Fractional Vacuum Distillation of Herring Oil Methyl Esters

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ABSTRACT

Methyl esters of a Canadian Atlantic herring oil containing 62% monoethylenic fatty acids were subjected to batch fractional distillation under vacuum on a pilot plant scale, to study the feasibility of fractionating fatty acid esters of marine oils of low iodine value into monounsaturated fractions with increased commercial value for industrial chemical uses. A total of 64 methyl ester fractions were collected and analyzed by gas liquid chromatography. Recoveries of the major saturated and monounsaturated acids were close to 100%, and some fractions contained over 90% of the desired 22:1 long chain monounsaturated acids. The short chain polyunsaturated acids were recovered in good yields, but the long chain highly unsaturated acids were recovered in yields of 60% or less due to oxidative and thermal decomposition in the particular apparatus employed. If small amounts of unsaturated acids are acceptable, fractional distillation of low iodine value marine oils could inexpensively supply fractions with high concentrations of methyl esters of longer chain (C_{20} and C_{22}) monounsaturated and shorter chain (C_{14}) saturated acid or (C_{16}) saturated-monounsaturated acid mixture.

INTRODUCTION

Triglyceride-based oils of marine origin differ from most vegetable oils in that they have a very complex fatty acid composition and contain acids with a wide range of chain lengths, unsaturations and positional isomers (1-4). Countercurrent solvent extraction of marine oil methyl esters followed by fractional distillation has been demonstrated to be a feasible method for preparing fractions with specific chain lengths and unsaturation on a pilot plant scale (5-7). This procedure is technically complex and was developed with the aim of recovering highly unsaturated fatty acids (18:4ω3, 20:5ω3, 22:5ω3, 22:6ω3) as the end products. The simpler approach of fractional distillation of methyl esters from low IV (iodine value) oils, such as oils from Canadian Atlantic herring (*Clupea harengus*) or Newfoundland turbot (*Reinhardtius hippoglossoides*) (IV of oils as low as 95 in extreme cases, typically < 125 [2]), appeared to offer the possibility of easy access to fractions containing modest proportions of these acids relative to the dominant 18:1, 20:1 and 22:1 acids of the longer chain lengths. Moreover the investigation of methyl esters of marine oils by molecular distillation had indicated that the presence of five or six double bonds increased the volatilities of the 20:5ω3 and 22:6ω3 acids sufficiently to distill them ahead of the corresponding 20:1 and 22:1 esters (J.C. Sipos and R.G. Ackman, unpublished results). It was not known if this same effect (compare [8,9]) would be operative in ordinary fractional distillation, although fractional distillation under vacuum is a well established procedure for separating and isolating individual components of fats and oils, both as an analytical tool on a laboratory scale and as an industrial process for continuous production of fatty compounds such as fatty acids.

The present study was designed to investigate the feasibility of fractionally distilling methyl esters of herring oil under vacuum in order to obtain fractions with defined fatty acid compositions for industrial applications. A total of 64 fractions were collected to study the segregation of fatty acids. However only a portion of the gas liquid chromatography (GLC) analyses are presented to show the specific groups from which component fatty acids could be isolated.

EXPERIMENTAL PROCEDURES

Preparation of Methyl Esters

The herring oil was obtained from British Columbia Packers Ltd. and was produced at their reduction plant at Pubnico, Nova Scotia. The analytical data obtained by AOCS procedures were: iodine value, 110; bound glycerol, 1.71%; saponification value, 186; free fatty acids, 1.35%; and nonsaponifiable materials, 1.2%. The methyl esters were prepared under nitrogen by a conventional batch-type process as described previously (7). The reaction was carried out in a 20 liter carboy at room temperature with constant stirring for 6 hr, using 75% excess methanol and 0.5% sodium hydroxide as catalyst. The liberated glycerol settled to the bottom overnight, and the methyl ester layer could be separated easily. The conversion was 99.2% based on the bound glycerol content of the methyl ester layer as determined on a chloroform extract by a standard method (10). The methyl esters were purified by stripping off the excess methanol under vacuum, cooling the esters to ambient temperature, and filtering in order to remove the precipitated traces of soap. A further washing with water was needed, usually to lower the soap content to 20 ppm or below, as estimated colorimetrically (10).

