Isopropyl Alcohol Extraction of Oil and Lipids in the Production of Fish Protein Concentrate From Herring

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Abstract

The extraction of lipid from fatty fish (herring) by the Halifax process for producing fish protein concentrate, using isopropyl alcohol (IPA), is virtually complete. The largest portion of the lipid is found in the first extract and high quality triglyceride oil is readily recovered by cooling this extract; under certain circumstances it can also be recovered from the second extract. The phospholipids are extracted without obvious degradation and together with free fatty acids are found mostly in the IPA-rich phase from the first extraction. Residual lipid in fish protein concentrate resembles the starting lipid of the fish. Detailed fatty acid compositions are given for various materials.

Introduction

The production of a stable and nutritious dietary supplement from fish (commonly referred to as fish protein concentrate or FPC) requires the almost complete removal of all oxidation-susceptible lipids (1–3). Initial work on FPC in North America (2,4,5) was largely based on fish sources low in fat, such as cod (*Gadus morhua*) muscle (<1% extractable lipid), and whole hake (the Pacific hake *Merluccius productus*). The common Atlantic red hake *Urophycis chuss* may contain up to 6% lipid, depending on season. Economically, the future of FPC may depend on the utilization of very low-cost raw materials of which the Atlantic herring (*Clupea harengus*) offers considerable potential, since landings of this species from Canadian waters are now virtually on a year-round basis (6). These herring contain fluctuating amounts of body fat, the range 4–20% encompassing most extremes, but 8–16% being more usual (7,8). The recovery of this fat from the FPC production process has received little attention, although good quality herring oil is readily marketable. Numerous solvents and combinations of solvents have been associated with FPC production but the present study is confined to the use of isopropyl alcohol (IPA) in the process originated in the Halifax Laboratory, Fisheries Research Board of Canada (4,5), as modified for fatty fish (9).

There is normally some post-mortem autolysis of lipids, especially phospholipids, in the flesh of fish during any period of storage including frozen storage (10,11) or storage in a preservative such as IPA (12). A comparison has therefore been made between fresh herring (about 4 hr out of water) and herring which had been held at −25 C (−14 F) for 8 months. The latter fish were an example of what might at times be used as raw material for FPC production.

Experimental Procedures and Results

Examination of Lipids From Fresh Herring

A representative 1 kg sample was collected from the ground whole herring prepared for FPC production. Quantitative extraction by the method of Bligh and Dyer (13) yielded 16.4% total lipid of yellow-brown color. The acid value of this lipid was 2.55, the iodine value (Wijs) 118. Part of the lipid was placed on a gel column (20 mm × 35 cm) of 200–400 mesh styrene divinylbenzene copolymer beads (Dow Chemical Co.) eluted with benzene. The course of this chromatographic separation (14) was monitored (Fig. 1–I) by a continuous recording differential refractometer. Fractions collected were weighed and examined by thin layer chromatography on silica gel (hexane-ether-acetic acid 70:30:1) with the following results: fraction 1 (6.9%) showed only phospholipids, fraction 2 (90.7%) showed only triglycerides, fraction 3 (0.9%) showed mostly free fatty acids with minor amounts of sterol ester and of diglycerides or sterol (cholesterol references), or both, fractions 4–7 (1.5%) were similar to each other and to fractions 3 but contained only traces of free fatty acids.

