acids were prepared for use in subsequent GLC analyses. Methyl esters were prepared by two methods: a semi-micro methanolysis adapted to the method of Gauglitz and Lehman (16), and the diazomethane method of Schlenk and Gellerman (17). For the latter, we generated diazomethane from N-nitroso-N-methylurea (18). Both methods were chosen because of their mildness and of their minimum destruction of double bonds.

The technique of preparing methyl esters from fish lipids was investigated for the purpose of determining any influence on GLC quantitation. For most lipids, both methanolysis and methylation of fatty acids with diazomethane gave equivalent results when analyzed by GLC. The conversion of lipids from the shellfishes and the fish livers was best, however, when the diazomethane method was used. In the case of the shellfish lipids, which were dark viscous oils, the methyl esters were purified by preparative silicic acid TLC (19) prior to GLC analyses.

Gas-Liquid Chromatography. Methyl esters of the fish oil fatty acids were analyzed with a Research Specialties Co. Series 600 gas chromatograph. The instrument was equipped with an argon ionization detector and strontium-90 source. The column used was composed of 3.7 mm ID by 173 cm borosilicate glass containing 5.10 g 7.0% (by wt) of diethylene glycol succinate polyester (DEGS from Wilkens Instrument and Research, Inc.) supported on 70–80 mesh acid-base washed and siliconized flux-calcined diatomateous earth (Anakrom ABS). Operating conditions were as follows: column temp, 170C; sample vaporizer temp, 290C; detector temp, 200C; outlet temp, 245C; and detector cell voltage, 750 v. A column inlet pressure of 7 psig argon was used, which measured 22.7 ml/min at the column outlet. Injected sample sizes were in the range of 0.15–0.25 μl.

The triangulation method was used to determine the corresponding peak areas from the GLC recorder curves. The fatty acid composition (in wt percentage) of each oil was calculated by multiplying each peak-area percentage by the corresponding mol wt of the fatty-acid methyl ester and subsequently dividing each product by the sum of the weighted products.

Qualitative GLC Technique. Methyl esters of pure fatty acids were used as reference standards for the C_{14} to C_{24} saturated acids, C_{16} to C_{24} monoenoic acids, plus linoleic, linolenic, arachidonic, eicosapentaenoic and docosahexaenoic acids. Also, conc of 16:2 (i.e., hexadecadienoic acid), 16:3, 16:4 and 18:4, obtained by fractional distillation and urea-inclusion compound fractionations, were used as reference standards. The above methyl esters were analyzed on three separate GLC columns, specifically diethylene glycol succinate polyester (as described above), butanediol succinate polyester and Apiezon L. As a secondary reference mixture, methyl esters from whole menhaden oil (12) were also analyzed on the three columns. From a plot of the logarithms of the retention times (relative to stearate) vs. the number of carbon atoms, nearly linear relations were observed for homologous series (12). Identifications were further verified by applying the graphical method of James (20) for analyses on the columns packed with DEGS and Apiezon L. These plots provided the necessary reference data for identification of the various fish lipids analyzed.

We conclude that the important fatty acids in fish lipids can be routinely identified by GLC analysis on a single column packed with DEGS, provided certain requirements are observed. Our requirements are that the column resolution for methyl stearate and oleate be at least 1.00 and that methyl eicosenoate

TABLE II

Equivalent Chain Lengths (ECL) of Methyl Esters of Fish-Oil Fatty Acids Separated on a DEGS Polyester Column at 170C

Fatty acid[a]	ECL	Fatty acid	ECL	Fatty acid	ECL
12:0	12.63	18.1	18 44	20:3(?)	21.72
13:0	13.47	16:4 + 19.0	18.97	20:4	22.13
14:0	14.22	18:2	19.22	22:1	22.34
15:0	15.16	18:2(?)	19.43	22:1(?)	22.66
15:1(?)	15.69	19:+(?)	19.81	20 5	23.12
16:0	16.06	20:0	20.00	22:4(?)	24.20
16:1	16 56	18:3	20.16	24:1	24.46
17:0	17.07	20·1	20.38	?	24.62
16:2	17 50	18:4	20.75	22.5	25 13
18·0	18.00	20·2(?)	21.22	22·6	?5.60

[a] Fatty acid designations as carbon number:double bonds.