Fractional Distillation

The distillation was carried out in an apparatus equipped with an all glass, perforated plate, Oldershaw column (3 in. diameter, 30 in. long) with a 61% plate efficiency when operated on total reflux (11,12). This column was mounted between a two neck 22 liter glass still pot and a 14 in. take-off section connected to a 26 in. vacuum jacketed distillation head with an air-cooled reflux condenser. A ground glass joint fitted with a glass capillary was inserted through the side neck of the still to promote smooth boiling by introducing a minute continuous flow of nitrogen. A glass take-off receiver containing two chambers with an intermediary stopcock was used for the periodic removal of the distillate, without interrupting the vacuum of the distillation as explained below. A 16 in. long air-cooled condenser with ground glass ball joints linked the receiver and the take-off section. All 3 in. connections were made with flat flanges fitted with Teflon and silicone rubber combination gaskets. Other joints were ground glass,

TABLE I

Weight Per Cent Composition of Fatty Acids in Selected Fractions from Vacuum Fractional Distillation of Herring Oil Methyl Esters[a]

Fatty acid	Fraction 1	5	9	13	17	21	25	29	Fatty acid	Fraction 33	37	41	45	49	53	57	61
	W_n (%)[b,c]									W_n (%)[b,c]							
	2.53	1.29	1.61	1.62	1.41	1.32	1.46	1.51		1.41	1.30	1.27	1.24	2.36	1.51	1.15	2.41
	ΣW_n(%)[d,e]									ΣW_n(%)[d,e]							
	2.5	8.2	13.8	19.6	25.5	31.3	37.2	42.9		48.4	53.9	59.4	64.8	71.1	76.7	82.2	89.3
12:0	4.09	2.78	.54	---	---	---	---	---	17:0	1.85	.14	---	---	---	---	---	---
13:0	1.00	---	---	---	---	---	---	---	17:1ω8	.25	---	---	---	---	---	---	---
14:0	82.33	65.08	12.06	1.06	.18	.14	---	---	18:0	5.85	3.62	.30	---	---	---	---	---
14:1ω5[f]	7.79	4.36	2.97	..34	---	---	---	---	18:1ω9[f]	68.66	32.53	1.67	.15	---	---	---	---
15:0	.50	1.98	2.92	.93	.26	---	---	---	18:2ω6	5.17	3.13	---	---	---	---	---	---
15:1	---	.40	1.10	.38	.15	---	---	---	18:3ω3	1.85	.36	---	---	---	---	---	---
16:0	1.89	12.10	36.24	48.81	52.03	49.00	12.14	.99	18:4ω3	3.57	1.54	---	---	---	---	---	---
16:1ω7[f]	2.40	12.10	35.39	36.92	35.26	23.59	2.88	.14	19:1	.46	---	---	---	---	---	---	---
16:2	---	---	2.57	3.16	2.47	1.70	.63	.35	20:1ω9[f]	9.00	40.28	64.13	67.59	57.94	21.86	3.48	---
16:3ω4	---	---	1.13	1.36	1.10	1.71	1.56	.85	20:2ω6	---	.50	1.01	1.11	.36	.13	---	---
16:4ω1	---	---	2.26	2.97	1.70	.79	---	---	20:4ω6	---	---	.84	.74	.18	---	---	---
17:0	---	---	.57	.75	.84	1.71	2.19	1.41	20:4ω3	---	---	.52	.95	---	.66	---	---
17:1ω8	---	.60	.57	.76	.74	.68	.63	.14	20:5ω3	2.88	12.71	20.97	12.06	6.34	4.18	---	---
18:0	---	---	---	---	---	.34	3.13	4.53	21:5	---	---	---	---	---	---	---	.26
18:1ω9[f]	---	.60	1.70	2.56	5.16	17.09	65.08	73.88	22:1ω11[f]	---	5.20	10.56	16.41	32.54	69.82	92.40	97.66
18:2ω6	---	---	---	---	.15	1.37	4.38	5.59	22:5ω6 and 3	---	---	---	---	---	.80	1.00	.56
18:3ω3	---	---	---	---	---	.17	1.75	2.47	22:6ω3	---	---	---	1.00	2.36	2.53	3.03	.70
18:4ω3	---	---	---	---	---	1.70	4.69	4.81	24:1	---	---	---	---	---	---	---	.82
19:1	---	---	---	---	---	---	.63	.53									
20:1ω9[f]	---	---	---	---	---	---	.31	4.31									
Saturated, %	89.81	81.94	52.33	51.55	53.31	51.19	17.46	6.93	Saturated, %	7.84	3.76	.30	---	---	---	---	---
Monounsaturated, %	10.19	18.06	41.73	40.96	41.31	41.36	69.53	79.00	Monounsaturated, %	78.37	78.01	76.36	84.15	90.48	91.68	95.88	98.48

[a]Total recovery is 11,860 g or 91.3% of a 13 kg charge.