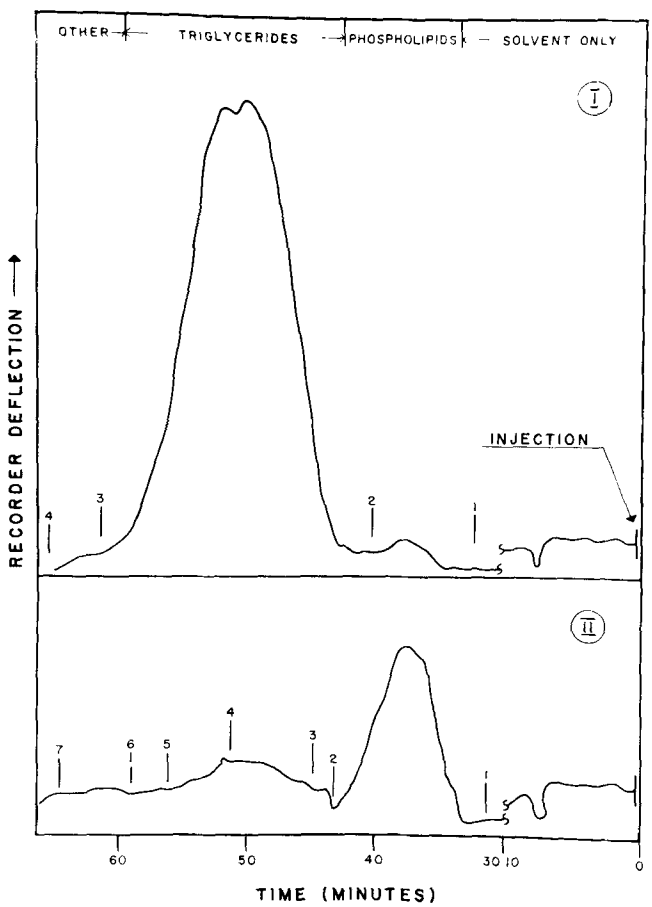

FIG. 1. Part of chart recording differential refractometer output of initial eluants from polystyrene bead column. Times given at bottom (part of solvent only omitted) and approximate regions corresponding to principal lipids noted at top. Numbers are those of fractions collected as discussed in text. I, 0.2036 g of total lipid extracted in the laboratory from fresh herring. II, 0.2154 g of IPA-soluble material from first extract after removal of triglyceride oil at 3 C.

TABLE I

Composition of Esters of Fatty Acids From Fractions of a Chromatographic Separation of Lipids Obtained From Fresh Herring by the Bligh and Dyer Method and From Extract I of the FPC Production Process

Fatty acid[a]	Weight per cent fatty acid					
	Bligh and Dyer extraction on raw fish			Extract I from FPC production process		
	Phospholipid	Triglyceride	Other lipids	Soluble phospholipid	Insoluble oil (3 C)	Other solubles
12:0	Trace	Trace	4.4	Trace	0.1	2.2
13:0	Trace	Trace	0.6	Trace	0.1	0.1
14:0	1.8	5.6	2.7	1.5	5.9	6.3
14:1[b,c]	0.2	0.7	1.1	Trace	0.4	0.7
15:0	0.3	0.4	1.2	Trace	0.4	0.6
15:1 + I 16:0[d]	0.2	Trace	1.2	Trace	0.1	0.2
16:0	21.4	12.5	19.5	23.1	12.9	15.9
16:1[e]	4.6	13.6	10.1	5.1	12.3	14.8
16:2 + 17:0[f]	0.7	1.4	1.3	0.8	0.6	1.6
16:3 + 17:1	Trace	0.8	0.4	Trace	0.5	0.7
16:4ω1	Trace	1.3	1.2	Trace	0.7	1.1
18:0	3.2	1.1	3.2	3.8	0.8	1.5
18:1[b]	13.0	15.5	11.3	12.1	14.7	13.4
18:2ω6	0.9	1.1	0.7	1.3	0.8	1.0
18:3ω6 + 19:1	0.1	0.2	0.2	Trace	0.1	0.3
18:3ω3	0.3	0.3	0.4	0.2	0.4	0.4
18:4ω3	0.2	1.2	0.4	0.3	1.2	1.3
20:0	0.2	Trace	0.1	Trace	0.1	?
20:1[b]	2.4	13.7	5.3	1.8	14.8	5.3
20:2ω6	0.1	0.2	Trace	?	0.1	Trace
20:3ω6	Trace	Trace	Trace	?	Trace	?
20:4ω6	1.4	0.3	Trace	1.0	0.1	0.7
20:4ω3	0.1	0.1	0.1	0.2	0.1	0.1
20:5ω3	12.2	6.8	13.1	15.6	6.4	12.3
21:5ω2(?)[g]	0.2	0.2	0.6	0.2	0.2	0.1
22:1[b]	1.6	19.4	2.9	0.9	21.5	4.6
22:5ω6	0.2	Trace	Trace	Trace	0.1	Trace
22:5ω3	0.8	0.2	0.6	0.2	0.5	0.6
22:6ω3	32.7	3.1	16.2	31.3	3.3	13.4
24:1	1.3	Trace	Trace	0.4	0.7	0.4

[a] Chain length, number of double bonds and position of nearest double bond relative to terminal methyl.
[b] Mostly with ω9 isomer predominating; other isomers present.
[c] Includes iso and anteiso 15:0 and possibly 4,8,12-trimethyltridecanoic acids.
[d] Includes pristanic acid.
[e] Includes iso and anteiso 17:0 acids.
[f] Includes phytanic acid.
[g] Includes traces of 22:4ω6.

