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Variability of the composition of fish oils: significance for the diet

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Photosynthesis by most algae and phytoplankton is associated with the production of *n*-3 polyunsaturated fatty acids (PUFA). These acids, members of the α-linolenic family, eventually pass through the food web and are incorporated into fish lipids and, thus, form an integral part of our diet either through consumption of fish, fish oils or the flesh of terrestrial animals subjected to a diet containing fish or fish products. Our consumption of fish has a long history. In palaeolithic times, man was already enjoying crustaceans and molluscs while fossilized remains of hand-caught fish date back 380 000 years. Methods of preservation, including smoking and drying, were developed thus allowing increased utilization of this food. Since the forging of a link between the consumption of *n*-3 fatty acids and benefits to health, many detailed studies have been carried out to investigate the influence of fish oils on various physiological processes. The term fish oil, used generically, embraces a complex matrix of components in which there are differences in composition depending on the species of fish from which the oil is obtained. This overview attempts to provide an insight into the range of molecular species present in fish oils and to emphasize the importance of characterizing the composition of products used in feeding trials and physiological studies.

NA MARA (OF THE SEA)

Oceans and seas cover more than seven-tenths of the earth's surface, providing a habitat for over 60 000 different species of fish, crustaceans and molluscs. Many of these are edible. Fish and shellfish were one of primitive man's main foods in his earliest days as a food gatherer (Connell & Hardy, 1982) although, in Scotland, the early inhabitants were not great fish-eaters and did not exhibit any marked seafaring tendency (Hope, 1987). Our ancestors did enjoy shellfish such as mussels, whelks and limpets but, apart from the occasional stranded whale or seal, this represented the limit of the Scottish marine diet. The introduction of small boats permitted the capture of ling and cod but the arrival of the Vikings with their large sea-going boats in the 8th century, introduced the Scots in the North and West to the bountiful supply of herring which were present in the seas around Scotland. Furthermore, the introduction of Roman Catholicism meant that fish was the chief permitted protein during Lent and at other times (Hope, 1987). Thus, commercial fishing soon became both necessary and profitable.

A major difficulty encountered with fish as a food is that it is highly perishable and rapidly spoils immediately after catching (Kelman, 1982). Thus, some means of preservation must be employed to counteract bacterial and oxidative spoilage. Demersal species including Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) together with flatfish, dogfish and others live on or near the sea bed. These fish tend to have a low fat content in the flesh (<20 mg/g), the bulk of the lipid being phospholipid (Bligh & Scott, 1966; Jangaard *et al.* 1967). As such, they lend themselves

Table 1. *UK imports and exports by fishery commodity groups**

Commodity group	Import (tonnes)		Export (tonnes)	
	1989	1990	1989	1990
Fish, fresh, chilled, frozen	279 796	302 316	393 700	371 688
Fish, dried, salted, smoked	2 879	3 842	7 839	6 910
Crustaceans† and molluscs‡	34 797	34 213	53 198	59 246
Fish, canned	133 528	126 733	13 774	13 825
Crustaceans and molluscs, canned	29 626	30 243	4 367	4 662
Oils	177 875	161 818	1 514	7 370
Meals	266 498	275 375	6 430	5 398
Total value (1000 US$)	1 627 924	1 911 161	794 293	961 982

* Food and Agriculture Organization (1992).
† Includes crabs, lobsters, prawns, shrimps and crayfish.
‡ Includes mussels, oysters, scallops, squid and octopus.

to a simple and effective cure merely by salting and drying in the sun and wind, yielding a bread substitute. Pelagic species, shoaling fish living in midwaters, include Atlantic herring (*Clupea harengus*) and mackerel (*Scomber scombrus*). These fish store most of their lipid in the flesh and under the skin (Love, 1982). Consequently, if simply hung up to dry, the flesh rapidly deteriorates due to oxidation of the highly unsaturated lipids. This problem was resolved in the 14th century with the introduction of a 'new process', the gutted and rinsed fish were packed in layers, interspersed with coarse salt, in barrels.

The historical consumption of fish in Scotland varied considerably according to location and availability. Cod, haddock and plaice were prominent in the diet of coastal areas while salmon and trout made a significant contribution to the diet in many inland areas (Steven, 1985). In the Northern Isles and the Hebrides coal-fish or saithe (*Pollachius virens*) were an important component of the diet while the liver oil was used as a fuel for lamps. Part of the staple winter and spring diet of the Shetlanders was dried saithe, cod and haddock, while shellfish boiled in milk was a nutritious stand-by. On the West Coast, herring was the most important fish, herring boiled over potatoes being considered very nutritious. Red herrings was a popular product in England. This heavily salted, hard-smoked product became popular during the 14th century but, in more recent times, mildly smoked products including the succulent 'Scots' kipper have become more popular, although the term 'kipper' initially referred to salmon (Cutting, 1955; Steven, 1985).