[b]W_n is the weight of the nth fraction of the distillate.

[c]W_n (%) is the percentage of W_n in the total sample for this distillation.

[d]ΣW_n is the accumulated weight from first to nth fractions of the distillate.

[e]ΣW_n (%) is the percentage of ΣW_n in the total samples used of this distillation.

[f]Total per cent includes all monounsaturated isomers; structure is that of dominant isomer.

TABLE II

Fatty Acid Composition of Hydrogenated Samples of Selected Fractions From Fractional Vacuum Distillation of Herring Oil Methyl Esters

	Fraction															
Fatty acid, wt %	1	5	9	13	17	21	25	29	33	37	41	45	49	53	57	61
12:0	3.80	2.58	2.40	---	---	---	---	---	---	---	---	---	---	---	---	---
13:0	.85	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Iso 14	.50	.42	.10	---	---	---	---	---	---	---	---	---	---	---	---	---
Anteiso 14 (?)	.35	.20	---	---	---	---	---	---	---	---	---	---	---	---	---	---
14:0	86.05	67.06	13.78	1.30	.21	.10	---	---	---	---	---	---	---	---	---	---
4,8,12-TMTD[a]	3.14	2.14	1.12	.20	---	---	---	---	---	---	---	---	---	---	---	---
Unknown	1.21	.7	.42	.10	---	---	---	---	---	---	---	---	---	---	---	---
Iso 15	.21	1.02	1.49	.41	.09	---	---	---	---	---	---	---	---	---	---	---
Anteiso 15	---	.38	.59	.18	---	---	---	---	---	---	---	---	---	---	---	---
15:0	.29	1.11	2.10	.82	.32	---	---	---	---	---	---	---	---	---	---	---
Iso 16:0	---	---	.20	.29	.43	.28	.11	---	---	---	---	---	---	---	---	---
Pristanic[a]	---	.15	.53	1.10	1.45	.92	.19	---	---	---	---	---	---	---	---	---
16:0	3.60	23.09	75.48	90.00	89.00	73.41	16.45	2.20	.30	---	---	---	---	---	---	---
7-MHD[a]	---	.30	1.09	2.34	2.17	1.59	.90	---	---	---	---	---	---	---	---	---
Iso 17	---	.09	.40	.27	.41	.63	.70	.51	.20	---	---	---	---	---	---	---
Anteiso 17	---	---	.18	.09	.11	.12	.10	---	---	---	---	---	---	---	---	---
17:0	---	.32	.62	.80	1.31	2.15	1.75	1.18	.81	.31	---	---	---	---	---	---
Phytanic[a]	---	---	---	.10	.60	1.30	1.72	1.81	1.38	.43						
Iso 18	---	---	---	---	---	.22	.27	.30	.21	.10	---	---	---	---	---	---
18:0	---	.44	1.50	2.01	3.00	19.28	77.31	90.04	84.75	40.36	2.06	---	---	---	---	---
19:0	---	---	---	---	---	---	.32	.39	.31	---	---	---	---	---	---	---
20:0	---	---	---	---	---	---	.18	3.57	12.04	55.11	87.80	81.04	65.07	26.80	3.40	---
22:0	---	---	---	---	---	---	---	---	---	3.69	10.00	18.24	34.10	72.25	95.41	98.90
24:0	---	---	---	---	---	---	---	---	---	---	---	---	---	---	.32	0.80
Unknowns I[b]	---	---	---	---	---	---	---	---	---	---	.15	.64	.41	.30		---
Unknown II[b]	---	---	---	---	---	---	---	---	---	---	.03	.11	.42	.35		---
Unknowns III[b]	---	---	---	---	---	---	---	---	---	---	---	---	---	.40	.67	---

[a]Branched chain fatty acids are specified by: TMTD = 4,8,12-trimethyltridecanoic acid; pristanic acid = 2,6,10,14-tetramethylpentadecanoic acid; 7-MHD = 7-methylhexadecanoic acid; phytanic acid = 3,7,11,15-tetramethylhexadecanoic acid.

[b]Unknowns I are three peaks between 19:0 and 20:0; unknown II is a single peak between 20:0 and 22:0; unknowns III are three peaks between 22:0 and 24:0.