Fraction 1 was examined by thin layer chromatography on silica gel (chloroform-methanol-water 70:30:5) and showed spots of roughly equal size for major components identified as phosphatidyl ethanolamine and phosphatidyl choline, with minor components identified as sphingomyelin and lyso-phosphatidyl ethanolamine. Fractions 1,2 and 3–7 (pooled) were respectively transesterified with methanol-boron trifluoride (15). The methyl esters were recovered and analyzed by gas liquid chromatography (16) with the results presented in Table I. The starting material total fatty acid composition is included in Table IV.

Examination of Lipids From Frozen Herring

A representative 1 kg sample was collected as the thawed herring were being ground. The lipid was extracted quantitatively. Recovery was 12.6% lipid of a very dark brown color, with an acid value of 13.1. This lipid was not examined in detail but the total lipid fatty acid composition was determined after transesterification (Table IV).

Manipulation of Extracts Recovered From Herring Processing

The processing involved three successive extractions. In addition to IPA a small amount of phosphoric acid was added to adjust the pH in the initial extraction. All extractions were carried out at 81 C (178–180 F) (9) and the hot slurry was centrifuged in a basket centrifuge. The recovered liquors were filtered through Whatman No. 1 filter paper (suction, Buchner funnel) to remove fine particles. The filter was rinsed with petroleum ether to recover adherent oils.

Extract I. The IPA added to the ground fish was intended to produce a 70:30 IPA:H_2O ratio. The extract from the stored herring was dark brown in color, that from fresh herring a lighter yellow-brown.

Extract II. The IPA added to the ground fish was 99% and the water content of the resulting extract was thus about 5%. This step differs from that used for lean fish where this extraction is normally performed with 70:30 IPA:H_2O. The extract from the stored herring was dark yellow in color, that from the fresh herring a slightly lighter yellow.

Extract III. The third extraction was carried out with 99% IPA and yielded pale yellow extracts.

The further treatment of the extracts from fresh herring followed the scheme of Figure 2. On cooling to room temperature (25 C) two phases formed in Extract I from stored herring and in Extracts I and II from fresh herring. The oil was found in the bottom layer and was removed in the case of Extracts I and II from fresh herring. Extract I from stored herring was examined for lipid content in the upper (IPA-rich) phase, and oil in the bottom phase was determined, but the two layers were not separated. Extract I from stored herring, and the IPA-rich upper phases from Extracts I and II from fresh herring, were cooled slowly to +3 C. Additional layers of oil separated from the latter extracts, and when Extract II from stored herring (homogeneous at room temperature) was similarly cooled to +3 C, an oil phase formed. In both Extracts II at +3 C the oil formed semisolid bottom layers (A) and in the upper IPA-rich layers there appeared crystals which could be filtered off as separate oil material (B). The A and B materials were recovered individually from the stored herring extract for examination but are otherwise taken as one phase, except in Table IV. Recovered B, after solvent removal, was a very pale oil, semisolid at room temperature and of i.v. 80.6. Recovered A was a clear yellow oil at room temperature, with i.v. 101.3. The ratio of B to A was 0.6 to 1, the combined oils having an i.v. of 94. The acid values of A and B were nominally < 0.1. There was no separation of an oil phase from Extract III in either fresh or stored

TABLE II

Recovery and Distribution of Oil (from IPA-Insoluble Phase) and of Other Lipids (in IPA-Rich Phase) From Extracts Prepared From Fresh Herring and From Herring Stored at −25 C (−14 F) for 8 Months

Material examined	Temperature (C)	Lipid fractions as percentages of raw material lipid			Lipid fractions as percentages of lipid in extracts		Concentration of lipid soluble in IPA phase
		Total lipid[a]	IPA-insoluble (oil)[b]	IPA-soluble[c]	IPA-insoluble (oil)	IPA-soluble	g/l
Fresh herring							
Extract I	25	70.6	59.6	11.0	84.5	15.5	8.0
Extract II	25	22.1	4.7	17.3	21.5	78.5	21.8
Extract III	25	2.7	.....	2.7	.....	100	3.4
Solution I[d]	3	11.0	1.1	9.8	1.6[e]	13.9	7.2
Solution II[d]	3	16.5	9.0	7.5	40.7[f]	34.1	9.6
Solution III[d]	3	2.7	.....	2.7	.....	100	3.4
Stored herring							
Extract I	25	64.0	46.1	17.9	71.9	28.1	12.6
Extract I	3	63.8	47.6	16.2	74.6	25.4	11.5
Extract II	3	28.0	10.4	17.6	37.0	63.0	15.0
Extract III	3	1.9	.....	1.9	.....	100	1.9