In the early 19th century, canning of fish products was introduced and canned fish became as much a standard item of the diet as pickled herring and dried-salted cod would have been in former times (Cutting, 1955).

In modern times, freezing has developed as a popular method of preserving fish. This technique, when successfully carried out, has the advantage of permitting the preservation of the fish, as it were, in the fresh state. A consequence of this is that dried fish or even pickled fish are no longer major products consumed in this country.

The present day UK trade in fish products is illustrated by examining the UK import and export figures for the seven fishery commodity groups (Table 1). Chilled, fresh or frozen fish represent the major fishery commodity with respect to both import and

export, but substantial quantities of canned fish, fish oils and fish meals are imported by the UK (Food and Agriculture Organization, 1992).

During 1991 about 488 878 tonnes of sea fish, excluding shellfish and livers, were landed in the UK, a substantial amount (83%) being caught in the North Sea or off the West Coast of Scotland. The bulk of sea fish (79%) was landed at Scottish ports where the percentage accounted for by pelagic species was slightly greater than that for demersal. Mackerel was landed in the greatest quantity in 1991 (124 955 tonnes) followed by herring (93 298 tonnes; Sea Fish Industry Authority, 1992).

THE SOURCE AND NATURE OF FISH OILS

Although water and protein are major components of fish flesh, fish oils, specifically cod liver oil, have long been held in high regard as being nutritionally beneficial (Bull, 1899). Since the early 1970s, there has been an intense scientific interest in the health benefits of fish and fish oils stimulated by the work of Dyerberg and co-workers on the incidence of coronary heart disease in native Greenland Eskimos (Dyerberg *et al.* 1975, 1978; Dyerberg & Bang, 1979; Dyerberg & Jorgensen, 1982).

Fish lipids are different from those of terrestrial origin in that the major unsaturated fatty acids belong to the *n*-3 family (Exler *et al.* 1975; Herold & Kinsella, 1986; Ballard-Barbash & Callaway, 1987). These long, straight-chain molecules contain a series of *cis* double bonds, the first of which is between C-3 and C-4 counting from the methyl- or ω-C. The fatty acids which have attracted most attention are *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA; timnodonic acid) and *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA) (Payan *et al.* 1986; Kinsella, 1987; Hirai *et al.* 1989; Yerram & Spector, 1989). Fish and fish dishes provide 14% of the average daily intake of *n*-3 fatty acids (Gregory *et al.* 1990). Other major sources include cereal products (17%), meat (19%) and vegetables (22%). Consumption of fish should help people to obtain the recommended provision of 7·5% of total energy by *cis*-PUFA (British Nutrition Foundation, 1992) but EPA and DHA can be consumed via fish oils.

The largest source of fish oils is that of body oils from pelagic species; 1 368 145 tonnes were produced in 1990 (Food and Agriculture Organization, 1992) corresponding to 97·9% of total fish oil production. Fish liver oils accounted for 1·7%, the remainder coming from marine mammals, squid and various other species. The major fish oil-producing species include anchovy (*Engraulis* spp.), capelin (*Mallotus villosus*), herring (*Clupea* spp.), horse mackerel (*Trachurus* spp.), menhaden (*Brevoortia* spp.), mackerel (*Scomber* spp), Norway pout (*Trisopterus esmarkii*), sand eel (*Ammodytes tobianus*), sprat (*Clupea spratus*), *Sardinops* spp. and others. Capelin and herring are found in polar or boreal waters, but the really large pelagic resources are found in temperate or subtropical waters. However, a very high proportion of the total catch of pelagic fish is taken over a very small proportion of the earth's surface, much of it close to land (Enser, 1991; Bailey, 1992).

Japan is the largest single producer of fish oils and fats, averaging 29% of total world production from 1988 to 1990, inclusive. There is little difference in the average production in Asia (31·7%) and South America (30·4%) over the same period (Food and Agriculture Organization, 1992). Production in Europe is dominated by Denmark, Norway and Iceland, with the United Kingdom contributing only 0·5% to total world production.

