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THE FATTY ACID COMPOSITION OF HERRING OILS

by

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INTRODUCTION

Fish body oils are prominent in the world production of oil from marine resources. According to FAO's yearbook of fishery statistics (1965) 545 thousand tons of fish body oils were produced in 1963 as compared to 65 thousand tons of fish liver oils and 411 thousand tons of whale and sperm oils (season 1962/63). Production of fish body oils is expected to increase in coming years with development of modern fishing methods in remote waters, and several new clupeiform species are expected to be exploited (FAO, 1961). Production of whale oils, on the other hand, will probably decrease as a result of reduced catches caused by heavy exploitation.

At least 90 % of all fish body oils are produced from different species of herrings (*Clupeoidea*). Herring fishes are caught in most fishing areas of the world, as can be seen from Table 1, which is based on FAO's yearbook of fishery statistics (1964). The total catch in 1963 was 14,77 million tons. The bulk of the herring oil production derives from five species. The South American Anchovy (*Engraulis ringens*) caught mainly in Peru gave 167 thousand tons or about a third of the total world production. The North Atlantic herring (*Clupea harengus*) accounted for the next third of the production giving 149 thousand tons. (Data from U.S.S.R. is not included). Third in importance ranks the menhaden (*Brevoortia tyrannus*) caught off the Atlantic coast of U.S.A., which yielded 83 thousand tons. The South African pilchard (*Sardinops ocellata*) gave 53 thousand tons. The last of the important species is the Pacific herring (*Clupea pallasii*) caught mainly by Canadian fishermen, which gave 30 thousand tons of oil.

Extensive differences in fatty acid composition may be expected. The herrings are all small species living in shoals and feeding on plancton, mainly crustaceans. They deposit fat in the muscle tissue and in fatty tissues around the intestines (mesentery). The livers are small and very meagre. During the time of spawning herrings do not take in food, while the body cavity fills up with the ripening gonads. The raw material for herring oil production thus varies according to: Species, fishing

Table 1. *World catch of herring fishes 1963.*[1])

Species of fish	Main fishing area or port of landing	Thousand metric tons	Percentage of total
Anchovies (*Engraulis*)		*7 830*	*53.0*
E. ringens	Peru	7 174	48.6
E. encrasicholus	Mediteranean	195	
E. japonica	Far East	459	
E. mordax	U.S.A. west coast	2	
Pilchards (*Sardinops*)		*1 137*	*7.7*
S. coerula	U.S.A. west coast	3	
S. sagax	Peru and Mexico	50	
S. melanosticta	Japan	56	
S. ocellata	South Africa	1 028	
Sardinells (*Sardinella*)		*137*	*0.9*
S. aurita	South Atlantic	67	
S. longiceps	India	70	
Herrings (*Clupea*)		*3 480*	*23.6*
C. harengus	North Atlantic	2 757	18.7
C. pallasii	North Pacific	723	
Menhaden (*Brevoortia*)			
B. tyrannus	U.S.A. east coast	809	5.5
Sardines (*Sardina*)			
S. pilchardus	Mediteranean, South Atlantic	456	3.1
Sprats (*Sprattus*)			
S. sprattus	U.S.S.R.	439	3.0
Various clupeoids		480	3.2
Grand total		*14 770*	*100.0*

[1]) Modified from FAO-Yearbook of Fishery Statistics Vol. 16 — 1963 (1964).

areas and season, state of feeding and reproduction cycle. The analytical values reflects this variance. The fat content of the whole fish varies from less than 2 % to above 25 %. The iodine values of the oil vary between 100 and 200. Evidently herring oils may vary as much in their composition as any fish oil. The complicated pattern of fish oil fatty acid composition has often been stressed. Methods of analysis have been correspondingly complicated. In recent times, the development of gas chro-

matography has resulted in quicker, more detailed and reliable results. The present paper reviews data from the literature on the fatty acid composition of herring oils and reports results from our own studies.