[a] Lipid loss (e.g., through manipulation) including lipids left in FPC was 4.6% of starting lipid in fresh herring and 6.3% in frozen stored herring.
[b] Total IPA-insoluble (oil) recovery was 74.4% of starting lipid in fresh herring and 58.0% in stored frozen herring.
[c] Total IPA-soluble lipid after final treatment was 20.0% of starting lipid in fresh herring and 35.7% in stored frozen herring.
[d] IPA phases separated from extracts at 25 C.
[e] Total of 10.3% of lipid in solution I.
[f] Total of 51.9% of lipid in solution II.

herring. Data for solution lipid contents and yields of oil are given in Table II. Further cooling to −18 C of IPA-rich phases from frozen stored herring after separation at +3 C yielded uneconomic amounts of lipid.

Properties of Lipids Recovered From Herring Processing Extracts and From FPC

The predominant lipid types in each fraction were determined by thin layer chromatography (Table III). The bulk of the solvent of the IPA-rich phases was removed with a rotary vacuum evaporator. Water was added and the lipid extracted into petroleum ether (four successive extractions). Chloroform gave similar weight recoveries of lipids. The water soluble materials were chiefly (94%) in Extract I, with some (6%) in Extract II and a trace in Extract III (experiment with stored frozen herring). The IPA-insoluble materials were washed two or three times with water to remove IPA and water soluble materials and dried over sodium sulphate. Acid values, iodine values (Wijs) and percentage of non-saponifiable materials (AOCS Official Methods) were determined on appropriate fractions (Table III). Details of fatty acid compositions for most materials are given in Table IV.

The final FPC filter cakes were extracted by the method of Bligh and Dyer (50 g, solvent ratios based on the addition of 80 ml water), with re-extraction of the filter with chloroform. The FPC was oven dried and bagged in polyethylene bags. After four days FPC samples were extracted quantitatively by the same procedure. FPC from the fresh herring yielded 0.28% lipid; that from stored frozen herring yielded 0.19% lipid. A thin layer chromatographic examination of the filter cake and final FPC lipids showed a strong similarity to the starting material lipids, but with additional emphasis on triglycerides and on phospholipids. Details of the fatty acid composition are given in Table IV.

Properties of Potential Phospholipid Product

The thin layer chromatographic investigations of the lipids soluble in IPA indicated a substantial concentration of phospholipid in the solution remaining from Extract I from fresh herring, after consecutive removal of the materials insoluble at 25 C and 3 C. A sample of this lipid was separated by gel chromatography as described above (Fig. 1-II) into 12 fractions. After thin layer chromatographic examination these were pooled in appropriate groups summarized as follows: fractions 1–2 (78.3%) were substantially pure phospholipid; fractions 3–4 (6.7%) contained roughly equal amounts of triglycerides and free fatty acid, with some sterol and diglyceride; fractions 5–6 (7.0%) were mostly free fatty acid, with sterol and sterol ester as minor components; fractions 7–10 (7.9%) were chiefly sterol and sterol ester. Fractions 1 and 2 were compared by thin layer chromatography with the phospholipids recovered from fresh herring lipids as described above. The pattern obtained was essentially identical except for a few trace spots of low R_f value. The fatty acid compositions of the phospholipids and of the balance of the lipid extract (fractions 3–10) are included in Table I.

TABLE III

Principal Lipid Types as Indicated by TLC and Acid Values, Iodine Values and Percentage Non-saponifiables Where Relevant, of Fractions Produced From Fresh (F) and Stored (S) Herring