In Europe, fish body oils are produced either from small fish such as sprat or sand eel or from larger fish such as herring when there is excess, or from fish offal (Windsor, 1982; Young, 1982). Fish meal and fish oil are generally made at the same time, fish oil being a byproduct of the meal industry. The most important factor in the production of a high-quality crude fish oil is the condition of the raw material at the start of processing. Prompt handling of the fish and fish offal is critical. The raw material is first cooked, for 15 min at 90°, to facilitate coagulation of the proteins, sterilization and separation of oil. The cooked fish is then conveyed through a perforated tube whilst being subjected to increasing pressure, normally by means of a tapered shaft on a screw conveyor. The oil and water mixture, known as press liquor, passes through the perforations while the solid, known as press cake, emerges from the end of the press. After screening to remove coarse pieces of solid material, the press liquor is centrifuged, in desludging or self-cleaning centrifuges, to separate the oil from the water. The fish oil is further refined by washing with water (100 ml/l oil) at 90–95° followed by centrifugal separation. At this stage the crude fish oil still contains a number of impurities. These include moisture, rust, dirt, proteins, free fatty acids, mono- and diacylglycerols, enzymes, soaps, trace metals such as Cu and Fe which promote oxidation, oxidation products, pigments, phosphatides, hydrocarbons, terpenes, resins, sterols, waxes, sugars and compounds containing S, N and halogens (Young, 1982, 1985, 1986). The impurities arise as a result of the natural phenomena of season, geographical location (giving rise to marine pollutants in the oil) and food, together with the freshness of the fish at time of extraction, and efficiency of the extraction process. Thus, the essentially 900–950 g triacylglycerol/kg oil (Young, 1986; Urdahl, 1992) is further refined as detailed in Table 2. The final product will still be subject to post-processing oxidation. The nature and concentration of the oxidation products will vary with time and are critically dependent on how well the oil is stored.

In the past, fish oils have been used for tanning, as water repellents, lubricants, plasticizers, corrosion inhibitors and as a fuel (Windsor, 1982). In excess of 95% of fish oil produced is now used for human food (Young, 1986). The oils are hydrogenated following refining to produce a solidified fat for use in margarines and shortenings. Total hydrogenation results in a product devoid of the vital *n*-3 PUFA and associated triacylglycerols containing the highly unsaturated molecules (Fig. 1). If, however, the oil is not hydrogenated, its composition and that of virtually all fish oils, can be described by reference to eight fatty acids: tetradecanoic acid (14:0, myristic acid), hexadecanoic acid (16:0, palmitic acid), *cis*-9-hexadecenoic acid (16:1 (*n*-7), palmitoleic acid), *cis*-9-octadecenoic acid (18:1 (*n*-9), oleic acid), *cis*-9-eicosenoic acid (20:1 (*n*-11), gadoleic acid), *cis*-11-docosenoic acid (22:1 (*n*-11), cetoleic acid), EPA and DHA (Fig. 2). The fatty acid composition of fish oils is further complicated by the presence of lesser amounts of pentadecanoic acid (15:0), hexadecadienoic acid (16:2), hexadecatrienoic acid (16:3), hexadecatetraenoic acid (16:4), octadecanoic acid (18:0, stearic acid), *cis*-11-octadecenoic acid (18:1 (*n*-7), asclepic acid), *cis*-9,12-octadecadienoic acid (18:2 (*n*-6), linoleic acid), *cis*-9,12,15-octadecatrienoic acid (18:3 (*n*-3), α-linolenic acid) *cis*-6,9,12,15-octadecatetraenoic acid (18:4 (*n*-3), moroctic acid), eicosanoic acid (20:0, arachidic acid), *cis*-11-eicosenoic acid (20:1 (*n*-9)), *cis*-5,8,11,14-eicosatetraenoic acid (20:4 (*n*-6), arachidonic acid), *cis*-8,11,14,17-eicosatetraenoic acid (20:4 (*n*-3)), and *cis*-7,10,13,16,19-docosapentaenoic acid (22:5 (*n*-3), clupanodonic acid). Furthermore, there are trace quantities of the odd carbon number fatty acids heptadecanoic (17:0) and

Table 2. *Unit processes utilized in the refining of crude fish oils (after Young, 1982, 1985)*

Stage	Procedure	Impurities reduced or removed
Crude oil storage	Oil left in tanks to settle. Good oil drawn off the top	Oil insolubles
Degumming	Treatment with phosphoric acid (800–850 ml/l) at 90°	Phospholipids, sugars, resins, proteinaceous compounds, trace metals and others
Neutralization	Treatment of hot oil (90–95°) with sodium hydroxide (4 mol/l)	Fatty acids, pigments, oil insolubles, water solubles
Washing	Treatment with soft water (<50 μg/g hardness expressed as Ca0) at 90–95°. Phosphoric or citric acid may be added to the final wash water	Soaps
Drying	Heated to 90° under vacuum with rapid agitation	Water
Bleaching	Treatment with activated clays (2–300 g/kg) under vacuum at 90–110°	Pigments, oxidation products (aldehydes and ketones), trace metals, trace soaps
Filtration	Various designs of filters	Spent bleaching earths
Deodorization	Steam distillation under vacuum	Fatty acids, mono- and diacylglycerols, aldehydes, ketones, hydrocarbons, sulphur compounds, pigment decomposition products