METHODS AND RESULTS

The first study of the fatty acids in herring oil was carried out by the Norwegian chemist H. BULL (1897). He found that the methods available for such studies were far from sufficient to tackle this problem, but could nevertheless prove the presence of monoenoic acids from C_{14} to C_{22} and the possible presence of polyenoic acids. BULL developed a better method of fractionation, distillation in vacuum of the fatty acid methyl esters, which he applied on cod liver oil (see LAMBERTSEN & BRÆKKAN, 1965). BONNEVIE SVENDSEN (1916) applied this principle to the analysis of herring oil. His results are reported as Analysis 1 in Table 3. This old study has been included as the results generally compare well with more recent studies. Only the value for oleic acid (C18:1) seems too low, and as should be expected, the technique resulted in somewhat higher residues of oxidized and polymerized acids than later, more advanced methods. LOVERN (1938) determined the fatty acid composition of 7 samples of herring fat collected through a year. This is the only study of seasonal changes in fatty acid composition, and for the sake of later discussion his results have been reported in detail in Table 2. The average values are given as Analysis 2 in Table 3. He applied a more refined system of vacuum distillation than BONNEVIE SVENDSEN (1916), and could calculate with higher accuracy. The values were corrected for losses during the analytical procedure (Calc. I.V. = determ. I. V.). BJARNASON & MEARA (1944) determined the fatty acid composition of herring oil as part of a study of the glycerides of herring oil (Analysis 3, Table 3). BLACK & SCHWARTZ (1950 b) used the distillation method in a study on the fatty acid composition of South African pilchard oil (Analysis 12, Table 3).

All the above authors applied the method of vacuum distillation of fatty methyl esters, which dominated fatty acid analysis up to 1950. The method could not separate the different polyunsaturated acids, except with quite complicated systems of group separations prior to distillation. Minor components could generally not be determined. Normally the results were reported as mean values of unsaturation, and the calculations were based on certain approximations (HILDITCH, 1947). The values reported in Table 3 for the unsaturated acids have been recalculated from the original data in the literature in order to obtain a better basis for comparison with the values from GLC-methods. The following

approximations were applied: Polyenoic acids of the C_{16}- and C_{18}- groups were taken as equal quantities of dienes, trienes and tetraenes, i. e. mean unsaturation 3.0. The C_{20}-polyenes were taken as 3 parts 20:5 and 1 part 20:4, mean unsaturation 4.75, and correspondingly C_{22}-polyenes were taken as 22:5.75.

The method of gas-liquid chromatography of fatty acid methyl esters now dominate this field of analysis. Nine of the analyses given in Table 3 are GLC-analyses. The method gives quick and detailed results, but certain problems of identification may arise, partly because of minor unsaturated or branched chain components, partly because of areas of overlapping «peaks» on the chromatograms. More detailed dicussions on modern methods of lipid analysis have been given by FONTELL *et al.* (1960) and LAMBERTSEN (in press). Only major fatty acid components and some which are necessary for discussion are given in Table 3. The polyenoic acids 20:4 and 20:5 are given as a sum, so are also the acids 22:5 and 22:6.

KLENK & EBERHAGEN (1962) analysed several fish oils for their fatty acid composition, using low-temperature crystallization followed by GLC. Among these oils were a herring oil (Analysis 4, Table 3), and a «peruvian sardinoil» (Analysis 10, Table 3). The latter was most probably a Peruvian anchovy oil. GRUGER *et al.* (1964) determined the fatty acids of several fish oils by GLC, among these were a Pacific herring oil (Analysis 6, Table 3) and a menhaden oil (Analysis 8, Table 3). AHRENS *et al.* (1959) prepared a menhaden oil for medical studies. The careful procedure is published in details together with a thorough GLC-determination of the fatty acid composition (Analysis 7, Table 3). No study seems to report on the body oil from the Californian sardine (*Sardinops coerula*), but LASKER & THEILACKER (1962) determined the fatty acid composition of the mesenteric fat («ister») of this species (Analysis 13, Table3).