(20:1) be resolved reasonably well from 18:3 and 18:4. As the column ages, the peaks for 20:1 and 18:4 gradually coincide, thus necessitating column replacement when they reach a column resolution of ca. 0.70.

Analyses of fatty acid chain lengths by GLC of hydrogenated methyl esters were used to verify peaks for which no standards were available. This verification was important for the analysis of striped mullet oil, which contained fatty acids of odd-carbon chain lengths in amt much greater than were found in the other species. Hydrogenation data for mullet indicated that much of the C_{17} group of fatty acids was comprised of 17:1.

Equivalent chain length (ECL) values, according to the procedure of Miwa (21), are reported in Table II for methyl esters of fish-oil fatty acids. The ECL values coincide with the order of elution from the 7% DEGS column at 170C and with the other conditions described above. These values are used as a basis for confirming the identity of the acids.

The fatty acids listed in Table II that are marked as questions were identified according to their best agreement to the straight line plot of logarithm of retention time vs. carbon number. For example, the 18:2(?) with an ECL value of 19.43 was not the $\Delta^{9,12}$-octadecadienoic acid known as linoleic acid.

No effort was made to isolate individual fatty acids and determine the positions of double bonds, because considerable information is in the literature on the structure of many of the unsaturated fatty acids from fish oils (12,22). It should be noted that whereas we have tentatively identified 22:4(?) to be present in fish oils, Klenk and Eberhagen (22) reported that 22:3 was present in their analyzed oils to the extent of 5–10 times more than 22:4. Also, Klenk and Eberhagen (22) point out that the 20:4 acids in fish oils is composed of the isomeric mixture of $\Delta^{8,11,14,17}$- and $\Delta^{5,8,11,14}$-eicosatetraenoic acid. The latter isomer is the arachidonic acid of mammalian lipids and, if present in fish oils, would be expected to be found in trace amt only (22). Stoffel and Ahrens report these latter isomers in menhaden oil in a ratio of 3:2, respectively (23).

Quantitative GLC Technique. In addition to the technique of preparing methyl esters, several other analytical variables were studied to determine their possible effects on the quantitative results. These variables included the influence on the polyunsaturates of high sample-vaporizer temp in GLC, the nature of the GLC column tubing and how the kind of tubing might influence possible catalysis toward thermal decomposition of polyunsaturates (24). Sample vaporization temp to 300C were found to be satisfactory. GLC columns of aluminum and of borosilicate glass had no influence on the results.

The linearity of the detector response was measured with the use of model mixtures of known methyl esters. These mixtures were used to measure the responses for the range of C_{14} to C_{22} chain length acids and for degrees of unsaturation of 0–5 double bonds. A model mixture containing pure hexaenoic acid methyl ester was not used because of its scarcity The results of four replicate analyses with standard deviations are given, for example, for one of the model mixtures, as follows: methyl palmitate, actual 10.41%, found 9.48 ± 1.06%; methyl stearate, actual 12.74%, found 12.83 ± 0.49%, methyl oleate, actual 20.21%, found 21.57 ± 1.62%; methyl linoleate, actual 29.05%, found 29.69 ± 1.13%; methyl linolenate, actual 11.53%, found 10.40 ± 1.48; and methyl eicosapentaenoate, actual 16.05%, found 16.00 ± 0.39%.

The average coefficient of variation for the analyses was found to be ca. 5%. Within the limits of error, our results confirm those reported by Vorbeck et al. (25) and Iden and Kahler (26) that the argon ionization detector gives peak area responses that agree reasonably well with mole percentage and that do not require special correction factors to compensate for nonlinearity.

The calculated peak-area percentages were converted to wt percentages. These conversions amounted to average deviations (from area % to wt %) that ranged from −19% for 14:0 to +15% for 22:6. Palmitate had a −9% deviation. These conversions are significant when compared with the 5% coefficient of variation noted above.

Results and Discussion

In this investigation, we confined our studies to fish of commercial importance in the United States. Species were chosen which contained body oils in a wide range: 0.7–3.3% in the low range, 6.4–9.2% in the medium range and 12.8–15.5% in the high range. Oils from shellfish, fish livers and fish eggs were included for comparisons with those from body oils of fish. No attempt was made to prepare composite samples representative of the particular species variations from one time of year to another, or from one catching area to another. In this respect, the sampling was definitely inadequate to be representative of the species under any conditions except those that prevailed at the time of sampling.