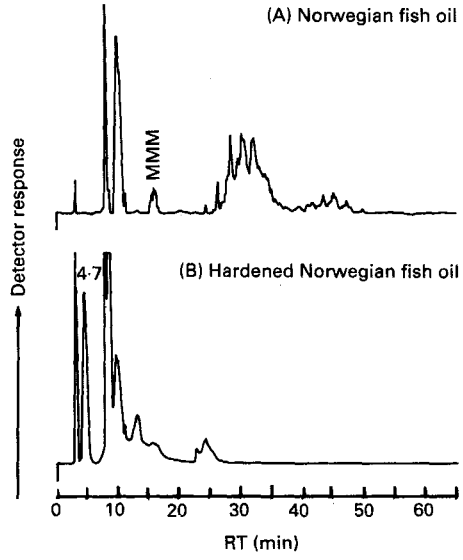

Fig. 1. Silver ion HPLC of the triacylglycerols from a Norwegian fish oil (A) and the hardened derivative (B). The oils (120 mg) were dissolved in 1,2-dichloroethane (10 ml). Injections (50 μl, 600 μg) were performed automatically to ensure reproducible reconditioning of the Nucleosil 5SA column. Detection was by means of a light scattering detector (McGill & Moffat, 1992). The original fish oil contained trisaturated triacylglycerols (retention time (RT) 3 min) and those with one or two monoenoic acids per molecule (RT 8 and 11 min respectively). Triacylglycerols containing three monoenoic acids (MMM) precede those with di-, tri- and more highly unsaturated fatty acids. Very highly unsaturated molecules (greater than twelve double bonds) are within the last group of peaks of chromatogram A. The peak at 4·7 min in the hardened Norwegian oil profile arises from triacylglycerols substituted by *trans*-acids.

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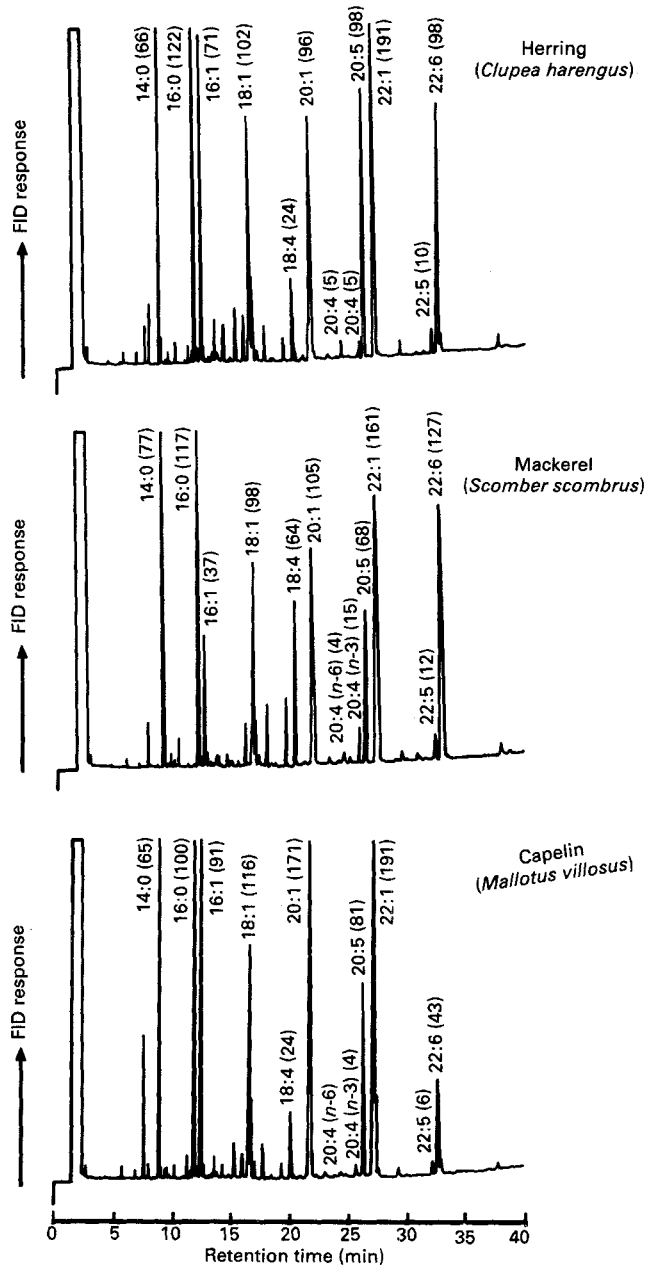