Our own analyses of commercial herring oils were performed in 1962, and included a Norwegian herring oil (Analysis 5, Table 3) and a menhaden oil (Analysis 9, Table 3). The method applied comprised saponification, methylation by borontrifluoride-methanol, hydrogenation of an aliquot and GLC on a vinylacetate column (unhydrogenated) and an apiezon column (hydrogenated). Calculation was performed by equalizing area (height x width at half height) with weight percentage of the hydrogenated aliquot.

In recent years Peruvian anchovy oil has gained in importance. Only one study on the fatty acid composition seems reported in the literature (KLENK & EBERHAGEN, 1962). By the courtesy of A/S Denofa og Lilleborg Fabriker, (Oslo), GLC-analysis of a commercial sample of a Peruvian fish oil was placed at our disposal (Analysis 11, Table 3).

In Table 5 are reported analyses of the fatty acid composition of the following four tissues of herring (*Clupea harengus*) : White muscle, red muscle, liver and roe. The fresh tissues were frozen, ground and extracted successively with methanol, ether, and pentane. The fat extracts were analysed for their fatty acid composition by the method outlined above for herring and menhaden oils.

Fig 1 illustrates the fatty acid composition of herring oils with different iodine values. The curves were obtained by modern temperature programmed GLC. These analyses were made possible by the courtesy of A/S Johan C. Martens & Co. (Bergen).

DISCUSSION

The differences of the fatty acid composition through a year in a single species, herring (*Clupea harengus*), has been summarized in Table 2 (Lovern, 1938). In spite of a rise in the total fat content from 4,6 % to 20,7 %, or about ± 60 % of the average content, the iodine values did not vary more than ± 10 to 15 %. These differences are further spred over many fatty acids with rather small variations within each. All samples can be recognized as herring fats (see below). Lovern (1938) states: «Whatever the cause of these variations in the proportions of the various acids, the variations themselves, although real enough, are not of a large order, and do not strikingly differentiate the samples». There is a general rise in unsaturation during the summer feeding period. One point in Table 2 may perhaps be of interest. A decrease in the content of 22:1 is parallelled by an increase in 20:4.75 and not in 22:5.75.

In Table 3 are compiled data on the fatty acid compositions in fat samples from 6 different herring fishes. Further informations regarding these analyses are given in Table 4. In the following discussion of the percentage values for the different fatty acids, reference has been made to the average values found for cod liver oil by Lambertsen & Brækkan (1965). Further has been applied a short-hand designation for the fatty acids. Each acid is represented by two figures, the first giving the chain length, the second the number of double bonds (i. e. linoleic acid is given as 18:2).

Two different principles of analysis have been applied, the «classical» vacuum distillation method, and the modern gas chromatographic method, each with different inherent types of error. Some differences must therefore be expected to derive from analytical failure rather than from real differences between the samples, in particular for the values of the

Table 2. *Seasonal changes in fatty acid composition of herring fat.*[1])

Fatty acid designation	Apr.	June	June	July	Oct.	Oct.	Apr.	Aver-age
14 : 0	8.0	7.3	7.5	8.3	7.3	6.6	5.8	7.3
14 : 1	—	0.6	0.3	0.5	0.8	0.2	—	0.3
16 : 0	15.7	16.7	12.8	12.1	13.0	13.7	12.4	13.8
16 : 1	3.9	6.2	5.2	4.2	4.0	3.9	3.5	4.4
16 : 3.0[1])	0.7	1.3	1.8	2.2	0.9	1.0	1.2	1.3
18 : 0	0.2	trace	0.1	0.3	trace	0.5	0.6	0.3
18 : 1	17.2	14.2	6.3	7.9	9.3	9.8	9.3	10.6
18 : 3.0[1])	5.0	6.9	14.8	13.1	11.4	6.5	8.5	9.5
20 : 1	16.4	17.1	17.2	15.1	19.7	19.5	21.6	18.1
20 : 4.75[1])	5.6	10.2	12.8	13.2	10.4	9.2	9.5	10.1
22 : 1	21.0	11.9	14.9	16.8	17.6	22.7	19.5	17.8
22 : 5.75[1])	6.3	7.6	6.3	6.3	5.6	6.4	8.1	6.7
% Fat	8.2	10.7	15.7	20.7	18.8	12.0	4.6	13.0
Iodine val.	115.5	144.2	154.3	152.5	138.6	129.9	147.9	140.4