TABLE III

Percentage Recovery of Principal Fatty Acids and of Chain Lengths in Fractional Distillation of Herring Oil Methyl Esters (total wt = 11,860 g)

Fatty acid	Wt % in starting material	Wt (g) content in herring oil methyl esters		Recovery, %
		Before distillation	In distillate	
Saturated esters	20.7 %	2457	2536	103
14:0	6.7	789	907	114
16:0	11.5	1368	1454	106
18:0	1.4	167	148	87
Monounsaturated esters	61.6%	7300	7110	97
16:1	8.6	1019	1026	101
18:1	14.4	1713	1818	106
20:1	14.3	1691	1781	105
22:1	22.0	2610	2405	92
Polyunsaturated esters	16.9 %	2003	984	49
15:2	1.6	186	145	80
18:2	1.2	146	129	88
18:3	0.9	112	71	63
18:4	1.45	172	132	78
20:4	0.86	102	68	67
20:5	5.62	666	395	59
22:5	1.08	128	45	35
22:6	3.35	397	65	17
Recovery by chain length				
C-14	7.50	890	974	109
C-16	22.58	2679	2661	99
C-18	19.46	2310	2224	96
C-20	21.05	2495	2228	89
C-22	26.53	3149	2513	80
Total	97.12	11523	10600	

lubricated with Dow-Corning high vacuum silicone grease.

The apparatus was connected to the vacuum system at the top of the distillation head and also at the upper receiving flask. The vacuum system was fitted with high vacuum Edwards-Saunders "Speedivalves" (Edwards High Vacuum Ltd., England), so that connections could be made either independently or together. The main vacuum was maintained by a gas ballast rotary vacuum pump fitted with a dry ice cold trap. An auxiliary vacuum pump was connected directly to the lower receiving flask to eliminate any pressure interruption when fractions were removed. With another "Speedivalve," these two pumps could be operated independently or together. The vacuum achieved was normally in the range of 1-2 mm, as continually measured at the top of the reflux system with a barometrically compensated dial vacuum gauge (Type CG-1, Edwards High Vacuum Ltd., England). This gauge was checked daily by a "Vacustat" McLeod vacuum gauge (Edwards High Vacuum Ltd., England).

The still pot was heated by a thermostatically controlled Isomantle with two 700 W heating elements. A well insulated column jacket was heated with an integral 300 W heating coil that was regulated by a variable transformer. This reduced the column heat loss to a minimum. A thermometer with an integral ground glass joint was inserted into the vapor in the take-off section to measure the distillation temperature.

Between 12 and 14 kg herring oil methyl esters was placed in the still for each distillation and carefully degassed under vacuum at room temperature. Initially nitrogen was introduced rapidly through a coarse capillary to promote agitation. This was reduced to less than 1 cc/min as heating commenced, and a stable total reflux condition was usually achieved in ca. 30 min. In distillations the reflux ratio was maintained at 10:1 by a proportionating reflux timer for the fractions containing the shorter chain methyl esters, and then changed to 7:1 after it was decided that most C_{18} methyl esters had been distilled, as determined from a preliminary distillation graph. The rate of distillation was initially 2 kg/hr, but was reduced to ca. 500-600 g/hr for the C_{20} and C_{22} methyl esters and varied slightly with day to day operating conditions. About 250 g of distillate was collected in each fraction and stored under nitrogen for iodine value determinations and for detail gas liquid chromatography (GLC) fatty acid composition studies. Column hold-up appeared to be 100-150 g, and the pressure drop through the column was estimated to be 5 mm Hg.

Gas Liquid Chromatographic Analysis

Analyses of the methyl ester mixture and hydrogenated esters of each fraction were carried out by GLC on two columns of differing polarity (13,14) (glass 3 mm ID x 2 mm length) packed, respectively, with 10.5% EGSS-X and 15.5% EGSS-Y organosilicone polyester on 100/120 mesh Gas-Chrom P (Applied Science Laboratories). These were installed in a Barber-Colman model 10, equipped with model 5121 flame ionization detectors, and the operating conditions were similar to those described previously (13). To assist in identifications in some fractions, a nitromethane extract of unsaturated esters (15) was used. GLC analyses of the hydrogenated esters of some fractions were also carried out on a Perkin-Elmer model 226 equipped with an open-tubular column coated with butanediolsuccinate polyester (0.01 in ID x 150 ft length) as detailed elsewhere (14,16). Urea complex enrichment (17) was used to identify the branched chain compounds in some fractions. Tentative identifications of peaks were made by the linear log plot and separation factor procedures (17), and appropriate detection correction factors were applied (18).

The presentation of the data to two decimal places is intended only to indicate the relative amounts of minor components and does not imply this degree of accuracy. GLC quantitation analyses of this type usually involve errors of the order of ±5% for major (>20%) components, ±10% for medium (5-20%) components, but may be subject to much larger errors for minor components (<5%).