	Material	Temperature (C)	Phase	Types of lipid present (adjudged from TLC)	Acid value	Iodine value	Per cent non-saponifiable
F	Extract I	25	Insoluble	Mostly triglyceride, some sterol	0.30	115	0.71
			Soluble	Mixture phospholipid, sterol, FFA, triglyceride	21.5	.....	.....
F	Extract II	25	Insoluble	Nearly pure triglyceride	0.05	110	0.21
			Soluble	Mostly triglyceride, some sterol and phospholipid	1.86	.....	.....
F	Extract III	25	Soluble	Mostly triglyceride, some sterol and phospholipid	1.60	.....	.....
F	Solution I	3	Insoluble	Mostly triglyceride, some sterol	0.79	114	0.69
			Soluble	Mixture phospholipid, sterol, FFA, triglyceride	23.7	.....	.....
F	Solution II	3	Insoluble	Nearly pure triglyceride	0.05	110	0.23
			Soluble	Mostly triglyceride, some sterol and phospholipid	4.70	.....	.....
S	Extract I	3	Insoluble	Mostly triglyceride, traces sterol, FFA, phospholipid	1.52	109	0.92
			Soluble	Mostly FFA, with large amounts of sterol ester and phospholipid	69.0	.....	.....
S	Extract II	3	Insoluble	Nearly pure triglyceride	0.10	81, 101 } 94	0.38, 0.34 } 0.36
			Soluble	Mostly triglyceride, some sterol and phospholipid	4.96	.....	.....
S	Extract III	3	Soluble	Mostly triglyceride, some sterol and phospholipid	4.34	.....	.....

TABLE IV

Fatty Acid Composition of Raw Material Lipids, of Various Processing By-product Lipids, and of Lipids Extracted From Final FPC Products

Fatty acid[a]	Raw material lipids	Weight per cent of fatty acids from lipids recovered from products made from fresh herring							Weight per cent of fatty acids from lipids recovered from stored frozen herring								
		Extract I 25 C		Extract II 25 C		Extract III	FPC filter cake extract	Final FPC extract	Raw material lipids	Extract I 3 C		Extract II 3 C			Extract III	FPC filter cake extract	Final FPC extract
		Insol.	Sol.	Insol.	Sol.					Insol.	Sol.	Insol. A	Insol. B	Sol.			
12:0	0.4	0.4	1.3	0.1	0.3	0.3	0.2	0.4	0.2	0.4	0.6	0.6	0.4	0.4	0.2	0.5	0.4
13:0	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.2
14:0	5.4	5.6	3.4	4.9	5.4	6.2	5.2	5.9	6.2	7.3	3.6	6.4	9.1	6.2	6.9	5.2	5.8
14:1[b,c]	0.5	0.5	0.4	0.4	0.5	0.5	0.7	0.5	0.4	0.7	0.4	0.7	0.5	0.4	0.6	0.3	0.6
15:0	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.5	0.3	0.6	0.5	0.4	0.5	0.4	0.4	0.1	0.5
15:1 + I16:0[d]	0.2	Trace	0.1	0.1	0.3	0.2	Trace	Trace	Trace	0.4	0.2	0.1	0.2	0.1	0.2	Trace	Trace
16:0	12.2	11.9	17.2	11.5	12.5	12.0	12.6	13.7	15.8	14.5	18.7	14.5	20.5	12.0	13.8	14.1	14.7
16:1[e]	12.8	13.6	8.3	11.5	13.9	12.6	11.9	12.8	10.6	12.5	9.0	9.5	8.0	12.9	11.4	9.9	10.3
16:2 + 17:0[f]	1.2	1.3	0.9	1.2	1.3	1.3	1.3	1.0	0.9	1.1	0.9	1.0	0.7	1.2	0.8	0.6	0.1
16:3 + 17:1	0.9	1.1	0.5	0.9	0.9	0.9	0.8	0.7	0.9	0.9	0.7	0.7	0.5	1.2	1.1	0.7	0.3
16:4ω1	1.0	1.0	0.8	1.0	1.0	1.1	0.7	0.7	0.5	0.8	0.8	0.6	0.5	1.0	0.7	0.5	0.3
18:0	1.2	1.0	2.8	1.2	0.9	0.9	2.4	1.8	0.8	0.8	1.8	0.9	1.2	0.7	0.9	1.3	1.1
18:1[b]	14.7	15.1	12.4	14.7	15.2	15.2	14.6	15.1	17.7	18.8	15.4	16.2	13.8	19.1	17.2	17.9	18.5
18:2ω6	1.0	1.0	1.0	1.2	1.0	1.0	1.1	1.1	1.3	1.2	1.2	1.1	0.8	1.3	1.2	1.1	1.0
18:3ω6 + 19:1	0.3	0.4	0.3	0.3	0.5	0.3	0.4	0.3	0.2	0.3	0.2	0.3	0.4	0.3	0.4	0.2	0.1
18:3ω3	0.3	0.5	0.3	0.3	0.3	0.3	0.3	0.3	0.5	0.4	0.5	0.3	0.5	0.5	0.4	0.5	0.3
18:4ω3	1.1	1.1	0.7	1.1	1.3	1.1	0.9	1.0	1.2	0.8	1.2	0.7	0.6	0.7	0.7	1.0	0.6
20:0	0.1	0.1	Trace	0.2	0.2	0.1	0.1	0.1	0.1	Trace	?	Trace	?	?	?	Trace	?
20:1[b]	12.7	13.2	5.0	15.5	12.3	13.5	11.5	13.1	11.6	13.0	4.0	15.1	14.5	12.9	13.8	12.0	13.3
20:2ω6	0.2	0.1	0.1	0.3	0.1	0.1	0.3	Trace	0.2	0.1	0.1	0.1	0.3	0.1	0.2	0.1	0.2
20:3ω6	0.2	Trace	?	?	0.3	?	0.1	0.1	Trace	?	?	?	?	?	0.1	0.1	0.1
20:4ω6	0.4	0.1	0.6	0.5	0.5	0.1	0.7	0.1	0.1	0.1	0.6	?	Trace	0.1	0.2	0.3	0.2
20:4ω3	0.2	0.1	0.2	0.1	0.3	0.1	0.3	0.2	Trace	?	?	Trace	Trace	?	0.1	Trace	Trace
20:5ω3	7.4	6.8	13.2	6.0	7.5	6.9	8.0	6.3	7.3	5.0	13.4	3.9	2.6	6.6	5.4	6.6	5.2
21:5ω2 ?[g]	0.1	0.2	0.2	0.2	0.5	0.2	0.1	0.1	0.2	0.1	0.1	0.1	Trace	0.1	0.1	0.1	0.1
22:1[b]	19.0	20.0	5.8	23.3	18.1	19.9	17.9	19.0	16.3	17.6	3.7	23.9	21.6	17.5	19.8	17.1	18.8
22:5ω6	Trace	Trace	0.1	?	?	?	?	?	Trace	?	0.2	?	?	Trace	?	0.2	0.2
22:5ω3	0.5	0.5	0.9	0.2	0.5	0.5	0.2	0.6	0.3	0.3	0.6	0.1	0.1	0.3	0.1	0.5	0.2
22:6ω3	4.6	3.1	22.4	2.4	3.6	3.8	6.7	4.3	5.9	2.0	21.5	2.0	1.3	3.6	2.9	8.0	6.3
24:1[b]	0.6	0.5	0.7	0.2	0.1	0.5	0.2	0.1	0.3	Trace	0.1	0.4	0.9	Trace	0.1	0.7	0.5