[1]) Modified from LOVERN, J. A. : Biochem. J. *32*, 676 (1938).

polyenoic acids. An example of such an unreliable value is 18:3.0 which consists of the acids 18:2, 18:3 and 18:4, together with their isomersand some 19-acids. Differences in the samples, on the other hand, must also be considered. Thus eight of the oils reported in Table 3 were extracted from the whole fish, either industrially or in the laboratory, whereas four were extracted from fillets or from gutted fish. Finally one sample represented mesenteric fat. Fat from different organs of the fish will differ in composition. This has been shown and discussed in connection with Table 5.

Myristic acid (14:0) seems to be present in about the same percentage in all samples, with values around 7,5 %. This is more than double the value of cod liver oil (3,2 %). This high value for 14:0 seems to be characteristic for body oils and derives from fish muscle triglycerides (LAMBERTSEN, in press).

Palmitic acid (16:0) shows values around 15—17 %, except for analyses No. 8, 9 and 13, which are unusually high in this acid. All values are clearly higher than the corresponding percentage in cod liver oil (11 %) Palmitoleic acid (16:1) shows an average value of 9 %, or similar to the value for cod liver oil (9 %). This acid does not seem to be characteristic for any particular fish oil. Generally herring (*Clupea harengus*) tends to give lower values for the sum of 16-acids, 20—25 % as compared to 25—35 %.

Table 3. *Fatty acid composition of different herring fats.*[2])

Fatty acid designation	*Clupea harengus*					*C.pall.*	*Brevoortia tyrannus*			*Engraulis ringens*		*Sardinops ocellata*	*Sardinops coerula*
	1	2	3	4	5	6	7	8	9	10	11	12	13
14 : 0	6	7.0	7.3	5.6	10.2	7.6	7.2	8.0	9.6	7.4	9.5	6.7	6.1
16 : 0	17	11.7	13.8	13.2	14.9	18.3	17.0	28.9	23.9	18.6	20.4	17.4	28.0
16 : 1	12	10.6	4.4	5.9	9.1	8.3	9.8	7.9	11.6	10.3	11.5	9.4	4.9
18 : 0	2	0.8	0.3	1.2	1.0	2.2	3.1	4.0	2.8	2.9	3.3	2.1	5.4
18 : 1	7.5	13.7	10.6	22.1	12.4	16.9	14.5	13.4	12.7	13.4	15.4	11.5	20.3
18 : 3.0	6.5	5.9	9.5	2.0	2.7	5.0	7.2	3.9	5.6	2.5	9.0	8.3	6.4
20 : 1	10	14.8	18.1	15.3	11.4	9.4	2.1	0.9	0.6	2.2	1.5	0.3	3.8
20 : 4.75 (4+5)	10 [1])	11.1	10.1	5.6	6.3	9.0	13.1	11.4	12.4	22.1	18.7	25.5	11.0
22 : 1	16	16.4	17.8	15.9	17.5	11.6	+	1.7	+	0.4	0.7	2.6	1.2
22 : 5.75 (5+6)	[1])	5.2	6.7	6.4	7.7	8.9	10.9	14.4	14.0	6.8	6.9	8.0	8.3
Others & residue	13	2.8	1.4	6.8	6.8	2.8	15.1	5.5	6.8	13.4	3.1	8.2	4.6

[1]) Incl. 22-unsat. [2]) For information regarding the analyses, see Table 4.