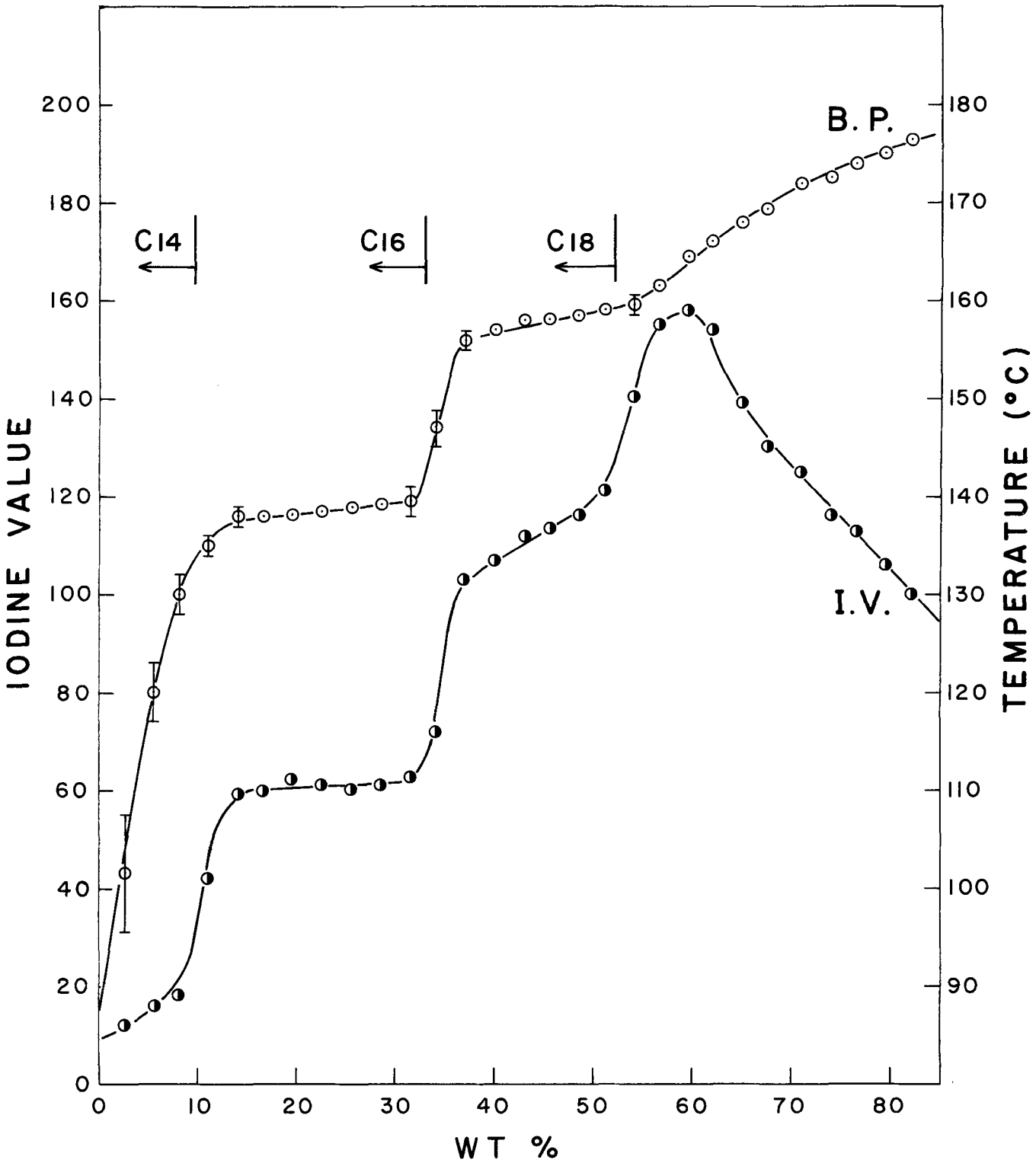

FIG. 1. The distillation curve and the curve showing changes of iodine value in distillates during vacuum fractional distillation of herring oil methyl esters.

RESULTS AND DISCUSSION

The fatty acid compositions of 16 selected fractions are given in Table I and of the hydrogenated acid esters from the same fractions in Table II. The simplified fatty acid composition of the herring oil starting material and the percentage recovery of the major component acid esters in the distillates are given in Table III.