Fig. 2. Fatty acid profiles for herring (*Clupea harengus*), mackerel (*Scomber scombrus*) and capelin (*Mallotus villosus*) body oils. The methyl esters were prepared and analysed as described by Moffat *et al.* (1991). The combined levels of *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA) and *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA) is 196, 195 and 124 mg/g fatty acids for herring, mackerel and capelin respectively. Other PUFA present include 18:3 (*n*-3), 18:4 (*n*-3), 20:4 (*n*-3 and *n*-6) and 22:5 (*n*-3). These oils contain significant amounts of 20:1 and 22:1. Values in parentheses are levels for individual fatty acids expressed as mg/g fatty acids. FID, Flame ionization detector.

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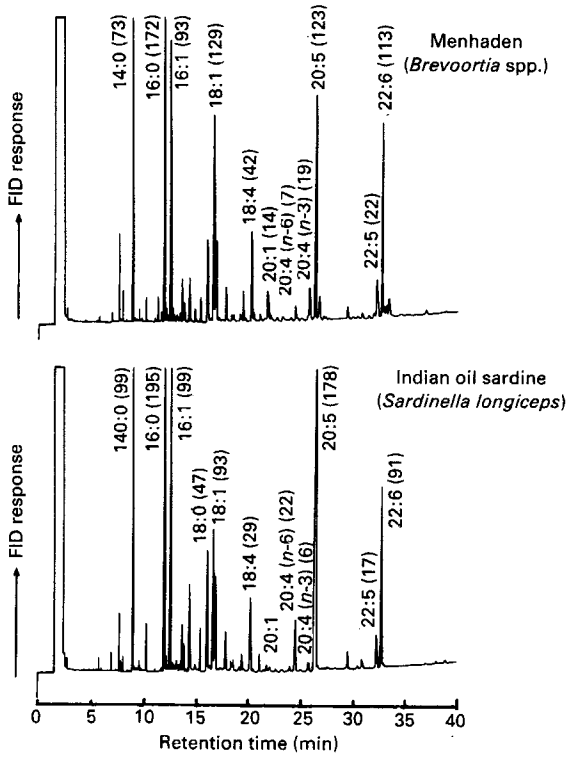

Fig. 3. Fatty acid profiles for menhaden (*Brevoortia* spp.) oil and Indian oil sardine (*Sardinella longiceps*). The combined amounts of *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA) and *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA) is 236 and 269 mg/g fatty acids respectively. These fish oils contain only small amounts of 20:1 and only traces of 22:1. Values in parentheses are levels for individual fatty acids expressed as mg/g fatty acids. FID, Flame ionization detector.

heptadecenoic (17:1) acid together with methyl-branched fatty acids (Ackman, 1980; Adolf & Emken, 1985; Ratnayake *et al.* 1989). The wider range of fatty acids found in fish relative to land animals arises because fish absorb and assimilate the wide range of fatty acids in their food. Pelagic species feed on the numerous floating populations of plankton species which contain significant concentrations of EPA and DHA. Fish can synthesize fatty acids *de novo*, but another source of 20:1 and 22:1 is thought to be the oxidation of the corresponding long-chain alcohols in the dietary copepod wax esters (Ratnayake & Ackman, 1979*a*,*b*). Thus, pelagic species, including herring which feed on the copepods, show large quantities of 20:1 (96–200 mg/g fatty acids) and 22:1 (150–320 mg/g fatty acids) in their depot triacylglycerols (Fig. 2). Mackerel and capelin oil are also high in 20:1 and 22:1 and contain reasonable quantities of EPA and DHA (Fig. 2).

In contrast, menhaden (*B. patronus* and *B. tyrannus*) which is also planktivorous (Ackman, 1989) and Indian oil sardine (*Sardinella longiceps*), both species which are found in much warmer waters, have an equivalent range of fatty acids in their oils (Fig. 3), but 20:1 and 22:1 are minor constituents or absent (Ackman, 1980; Grundy, 1986). Although not regarded as a classical division, it is convenient to subdivide fish oils into those containing significant amounts of 20:1 and 22:1 and those with only trace quantities of these fatty acids. Thus, a major interspecies variation is immediately apparent.