In the case of large fish, we chose only to analyze sections of the bodies rather than the entire bodies. Attention was given to select sections of the edible species that represented common edible portions, such as fillets and steaks.

The fish oils, with one exception, were composed of the extractable lipids based on the method by Bligh and Dyer (14). Attention was given to the prescribed terniary mixture of chloroform-methanol-water. A second chloroform extraction of the sample tissues was sufficient to produce quantitatively all extractable lipids. Lipids were not recovered in instances where their lipoprotein complexes resisted chloroform-methanol extraction (27). The amt of such unrecovered lipids are assumed negligible for the purpose of this investigation.

The oil from the eggs of pink salmon represented the free oil droplet, which is only one-third of the total oil in the eggs (15). Kyte reported that this oil droplet consists essentially of fatty acid triglycerides (28).

Fish-Oil Composition. The fatty acid composition of the various oils are listed in Table III. The data

TABLE III

Fatty Acid Composition of Fish Oils

Fatty acid chain length	Double bonds	Concentration of fatty acids in:																							
		Saltwater fishes and components																		Freshwater fishes			Shellfishes			
		Atlantic cod	Atlantic cod liver	Spiny dogfish	Spiny dogfish liver	Pacific halibut	Pacific herring	Mackerel	Menhaden	Striped mullet (A)	Striped mullet (B)	Ocean perch	Rockfish	Sablefish	Chinook salmon	Chum salmon	Coho salmon	Pink salmon	Pink salmon egg	Lake herring	Rainbow trout	Lake whitefish	Blue crab	Littleneck clam	Pacific oyster	Sea scallop
No. C atoms	No. per molecule	Weight per cent of total fatty acids																							
14	0	1.8	2.8	2.0	1.6	2.8	7.6	4.9	8.0	4.6	7.5	4.6	4.1	4.6	3.7	2.2	3.7	3.4	2.9	5.5	2.1	2.2	2.2	3.2	2.7	1.9
15	0	0.5	0.4	0.5	0.3	0.3	0.4	0.5	0.5	6.3	4.5	0.6	0.6	0.4	0.4	0.6	0.5	1.0	0.5	0.4	0.8	0.3	0.9	0.8	0.9	0.7
	1(?)		0.2		0.4					0.5	1.1	0.3	0.4				0.3			0.3	0.3	0.3	0.4			