[a] Notation for chain length:number of double bonds and position of nearest double bond relative to terminal methyl.
[b] Mostly with ω9 isomer predominating; other isomers present.
[c] Includes iso and anteiso 15:0 and possibly 4,8,12-trimethyltridecanoic acids.
[d] Includes pristanic acid.
[e] Includes iso and anteiso 17:0 acids.
[f] Includes phytanic acid.
[g] Includes traces of 22:4ω6.

Discussion

The choice of IPA as the solvent for extraction of lipids from raw fish stems from a thorough investigation of the influence of solvent-water ratios (17–19) and appreciable development work (2). Various views on the economic implications of FPC production will be found in the proceedings of a recent symposium (20). Unpublished observations from U.S. Bureau of Commercial Fisheries Laboratories stated that an oil could be recovered from IPA extracts from whole fish of even low fat content by simply cooling the hot extract. These observations suggested a favorable prospect for recovery of a good quality oil from fatty fish. In preliminary tests in this laboratory, attempts to remove most of the IPA by distillation led to degradation of the extracted lipid, therefore this procedure might also contribute to accumulation of undesirable flavor components in the IPA (21). Rapid removal of as much lipid as possible by continuous centrifugation might facilitate recycling of solvent fractions in a countercurrent FPC production process. This report is concerned primarily with lipid fractionation and the proportions and quality of oils and lipids produced. The economics of recovering IPA from the oil phase, or from the IPA-rich phases are not included.