Table 3. *Fatty acid composition of Atlantic herring (*Clupea harengus*) oils and cod (*Gadus morhua*) liver oils (after Ackman & Eaton, 1966 and Jangaard* et al. *1967 respectively)*

	Wt (mg/g fatty acids)	
Fatty acid	Herring oils (range of twelve)	Cod liver oils*
14:0	46– 84	15– 48
16:0	101–150	110–188
18:0	7– 21	17– 45
16:1	63–120	68–119
18:1	93–214	171–314
20:1	110–199	40–146
22:1	148–306	8–123
18:2 (*n*-6)	6– 29	8– 21
18:3 (*n*-3)	2– 11	3– 11
18:4 (*n*-3)	11– 25	5– 17
20:4 (*n*-6)	2– 5	5– 28
20:5 (*n*-3)	39– 88	59–151
22:5 (*n*-6+*n*-3)	6– 17	9– 35
22:6 (*n*-3)	20– 62	76–192

* Range of eighteen samples each of male and female cod collected over 18 months. Each sample constitutes four livers.

The EPA content of fish oils may range from 20 to 100 mg/g fatty acids for Atlantic herring oil (Ackman, 1980; McGill & Moffat, 1992), through 60–120 mg/g fatty acids for capelin oil (Ackman, 1982), to 170–250 mg/g fatty acids for anchovy oil (Ackman, 1982, Moffat *et al.* 1993). Again, major interspecies variation is evident but, at the same time, it is obvious that intraspecies variation is also extensive, this being associated with season (Hardy & Keay, 1972; Ackman, 1980, 1982; Opstvedt, 1985; Young, 1986), sex (Hardy & Keay, 1972) and catching area (Joseph, 1985; Urdahl, 1992). All the fatty acids are subject to this variation as illustrated for Atlantic herring oil (Table 3). Seasonal variations can result in as much as a 90% decrease in the EPA content of a fish oil (Ackman, 1982), but a 75% decrease over a season is more common. Similar changes are observed for DHA. Seasonal variations have been demonstrated in male mackerel where the concentration of 20:1 and 22:1 increased seasonally from approximately 30 to 90 and 130 mg/g fatty acids respectively (Hardy & Keay, 1972). Such variations were not observed for female mackerel, the concentration of 20:1 remaining at 63–64 mg/g fatty acids, while that of 22:1 increased from 83 to 87 mg/g fatty acids between December and June.

Extensive seasonal and geographical variations in fatty acid composition have been demonstrated for menhaden oils produced from fish obtained from two discrete catching areas, the Gulf of Mexico (*B. patronus*) and the Atlantic coast of Virginia (*B. tyrannus*). Gulf coast fish contained, on average, lower amounts of 22:6 and 20:5. Furthermore, in addition to geographical variations there were statistically significant differences in the mean levels for many of the fatty acids from oils obtained from 1982 fish relative to 1983 fish (Joseph, 1985).

The critical point is that although the range of major fatty acids remains constant, the concentration of each acid shows extensive inter- and intraspecies variation.

SILVER-ION HPLC OF FISH OIL TRIACYLGLYCEROLS

Ag^+-HPLC of fish body oils (Laakso *et al.* 1990; Laakso & Christie, 1991; McGill & Moffat, 1992) produces a triacylglycerol profile for the oils. Mackerel, herring and capelin oils, those containing high concentrations of 20:1 and 22:1, give similar overall patterns. Trisaturated triacylglycerols and those composed of one or two monoenoic acids with the appropriate number of saturated acids are present in the three types of oil. The trimonounsaturated triacylglycerols are especially evident in capelin oil (McGill & Moffat, 1992). As the triacylglycerols become more highly unsaturated so baseline resolution is lost in the Ag^+-HPLC profiles, but fractionation of these oils by Ag^+-HPLC reveals a progressive introduction of tri-, tetra-, penta- and hexaenoic acids into the triacylglycerols. Very highly unsaturated triacylglycerols (greater than twelve double bonds) are present at low concentrations in these oils.

The oils from menhaden, South African anchovy and Indian oil sardine contain lesser amounts of monoenoic acids. These fish oils still contain trisaturated triacylglycerols but do not show significant quantities of triacylglycerols substituted by two or three monoenoic acids (McGill & Moffat, 1992). The PUFA-containing triacylglycerols show an increased range of molecular composition with the presence of some very highly unsaturated triacylglycerols composed of a mixture of tetra-, penta- and hexaenoic acids. Triacylglycerols containing 520 mg EPA and 270 mg DHA/g fatty acids have been isolated. Thus, triacylglycerols with at least sixteen double bonds are present in fish body oils (McGill & Moffat, 1992).

COD LIVER OIL

The livers of demersal species can comprise up to 750 g lipid/kg, triacylglycerols being the major constituent. Cod liver oil contains a range of fatty acids similar to that of the pelagic body oils, but specific concentrations are different and vary (Table 3). The concentration of 20:1 and 22:1 is generally intermediate between that for typical herring and mackerel oils and the likes of menhaden and anchovy oils. Furthermore, the concentration of 22:6 is often greater than for 20:5 and can be as high as 230 mg/g fatty acids (C. F. Moffat, unpublished results).