Table 4. *Information regarding analyses in Table 3.*

No.	Fat	Method	Author(s)	Year	I.V.	% Fat
1.	Herring — Norway Lab. extr. filet	Dist. fract.	BONNEVIE SVENDSEN	1916	134.3	
2.	Herring — North Sea Lab. extr. gutted fish Mean of 7 anal.	Dist. fract.	LOVERN	1938	140.4	
3.	Herring — Iceland Comm. oil	Dist. fract. Glyc. fract.	BJARNASON & MEARA	1944	140.0	
4.	Herring — Norway? Comm. oil	Freez-fract. GLC	KLENK & EBERHAGEN	1962	119	
5.	Herring — Norway Comm. oil	GLC	Present work	—		
6.	Herring — Pacific Lab. extr. filets	GLC	GRUGER *et al.*	1964		12.8
7.	Menhaden — U.S.A. Small-scale prod. oil ÷ head and viscera	GLC	AHRENS *et al.*	1959	179	
8.	Menhaden — U.S.A. Lab. extr. whole fish	GLC	GRUGER *et al.*	1964		15.5
9.	Menhaden Comm. oil	GLC	Present work	—		
10.	Anchovy — Peru Comm. oil «Peruanischer Sardinen öl»	Freez-fract. GLC	KLENK & EBERHAGEN	1962	199	
11.	Anchovy — Peru Comm. oil	GLC	A/S DENOFA og LILLEBORG FABRIKER	1965		
12.	Pilchard South Africa Lab. extr. oil	Dist. fract.	BLACK & SCHWARTZ	1950	196,5	15.7
13.	Sardine — California Lab. extr. mesenteric fat	GLC	LASKER & THEILACKER	1962		

Stearic acid (18:0) deserves some comments. Herring oil (*C. harengus*) shows an average value of only 1 %, whereas all other species in Table 3 show values above 2 %, with an average of c. 3 % as compared to 2.5 % for cod liver oil. Oleic acid (18:1) is very low in all samples except in the mesenteric fat, average value 14 %, as compared to 24 % in cod liver oil. The polyenoic acids of chain length 18 can not be properly considered because of the above mentioned analytical complexity.

The monoenoic acids of chain length 20 and 22 are both exceptionally high in oils from the *Clupea*-species, 20:1 shows an average value of 13 % and 22:1 an average value of 16 %. The corresponding values for cod liver oil are 11 % and 5.5 %. In particular the value for 22:1 seems to be a «trade mark» for herring in colder area (*Clupea*-species). The other species in Table 3, caught in warmer climate have characteristically low percentages of 20:1, usually below 2,5 %. This great difference in the contents of monoenoic acids is the main cause of the difference in iodine values, 110—150 for *Clupea*-oils as compared to 180—200 for menhaden, anchovy and sardine oils. The polyenoic acids of the 20-series consist mainly of 20:5. The average value for this acid in *Clupea*-oils is 9 %, or the same as in cod liver oil. Menhaden oils, however, have somewhat higher proportions of 20:5, and the samples of anchovy oil and South African pilchard oil are unusually high. A final conclusion with regard to 20:5 as characteristic of such oils must await further analytical data.

Polyenoic acids of chain length 22 consist mainly of 22:6. The content of this acid is in the order of 7 %, except for menhaden oils with 13 %. The average value for cod liver oil is 10 %.

Although the analytical material is rather limited, a few general characteristics may be pointed out with regard to the relative values for some of the fatty acids: *1. Clupea*-oils have high values for 20:1 and 22:1 and low values for 18:0. *2.* Menhaden, anchovy and sardine samples have all low values for 20:1 and 22:1. *3.* Menhaden oils are relatively high in 20:5 as well as 22:6. *4.* Anchovy oils and South African pilchard oils are very high in 20:5, while normal in 22:6.