16	0	33.4	10.7	21.2	13.2	15.1	18.3	28.2	28.9	17.3	13.9	12.6	14.9	18.1	16.6	17.0	10.2	10.2	9.5	17.7	11.9	12.1	15.2	23.8	21.4	23.0
	1	2.4	6.9	6.0	5.7	8.9	8.3	5.3	7.9	11.0	15.5	8.0	6 6	8.0	9.2	4.1	6.7	5.0	7.0	7.1	8.2	10.5	11.2	9.6	4.6	2.0
	2[a]	0.6	1.0	0.9	1.0	0.8	1.0	0.7	0.8	3.8	6.0	0.9	1.5	1.0	1.1	0.8	1.2	1.7	1.2	0.7	1.2	1.2	1.8	0.8	1.6	0.5
17	0	0.9	1.2	1.2	1.0	0.7	0.5	1.0	1.0	0.8	1.0	1.0	2.6	0.8	1.1	1.1	0.9	1.6	0.8	0.6	1.5	1.1	1.9	1.3	1.4	0.8
18	0	4.0	3.7	2.7	4.3	3.4	2.2	3.9	4.0	5.0	5.1	3.6	6.0	3.1	5.8	3.2	4.7	4.4	2.9	3.0	4.1	4.0	7.2	5.4	4.0	5.3
	1	11.8	23.9	27.5	28.5	25.7	16.9	19.3	13.4	8.4	9.1	22.0	20.8	20.4	29.1	21.4	18.6	17.6	20.5	18.1	19.8	27.2	17.6	10.8	8.5	5.2
	2	1.2	1.5	1.3	0.7	0.9	1.6	1.1	1.1	3.2	2.2	1.5	1.6	0.8	1.1	2.0	1.2	1.6	1.5	4.3	4.6	5.5	1.9	1.4	1.2	0.6
	3	0.8	0.9	0.6	0.6	0.3	0.6	1.3	0.9	1.4	1.0	0 6	0.8	0.5	0.9	1.0	0.6	1.1	1.2	3.4	5.2	3.7	1.2	1.6	1.6	0.3
	4	1.2	2.6	0.7	0.8	3.6	2.8	3.4	1.9	3.0	3.1	1.6	1.3	1.3	1.5	2.0	2.1	2.9	1.8	1.8	1.5	1.0	0.6	3.0	4.3	1.8
19	0[b]		0.6		0.7		+		0.9	1.5	1.6	0.8	0.9	1.1	1.2		1.8	0.7	0.7					+	+	0.4
20	1	1.6	8.8	5.8	10.5	8.0	9.4	3.1	0.9	0.7	0.6	8.0	1.4	5.6	4.7	5.4	8.4	4.0	1.1	1.2	3.0	2.1	1.9	3.5	+	1.7
	2(?)		0.5		0.4				0.5	1.0	0.8		0.5	+	0.4	0.7	0.4	0.6	0.5	0.9	0.6	0.8	1.1	1.2		0.5
	3(?)	0.4	0.1		0.2			0.5		+	0.4		0.2		0.1		0.1	0.1	0.4	0.1	0.4	0.6	0.4	0.4		
	4	3.2	1.0	2.5	0.8	2.5	0.4	3.9	1.2	2.6	3.6	0 8	1.5	0.8	0.5	0.9	0.9	0.7	1.5	3.4	2.2	3.9	4.1	1.7	1.9	4.5
	5	12.4	8.0	7.9	3.7	10.1	8.6	7.1	10.2	7.5	11.8	9.3	11.7	8.5	8.2	6.7	12.0	13.5	20.6	5.9	5.0	6.4	13.4	10.0	21.5	21.3
22	1	0.7	5.3	4.1	10.3	5.1	11.6	2.8	1.7	0.7		8.7	0.8	8.9	3.6	9.4	5.5	3.5	0.4	2.8	1.3	0.5	1.5	2.6	2.6	0.2
	1(?)		1.1		0.7						0.8	0.7	0.5		1.1		1.1	1.8	2.8		2.1	0.6				
	4(?)		0.3	1.0	1.3	+	+		0.7	1.0	1.0	0.4	0.6	0.6	0.6	0.6	0.6	0.6	0.5	1.2	0.6	1.1	1.0	1.2	0.5	1.5
	5	0.6	1.3	2.3	3.1	1.6	1.3	1.2	1.6	3.9	3.2	0.6	1.6	1.8	2.4	2.3	2.9	3.1	4.6	3.3	2.6	3.3	1.1	1.7	1.0	1.0
	6	21.9	14.3	10.4	6.5	7.9	7.6	10.8	12.8	13.4	3.2	12.0	17.4	12.1	5.9	16.1	13.8	18.9	16.0	13.3	19.0	8.8	11.0	14.5	20.2	26.2
24	1(?)[c]		0.5	0 8	1.9	1.0	0.9	0.8	0.9	1.5	0.7	0.5	0.5	1 4	0.7	1.5	0 6	1.1	0.2	4.4	0.7	1.2	1.0	0.7		0.6