A typical distillation curve and the corresponding iodine value of the fractions collected are shown in Figure 1. The C_{16} and C_{18} fractions can be identified from both the boiling point and iodine value curves. The iodine value curve drops rapidly as the content of 22:1 increases in the last fractions. In Figure 2 the changes in the percentage of the chief fatty acids in the various fractions are plotted against the weight percentage of total distillate. It is shown that fractions containing a high percentage of C_{18}, C_{20} and C_{22} monounsaturated acids can be produced by simple distillation of methyl esters of low iodine value marine oils. A tentative segregation scheme according to type of unsaturation and chain length is presented in Figure 3. The bar graphs in Figure 3A indicate the expected composition of broader cut fractions according to saturation and in Figure 3B according to chain length. The chain length data

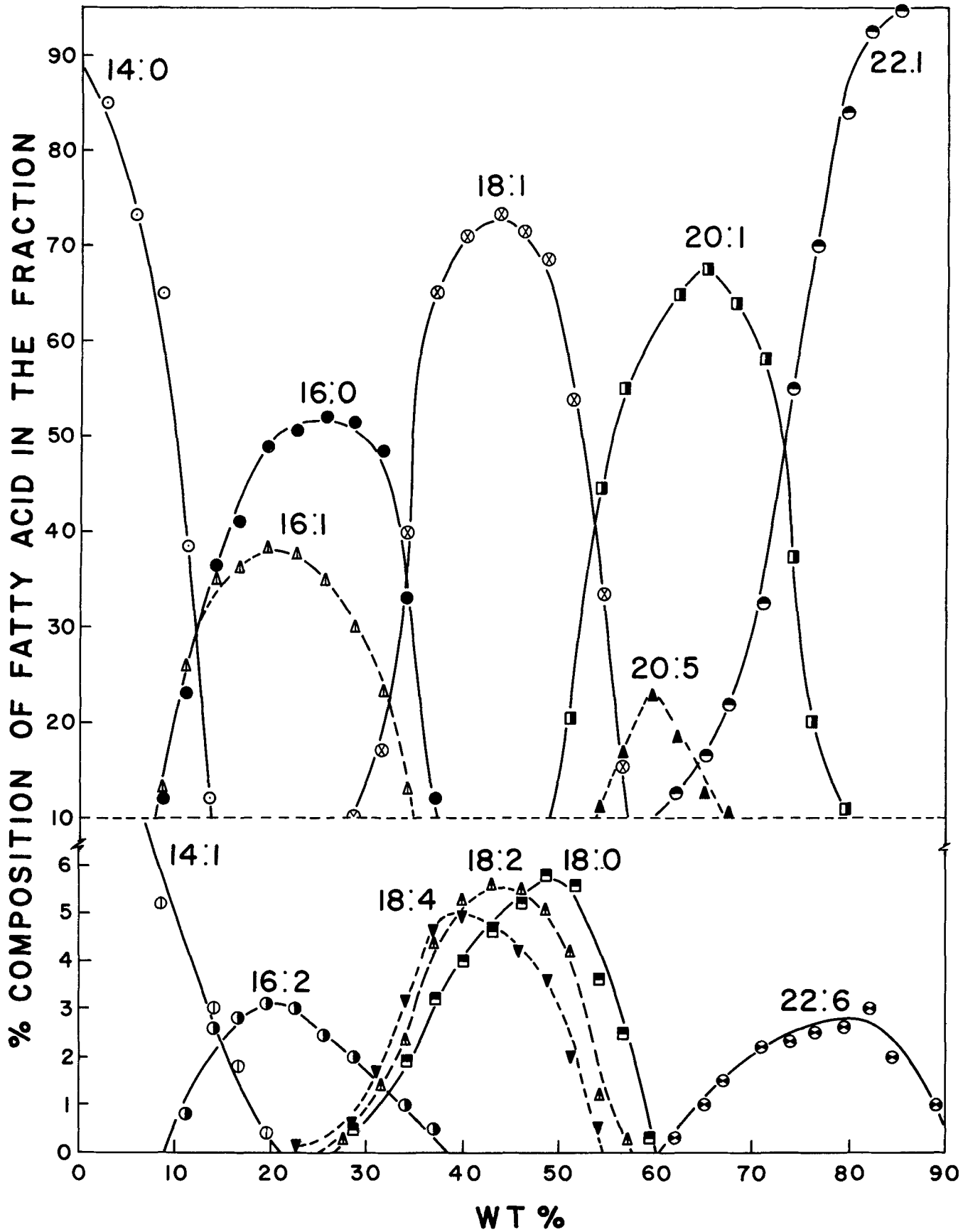

FIG. 2. The compositon of principal fatty acids in selected fractions in the distillation of herring oil methyl esters.

are also summarized schematically in a different way in Figure 3C.