The commercially produced herring oils are body oils from the whole fish. The fat content of the fish may vary widely. Data reported in the literature for different species of clupeoid fishes giving variations between 2 and 25 %. (Flood, 1958, Bramsnæs *et al.*, 1954; Black & Schwartz, 1950a) The fatty acid composition of an oil may vary with the fat content of the raw material used in the production. Lower total fat content of the fish will result in an oil with a higher proportion of fat from the intestines and from stomach content. The importance of this point is demonstrated by the study reported in Table 5. The most pronounced difference between muscle depot fat and the fats from the liver

Table 5. *Fatty acid composition of fat from different organs of herring (Clupea harengus).*[1])

Fatty acid designation	White muscle	Red muscle	Liver	Roe
14 : 0	7.1	10,7	3.0	3.0
16 : 0	14.2	18,6	21.5	17.0
16 : 1	4.6	7,8	3.5	3.0
15 : —17 : — / 16 : unsat	0.8	3,4	2.0	1.0
18 : 0	0.5	0,5	2.5	1.0
18 : 1	15·3	17,1	17.0	12.5
18 : 2,3,4	3.9	3,1	2.5	4.0
20 : 1	17.4	11,5	2.5	2.0
20 : 2,3,4, / 21 : —	0.8	0.4	1,5	1.5
20 : 5	6.9	9.5	13,5	14.0
22 : 1	22.1	10.5	2,5	3.5
22 : 2,3,4,5,	1.0	0.6	1,5	2.0
22 : 6	5.4	6.3	26,5	35.5

[1]) Tho herring was caught off the coast of Norway in the spawning season.

and roe is found for the 20- and 22-acids. High values for monoenoic acids and low values for polyenoic acids are typical for the muscle fats, whereas the fats from liver and roe show the opposite relation. Especially may be noted the very high value for 22:6 in herring roe, 35,5 %. The total fat content of the roe, however, is usually low, in the order of 4 % (Brækkan & Propst, 1953). Further may be pointed out the low values for 14:0 in intestinal fats as discussed above, and the low 18:0 values for muscle fat. Comparison of the fatty acids of the red muscle with those of the white muscle show the same general distribution, but certain trends are of interest. The monoenoic acids 20:1 and 22:1 are lower in the red than in the white muscle, while the polyenoic acids 20:5 and 20:6 show somewhat higher percentages for the fat of the red muscle. On these points the fatty acid composition of the red muscle tends towards the relations found in the liver. This may reflect the metabolic function of the red muscle as suggested by Brækkan (1956).

The oil industry using herring oils as raw material for the production of hydrogenated food fats, need a quick means for the identification of different fish oils. Iodine values are not sufficient for this purpose. Gas chromatography provides a quick and simple method of analysis. A preliminary transmethylation of the fat is sufficient to prepare a sample for GLC. «Polar» columns are usually preferred for fatty acid determinations as they separate according to unsaturation as well as to chain length.