[a] Combined critical pair of 17:1 and 16:2; 17:1 for mullet oil.

[b] Combined critical pair of 19:0 and 16:4.

[c] Amt agree well with C_{24} acid (lignoceric acid) from chain-length analyses of hydrogenated methyl esters.

shows that the amt of constituent fatty acids varied widely among some species. This fact is illustrated by the wide range of the often most prominent fatty acids in the fish oils: notably, 1.6–8.0% myristic, 9.5–33.4% palmitic, 2.0–11.2% palmitoleic, 5.2–29.1% oleic, 0.7–10.5% eicosenoic, 5.0–21.5% eicosapentaenoic, 0.2–11.6% docosenoic, and 5.9–26.2% docosahexaenoic acids.

An attempt was made to relate the amt of eicosenoic and docosenoic acids to the species having low, medium and high levels of oil. A general correlation, however, of the composition of these and other fatty acids to the oil content of the species was not possible.

Certain differences are apparent in the fatty acid composition of the freshwater fish—lake herring, rainbow trout and lake whitefish—compared with the marine species. The linoleic acid content was higher for the freshwater fish, averaging 4.8% (range 4.3–5.5%), as compared with an average for the marine species of 1.5% (range 0.7–3.2%). Concurrently, the content of eicosapentaenoic acid (20:5) in the freshwater fish was lower, averaging 5.8% (range 5.0–6.4%), than for the marine species—averaging 9.7% (range 6.7–13.5%). It was also noted that the percentage of the total polyunsaturated fatty acids which contained 4, 5 and 6 double bonds was lower in the freshwater fish, average 70% (range 65–74%), than for marine fish, average 88% (range 84–92%).

These differences in polyunsaturates are probably due largely to differences in the respective feeding environments and diets of the species. Recent studies by Kelly et al. (29) and by Mead et al. (2) have demonstrated definite dietary effects of unsaturated lipids on the composition of depot fat of fish. It was pointed out by Reiser et al. (30) that freshwater and marine fishes differ from each other and from other animals only in their diet, and not in the mechanistic nature of their fatty acid metabolism.

The analyses of Pacific oysters and sea scallops indicated unusually large amt of eicosapentaenoic and docosahexaenoic acids—over 40% of the total fatty acids. Blue crab, sea scallop, mackerel and lake whitefish were shown to have ca. 4% eicosatetraenoic acid, which is high for fish lipids.

Striped mullet oil was one of the most interesting oils studied. Data in Table III give the analytical results for two separate mullet samples. Analysis A is for oil from mullets caught in December 1959, and analysis B is for oil from mullets caught in July 1960. The analyses of this species caught in the same general area seven months apart illustrate the possible wide variation in the relative amounts of particular fatty acids. In analysis A of mullet oil, there was nearly half as much of the 16:2 and 17:1 components and four times as much of the 22:6 acid as was found in analysis B. Also, analysis B showed this particular sample of oil to be the only one of all the samples analyzed in this investigation that had palmitoleic acid in excess of palmitic acid. Both samples showed the lowest ratio of oleic to stearic acids of all species investigated, except the oyster and the scallop. Large amt of odd-numbered carbon acids, mainly C_{15} and C_{17}, were found in the mullet oils. Comparisons of nonhydrogenated samples and samples hydrogenated for chain-length analyses indicated that the C_{17} fatty acids were largely 17:1 with relatively less 17:0. Data reported by Reiser et al. (30) revealed that older and larger mullets contain much large amt of 18:2, 20:4, 22:3 and 22:6 acids, and only minor amt of 16:1 acid compared to younger and smaller mullets. A thorough study of mullet-oil fatty acids, including structure determinations, was recently reported by Sen and Schlenk (31).

Differences were found in body and liver lipids from the Atlantic cod and the spiny dogfish. There was more 20:4 acid in the body lipids than in the liver lipids. The cod body lipids showed the highest amt of palmitic acid (33%) of any of the samples investigated. The liver lipids from both species showed much higher amt of 20:1 and 22:1 acids than were found in the body lipids; however, the difference was more dramatic in cod as compared with dogfish. The large amt of 20:1 and 22:1 that were found in the dogfish livers agree well with the results of Malins and Houle (32).

The fatty acid composition of the triglyceride fraction of the lipids from pink salmon eggs showed that 20:5, 22:5 and 22:6 acids constituted more than 40% of the fatty acids. The total amt of C_{20} and C_{22} fatty acids found here agrees with the findings of Kyte (28). Our results indicate that salmon egg oil is a rich source of docosapentaenoic acid (i.e., clupanodonic acid) when the oil is produced in the manner described by Sinnhuber (15).

Although not reported in Table III, analyses of nearly all hydrogenated samples showed traces of C_{21} acids. Also, trace amt of branched chain C_{15}, C_{17} and C_{19} acids were indicated from chain length analyses. It was not our intention, however, to study each oil completely for these trace components but rather to find possible major differences among the oils.

Regarding the use of the data reported here, a word of caution is in order. Various biological differences do occur in natural products that can influence the significance of reported values as possible new standards for composition (33). For fish oils, there are the often uncontrollable variables due to geographical location of catch, season of the year (like the illustration shown), sex, age and feeding habits of the fish (34). To establish standards for composition, one therefore needs much greater sampling of individual species than was possible in the case of those reported here. This investigation, however, has given needed information that fills a gap in the knowledge of fish-oil composition.