Raw Material and Lipids Recovered From Herring

The moderate difference in the fat contents of the two lots of herring tested is typical of seasonal variations, or of different schools of fish taken at the same time of year. The fresh herring were caught in April 1967 near Halifax, N.S. The stored frozen herring had been taken the previous August. The formation of free fatty acids during frozen storage is probably largely from phospholipids, but some hydrolysis of triglycerides would be expected on prolonged storage (11). Some herring are taken when not feeding, others are gorged with food (small crustaceans). It is probable that the latter herring would exhibit more rapid lipid hydrolysis, but such fish would be undesirable for FPC production after frozen storage since protein autolysis would also occur rapidly, lowering yields of FPC. The total yields, relative to starting lipid, of IPA insoluble lipid were 74.4% for fresh herring (82% of available triglyceride) and 58% for stored herring. This clearly indicates that fresh herring are also preferable from the oil yield as well as oil color point of view. The balances for residual lipids (Tables II-III) show that the diminution in yield is largely due to the free fatty acids present and is not associated with starting lipid or fat level in the herring. The concentration of these free acids in the IPA-rich phase from the first extraction would facilitate their recovery, but they are of little potential value compared to the triglyceride oil. Throughout this study the oils and extracts from fresh herring were invariably lighter in color than those from the stored herring. Most of the color from both raw materials was however concentrated in the IPA-rich phase of Extract I. The concentration of phospholipid in the same fraction is also of interest if high quality marine phospholipid could be marketed. Otherwise the combination of fatty acid and phospholipid could produce a material combining emulsifying characteristics with oily properties and hence perhaps useful at a technical level for leather dressing (3) or as a carrier for spraying of insecticides, weed killers, etc. (compare 22).

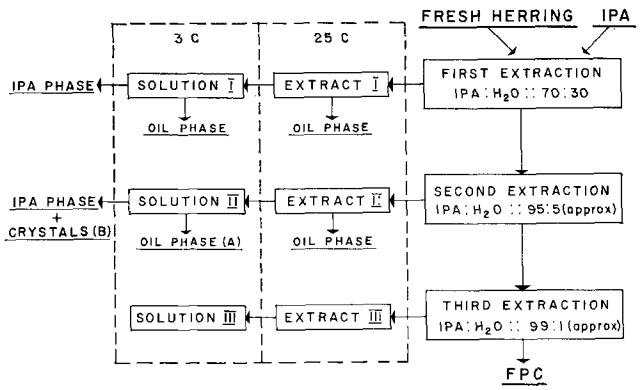

FIG. 2. Outline of extraction and separation steps employed in studying recovery of triglyceride oil from FPC production based on fresh herring. The extraction procedures were the same with the frozen stored herring, but treatment of the extracts differed somewhat as was described in the text.

The recovered IPA-insoluble lipids are triglyceride oils of high quality (Table III). The low level of cholesterol, normally the dominant non-saponifiable material in herring oils (ca. 0.5–1.5%), suggests the possibility of specialty dietary products. It is also probable that the partition process would carry certain other objectionable materials, such as sulphur-containing amino acids which inhibit hydrogenation processes, into the IPA-rich phase. The extracted oils might therefore be more easily hydrogenated than normal commercial oils. The fatty acid compositions of the various major oil fractions show only modest differences for particular fatty acids; it is not at present possible to associate these systematically with the solubility of different triglycerides. However the reduction in iodine values of oils recovered from Extract II in relation to Extract I (Table III) indicates that a slight triglyceride partitioning effect is operative, with the more highly unsaturated materials being marginally more soluble in the IPA containing 30% water. The fresh herring triglycerides separated at 3 C after prior removal of an oil-rich layer at 25 C had the same iodine values and showed negligible differences from the latter in details of fatty acid composition. These differences were also those expected, with generally higher levels of polyethylenic acids and lower levels of long-chain monoethylenic acids. The method of separation of lipid from both Extracts II is difficult to explain in detail, but the crystalline material (B) observed in the IPA-rich layer is a classical stearine rich in myristic and palmitic acids (Table IV). Comparison of fatty acid compositions for the triglyceride oils with normal commercial herring oils (16,23) reveals no obvious differences which might not be to local variations in "condition" of the herring.