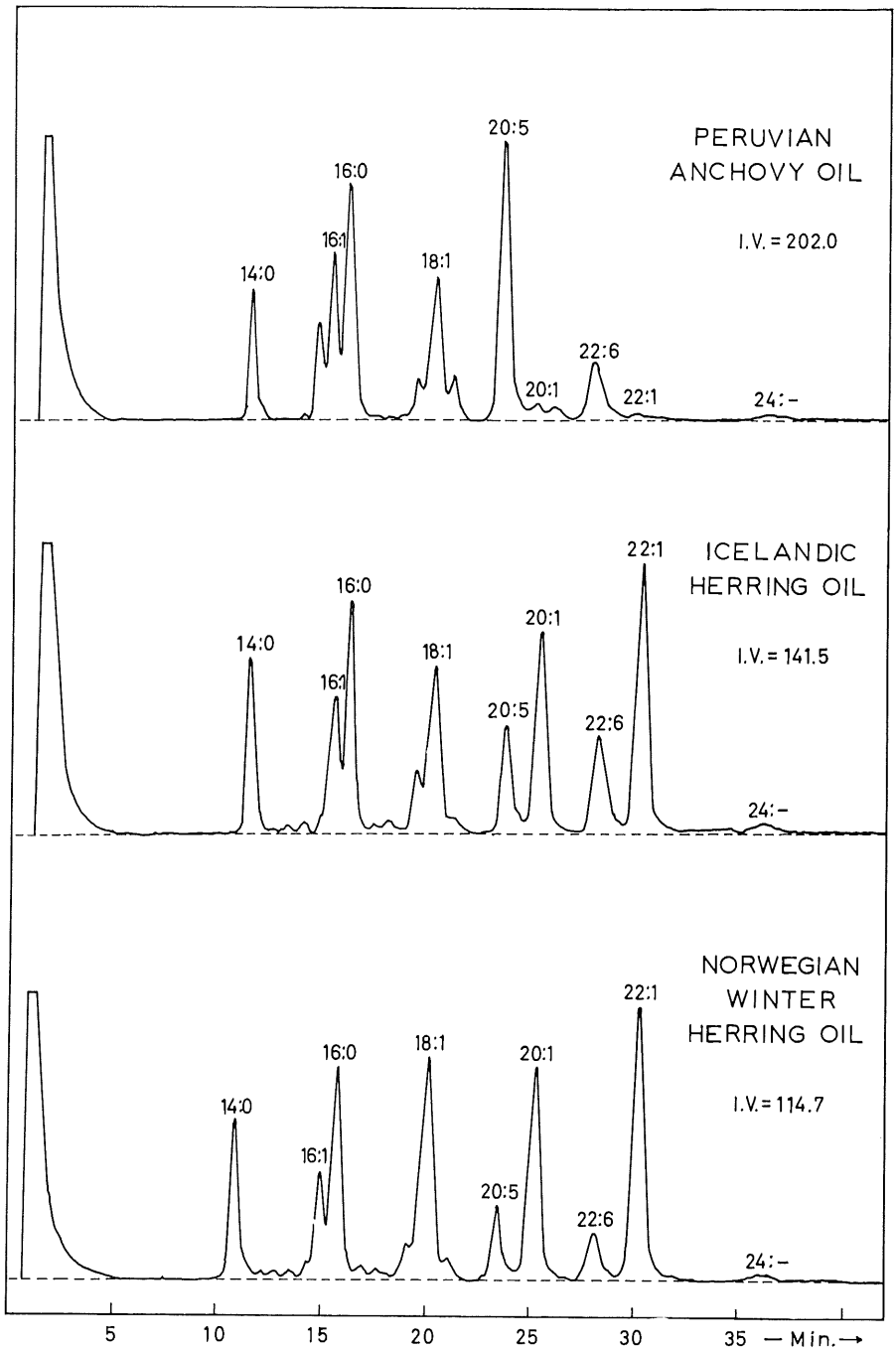

Fig. 1. GLC-chart of three different herring oils.

Instrument: Wilkins Aerograph with dual columns and flamedetector. Filling 10 % Apiezon L on celite. Temperatureprogrammed with 2 per min. from 160 C. Current: 25 ml N_2 per min.

For fish oils such separations are complicated by the simultaneous presence in substantial amounts of the following four acids: 18:4, 20:1, 20:5 and 22:1. On the chromatograms they will be found as the pairs: (18:4 and 20:1) and (20:5 and 22:1). Certain columns result in complete overlapping within these pairs. A «nonpolar» column does not give this type of problem. Minor components which are lost on such columns do not matter for identification analysis. Moreover, non-polar columns are stable in use and have high theoretical plate values. A particular convenient chart may be obtained on a modern temperature-programmed gas chromatograph. Fig. 1 shows GLC-charts of the fatty acids from 2 Norwegian herring oils and a Peruvian anchovy oil. A clear relation between the content of the major fatty acids and the iodine values of the oils can be observed.

SUMMARY

Herring oil production in different areas of the world has been reviewed, and the possible variations of the fatty acid composition has been discussed.

The literature on the determination of fatty acids in such oils has been reviewed, and results for six different species have been compiled together with some results from the present study.

The fatty acid compositions of fat from four different organs from herring (*Clupea harengus*) have been reported.

A gas chromatographic method suitable for the identification of different herring oils has been recommended.

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