In order to determine the contents of branched chain fatty acids and to compare the fatty acid composition by chain length, the same fractions were hydrogenated and analyzed by GLC with a BDS open tubular column with the results shown in Table II. It can be seen that several fractions contain 90% or more fatty acids of one chain length. Some of the branched chain fatty acids normally present in marine oils in trace amounts only (ca. 0.1%) are

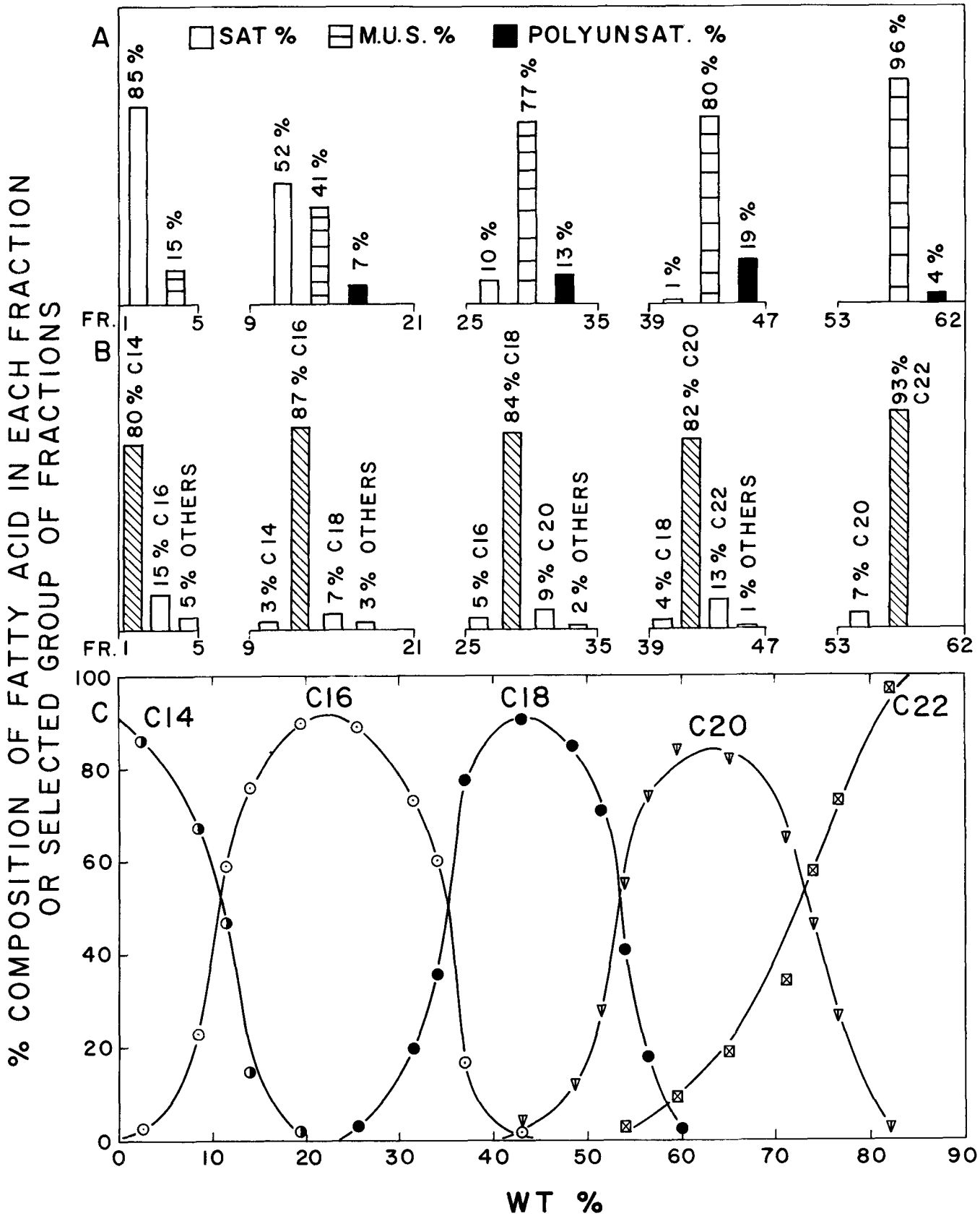

FIG. 3. Fatty acid compositions calculated by degree of unsaturation and by chain length of fatty acids for group fractions of distillates. (M.U.S. = Monounsaturates.)

present in some fractions in amounts up to 2-3%. No branched chain fatty acids were detected except those described in earlier publications (14). However a few minor unknown compounds were detected between 19:0 and 24:0 on the chromatogram. It is possible that these were cyclization or other artifacts produced by oxidative or thermal action on the unsaturated, longer chain fatty acids, although unknown II was likely 21:0.