ENCAPSULATED FISH OILS

Direct consumption of fish and other oils is restricted because many people find 'oiliness' unacceptable and have difficulty in swallowing the oil. The introduction of oils encapsulated in a soft gelatin shell has overcome this problem, leading to UK sales in 1991 worth £133 million, fish oils capturing 55·5% of this market (Market Research GB, 1992). A variety of products are readily available from supermarkets, pharmacies and healthfood shops. Cod liver oil is a major product. Not surprisingly, the fatty acid composition of encapsulated cod liver oils (Table 4) is not constant. The triacylglycerol composition of encapsulated cod liver oils shows the same broad spectrum of molecular structures as the pelagic body oils (Fig. 4). Trisaturated triacylglycerols and those with

Table 4. *Fatty acid composition (mg/g nominated fatty acids*) for various encapsulated fish oil products*

Fatty acid	Cod liver oil	Salmon oil	Fish oil concentrates
14:0	40–50	67	2–117
15:0	3–5	4	Tr
16:0	112–122	156	22–132
16:1	74–91	82	5–113
16:2	2–8	8	Tr–12
16:3	2–3	7	Tr–9
16:4	4–9	19	Tr–37
18:0	20–27	24	24–26
18:1	238–259	140	118–119
18:2 (*n*-6)	23–42	15	9–11
18:3 (*n*-6)	Tr–2	3	Tr–1
18:3 (*n*-3)	12–20	8	3–8
18:4	24–28	28	16–23
20:0	Tr–2	2	3–7
20:1	71–110	18	8–60
20:4	14–15	27	13–42
20:5 (*n*-3)	100–104	194	289–323
22:1	47–66	26	6–41
21:5	4–6	7	8–12
22:5 (*n*-3)	14–26	28	26–45
22:6 (*n*-3)	96–114	140	54–247
Saturates†	179–204	253	63–280
Monoenoic	447–492	266	224–246
Dienoic	27–50	23	11–21
Trienoic	16–23	18	9–12
Tetraenoic	43–62	74	65–66
Pentaenoic	123–132	229	323–380
Hexaenoic	96–114	140	54–247

Tr, trace (<1 mg/g fatty acids).

* 18:1, 20:1, 20:4 and 22:1 – collective sum for isomers where appropriate.

† Sums of saturated, monoenoic, dienoic, trienoic, tetraenoic, pentaenoic and hexaenoic acids to assess the overall double-bond distribution.

The range of values, where shown, are indicative but not definitive of the variation in sample composition.

greater than twelve double bonds per molecule are present. The trimonounsaturated triacylglycerols (15 min, Fig. 4) are a major component.

Increasingly, fish and plant oil mixtures are being sold as healthfoods. The major effect is that the plant oil enhances the concentration of linoleic acid by, for example, a factor of five, compared with cod liver oil. A series of sharp peaks eluting after the trimonounsaturated triacylglycerol peak, but before the typical unresolved mixture associated with the introduction of fatty acids with more than three double bonds in the Ag^+-HPLC profile, is indicative of the plant oil components (Fig. 4C).

Salmon oils can contain EPA and DHA at concentrations similar to those of anchovy and menhaden oils, with associated low levels of 20:1 and 22:1 (Table 4). As with all fish oils, the composition will be variable; in this case the encapsulated oil contained

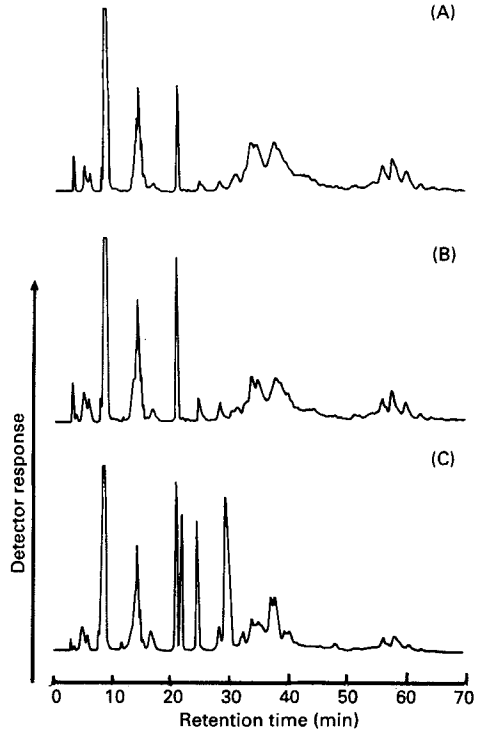

Fig. 4. Silver ion HPLC of cod liver oils (A and B) and a mixture of cod liver and evening primrose oils (3:1, w/w; C). The cod liver oils showed a complex range of triacylglycerols including trisaturated and highly unsaturated molecules. The evening primrose oil gave rise to a series of triacylglycerols which present as a set of sharp peaks eluting between 20 and 32 min.

significant quantities of very highly unsaturated triacylglycerols (Fig. 5). Spiking the oil with appropriate triacylglycerol standards gives a clear indication of the relative unsaturation of triacylglycerols in specific areas of the Ag^+-profile. The presence of a peak with an equivalent retention time to cholesterol (Fig. 5) illustrates the presence of compounds other than triacylglycerols in these oils although the estimated concentration is less than 1%.