The Extraction Procedure

We are unaware of any comparison of the basic IPA extraction process of Dambergs (17,18) and the Bligh and Dyer chloroform-methanol-water system (13) in terms of the types of lipids extracted, and of any alterations in their fatty acid composition due to differential selectivity of extraction. Table I indicates that there is no notable difference in the fatty acid composition of the total phospholipids from the laboratory extraction or the FPC process, which supports the thin layer chromatographic evidence for the similarity of the two extractive phospholipid systems in terms of types of phospholipid. The extracted triglycerides are also essentially similar, but somewhat more variation is apparent in the fatty

acid compositions of the combined "other lipids." A small amount of triglyceride was included in the other lipids from the IPA extract, but some products such as free fatty acids and diglycerides formed by lipid degradation during the IPA extraction process could also account for the differences which suggest fatty acids of triglyceride origin. Pending a detailed laboratory comparison of individual phospholipids and other lipids we suggest that the two extraction processes recover essentially the same lipids and that any small differences in weight recoveries reported under laboratory conditions (13,18,19) reflect inclusion of some undegraded lipoproteins or similar material. A large-scale source of high quality phospholipid is therefore available in connection with FPC production from fresh herring. The composition is evidently similar, in respect to types of phospholipids and total fatty acids, to other marine phospholipids (10,24,25).

Commercial practices in FPC production based on IPA will probably be based on an IPA-water azeotrope containing about 90% IPA (2). This need not materially alter concepts of fat recovery or lipid distribution based on the results given in this paper since the first extraction which removes the bulk of the lipids (Table II) would be effectively the same. A countercurrent process would perhaps particularly benefit from the use of this azeotrope since it is apparent that in Extractions II and III the additional water would result in separations of additional oil at reduced (room) temperature without impairment of extraction efficiency for all lipids at the higher operating temperature of the extraction. Solubility data for triglyceride oils in IPA-water systems is available (Dyer, private communication). It should be noted that reduction of temperature from 25 C to 3 C has no especial advantage in oil recovery.

Residual Lipids in FPC

The comparison of the fatty acids extracted from the fresh FPC filter cake and from the dried FPC after brief storage indicates that some autoxidation has taken place, since the chief polyunsaturated acids, 20:5ω3 and 22:6ω3, are markedly reduced in the FPC extract. The fatty acid compositions (Table IV, compare triglycerides, phospholipids and other lipids, Table I) clearly indicate that the residual lipid which is extractable from FPC by the process described is to a large extent triglyceride in character, confirming the results of the thin layer chromatographic examination. The high levels of 20:1 and 22:1 are particularly indicative of this relationship. The spot for phospholipids was at the origin in the solvent system employed and may include partially degraded phospholipids which would not contribute much to the fatty acid analysis. The U.S. Bureau of Commercial Fisheries has supported a thorough examination of the lipids recovered by an exhaustive extraction of FPC products, including red hake and menhaden FPC produced by an IPA process (26,27). The lipids extracted from hake FPC had neutral lipids and a substantial proportion (20–35%) of phospholipids, although these may have been partially degraded [Note that Table V in the original publication (26) requires the insertion of a C_{24} in the fatty acid column and raising of the fatty acid notations $C_{20:2}$–C_{23} by one line. This material is also reprinted in part in (27)]. The fatty acid compositions for the hake FPC lipids are indicative of an origin in phospholipids, because proportions of 18:0 and 18:1 are high. On the other hand 16:1 is very low compared to 18:1 and 16:0, and 20:1 and 22:1 are apparently negligible (24). The authors mention relatively low proportions of 20:5ω3 and 22:6ω3 in comparison with the lipids of fresh hake, but these acids would be particularly affected by autoxidation, and this would extend to damage done during the lengthy extraction period. It is therefore curious that in the similar study of FPC produced from fresh menhaden (*Brevoortia tyrannus*), these acids are reported in proportions similar to those in literature data on menhaden lipid composition (27). Menhaden, like herring, are classed as fatty fish, and the fatty acid composition of the extracted lipid (0.15%) confirms lipid chromatography results indicating that the lipid of menhaden FPC was largely triglyceride. The total lipid recoveries from several samples of IPA-produced hake FPC were in most cases about one half those recovered by brief laboratory extraction in the present study, although elsewhere a range of 0.13–0.22% is reported (28). There are thus indications that in FPC from either fatty or lean fish the residual lipids reflect the predominant types of starting lipid, i.e., triglycerides for the herring and menhaden, and phospholipids for the hake, and this is likely to be independent of actual lipid level in the FPC. The presence of unaltered polyunsaturated acids after air drying or prolonged storage of FPC suggests that the residual lipid is protected to some extent from oxygen, either by enclosure in physically contracted dried fish muscle or by the proposed membrane effect (29,30).

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