The 64 fractions of the distillate from whole-oil methyl esters that were collected represented ca. 90% of the starting material. It generally took at least two 10 hr days to complete a run. It is obvious from Tables I-III that the

losses of highly unsaturated acids were very high and that they were proportional to the chain length and degree of unsaturation. This fact has of course been pointed out earlier by many authors (8, 19-22), but the degree of thermal and oxidative decomposition and polymerization that can be expected in a vacuum distillation on a pilot plant scale has not to our knowledge been followed in detail before. Only 17% of docosahexaenoic acid (22:6) present was recovered in the distillate, whereas the recovery of most of the major constituent saturated and monounsaturated acids was close to 100%. However, since the 22:6 only comprised 3.4% of the starting material, the actual loss by weight was not a great part of the starting material.

In our opinion the Teflon-to-glass-flange seals, which were shown to leak to an undetermined extent by a halogen-sensitive leak detector when the apparatus was being assembled, permitted access of air in sufficient amounts to cause oxidative destruction of the polyunsaturated fatty acids. The overnight interruption also enhanced total degradation. It is remarkable that the monoethylenic fatty acids were very much less susceptable to oxidation (compare [16]). Modern industrial plant technology is capable of circumventing this problem (23,24).

It has been suggested that these losses of polyunsaturated acids could be minimized by rapidly distilling off most of the C_{14}-C_{16} esters, and subjecting the residue to countercurrent solvent extraction with nitromethane (6). This procedure would be especially useful for marine oils of medium iodine value (ca. 150) to high iodine value (ca. 180), in that concentrates of highly unsaturated esters would be obtained where desired for certain applications (1,25,26). The proportion of 20:1 to 20:5ω3, and of 22:1 to 22:6ω3, may be so low in some marine oils that only distillation would be necessary to obtain 70-80% concentrations of the polyunsaturated acids. Menhaden (*Brevoortia tyrannus*) and South American anchoveta (*Engraulis ringens*) are suitable for this purpose.

Unfortunately the losses of oxidation-sensitive materials in our study prevented an assessment of the relative distillation rates, respectively, between 20:1 and 20:5ω3, and between 22:1 and 22:6ω3. The Table I data in fact suggests that no such enrichment in the lower molecular weight materials occurred, but instead a nearly constant proportion of the two types of acids of interest in the C_{20} and C_{22} chain lengths was obtained. Molecular weight can be an important factor in molecular distillation (8,9), but it is less important in conventional distillation. However, although alcohol acetate codistillation data (3,27) show progressively less 20:5ω3 relative to 20:1 in early successive distillate fractions for C_{20} materials from a spinning band column, the effect of carrier acetates is an enigmatic complication.

Privett and coworkers (3,27) carried out this high vacuum fractional distillation of tuna oil methyl esters with a spinning band column on a laboratory scale, using the carrier of long chain acetates in order to avoid thermal changes in the unsaturated fatty acids and to promote sharper chain length fractionation. Using 10 g samples, these authors were able to collect fractions containing up to 84% docosahexaenoic acid and obtain 105% total recovery. However no further studies of this technique on a pilot plant or industrial scale have appeared. The data for 18:4 relative to 18:1 (Table I, Fig. 2), if oxidative losses were not taken as too serious (net recovery of 18:4ω3, 78%), suggest that 18:1 is in fact more volatile than 18:4ω3, as a higher relative proportion of 18:1 is found in successive fractions.

With increasing amounts of low iodine value marine oils available (from herring, Newfoundland turbot and capelin [*Mallotus villosus*]) and the current lack of commercial interest in highly unsaturated acids, it appears possible that a straight fractional vacuum distillation might be a low cost method for producing fatty acid fractions with specific properties and compositions. GLC is now used routinely and in many cases installed in automated systems to continuously check the composition of distillates and thereby make possible the collection of fractions with defined fatty acid compositions. With highly automated systems, batch operations similar to that described would require much less attention than our work indicates. The contents of the chief saturated and monounsaturated acid esters can reach high proportions in certain fractions. In Table I it is shown that 16:0, 18:1, 20:1 and 22:1 have been concentrated to 52%, 74%, 67% and 98%, respectively, in specific fractions. Possibly controlled oxidation could be combined with reflux and distillation to promote the enrichment. This amount of segregation might be sufficient to produce technical fatty ester or acid fractions to be used as raw materials for the chemical industry. If it were necessary to remove the final contaminants of highly unsaturated acids, this could be done by solvent extraction or by partial hydrogenation.

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