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Minor Component Fatty Acids of Common Vegetable Oils

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Abstract

A combination of gas-liquid chromatography (GLC) and oxidative cleavage on fractions isolated by mercury derivative chromatography has shown the presence of previously unreported minor component fatty acids in olive, soybean, cottonseed, corn, peanut, rapeseed and safflower oil. All of the oils examined contain small amt of saturated acids above arachidic, some as high as hexacosanoic acid. *Cis*-11-octadecenoic acid was found in amt ranging from 0.5–2.0%. *Cis*-11-eicosenoic acid is present in the 0.04–1.4% range (rapeseed oil excluded). The tetracosenoic acid present in rapeseed (0.4%) and safflower oil (0.1%) has been identified as the *cis*-15-tetracosenoic acid. No unusual polyenoic species were detected with the exception of those in rapeseed oil, which contains 0.6% of both 11,14-eicosadienoic and 13,16-docosadienoic acid.

Introduction

THE SUGGESTION THAT the unsaturated acids of common vegetable oils contain isomers or homologs of oleic and linoleic acid has appeared sporadically in the literature (1,4,6,14,19). These reports have attracted little attention, most probably because of the limited amt of supporting data presented.

A few well-documented reports of unusual fatty acids in common oils have appeared. The eicosenoic acid (1–2%) present in peanut oil has been identified as *cis*-11-eicosenoic acid (7,8). The presence of 15% *cis*-11-octadecenoic acid and 10% *cis*-9-hexadecenoic acid in milkweed seed oil has been shown (3). Although not a common oil, milkweed seed oil is a good example of an oil whose major unsaturated components had been thought to be oleic and linoleic acid. Also pertinent to the present work are the recent reports by GLC of tetracosenoic acid (1%) and eicosadienoic acid (0.5%) in rapeseed oil (13,15) and eicosenoic acid (trace) in safflower oil (9).

The ability of present-day separation and analysis techniques, when used in a co-ordinated analytical scheme, to detect small amt of geometrical and positional isomers prompted a new look at common edible oils whose fatty acid composition has been accepted for many years. Olive, soybean, cottonseed, corn, peanut, rapeseed and safflower oil were analyzed. The oils were randomly selected as representative of high quality edible products but their exact origin is unknown.

Experimental

General Analysis Scheme. The methyl esters of the oils (all refined and bleached except olive oil) were prepared by transesterification (10). Mercury derivatives of the methyl esters were prepared and subsequently separated into saturated, monoenoic and polyenoic fractions by column chromatography as previously described (11). The resulting fractions were analyzed by IR spectroscopy (2), GLC and oxidative cleavage. In many cases the monoenoic methyl esters were subjected to further column chromatography on silver nitrate-silica gel adsorbent, using a variation of de Vries' technique (5), to obtain enrichment of a minor component.

GLC Analysis of Fractions. Three types of columns were used to analyze the fractions isolated by column chromatography. Column A was a 24 x ¼ in. stainless steel column packed with 20% Dow-Corning 200 silicone fluid (12500 centistokes) on 60–80 mesh acid-washed Chromosorb W and was operated at temp of 230 or 280C with a helium flow rate of 95–100 ml/min at STP. Column B was a 72 x 3/16 in. aluminum column packed with 15% organosilicone-ethyleneglycol succinate on 100–200 mesh Gas-Chrom P (Applied Science Laboratories, Inc. EGSS-X) and was operated at a temp of 180–190C with a helium flow rate of 35–40 ml/min at STP. Column C was a 120 x ¼ in. stainless steel column packed with 15% stabilized diethyleneglycol succinate (Analabs, Inc. C6) on 60–70 mesh Anakrom ABS and was operated at 170C with a helium flow rate of 34–36 ml/min at STP. All chromatograms were obtained on an F&M Scientific Corp.

TABLE I

Analysis of Oils by Mercury Derivative Chromatography

Oil	Olive	Soybean	Cottonseed	Corn	Peanut	Rapeseed	Safflower
Weight %							
Saturated acids	14.1	15.9	26.2	13.8	19.4	6.8	10.3
Monoenoic acids	78.2	28.3	18.2	27.4	52.4	70.5	15.0
Polyenoic acids	7.7	55.8	55.6	58.8	28.2	22.7	74.7