By careful winterization and blending or by solvent crystallization and/or molecular distillation, it is possible to produce encapsulated products with a combined EPA and DHA concentration of 300 mg/g. Many such products are available which contain 180 mg EPA/g and 120 mg DHA/g (Ackman, 1988). A fish oil concentrate is available with an EPA concentration in excess of this figure (289 mg EPA/g fatty acids), but it contains a significantly lower concentration of DHA (54 mg DHA/g fatty acids). This oil still contains trisaturated triacylglycerols together with the predominantly monoenoic acid containing molecular species, but the concentrations of the very highly unsaturated triacylglycerols are significantly greater than those of crude body oils (McGill & Moffat, 1992).

Encapsulated oils containing concentrations of EPA and DHA totalling above 300 mg/g are available (Table 4). Many of these products are either methyl or ethyl esters. Such products are highly processed and are no longer in the natural form. Ackman

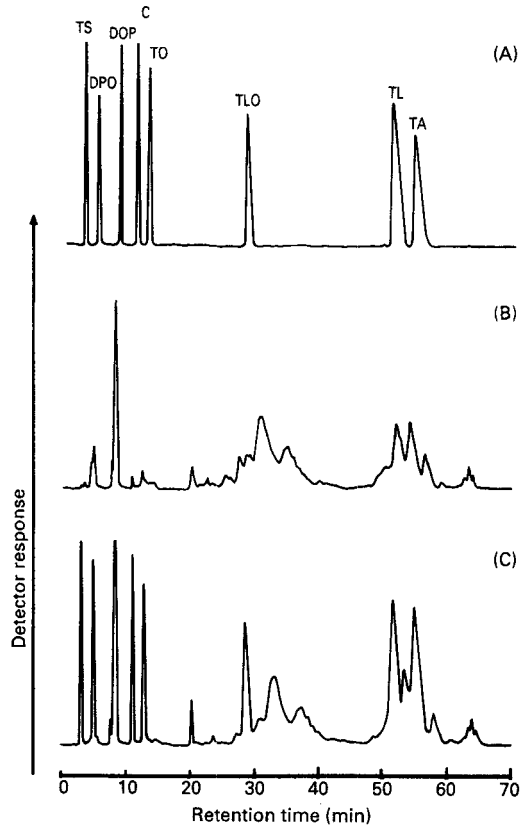

Fig. 5. Silver ion HPLC of standard triacylglycerols and cholesterol (A), salmon oil (B) and salmon oil spiked with standards (C). TS, tristearin (31·2 μg); DPO, 1,2-dipalmitoyl-3-oleoyl-*rac*-glycerol (24·3 μg); DOP, 1,2-dioleoyl-3-palmitoyl-*rac*-glycerol (28·4 μg); C, cholesterol (31·5 μg), TO, triolein (35·6 μg); TLO, trilinolein (42·2 μg); TL, trilinolenin (75·7 μg); TA, triarchidonin (61·4 μg).

(1992) has recently reviewed the absorp- tion of fish oils and concentrates, including esterified and free acids, and highlights the problem of data comparison, when different forms of material have been administered.

Some encapsulated fish oils are sold as vitamin supplements. Halibut liver oil, for example, is sold chiefly as a source of vitamin A, and as such may be diluted with soya-bean oil and other types of fish oil to achieve the designated concentration of the vitamin. Consequently, these oils can contain concentrations of EPA and DHA totalling between 40 and 90 mg/g fatty acids and concentrations of linoleic acid between 340 and 400 mg/g fatty acids (C. F. Moffat, unpublished results). Components of both liver oil and diluent plant oil are apparent in the triacylglycerol distribution (Fig. 6).

NON-TRIACYLGLYCEROL COMPONENTS IN FISH OILS

The beneficial influences of fish oils on health have been universally accepted but, as mentioned earlier, detailed knowledge or even a perception of what a fish oil actually contains is often very limited. The enormous variation in triacylglycerol structure and fatty acid composition has been illustrated, furthermore not all fish oils are tri-

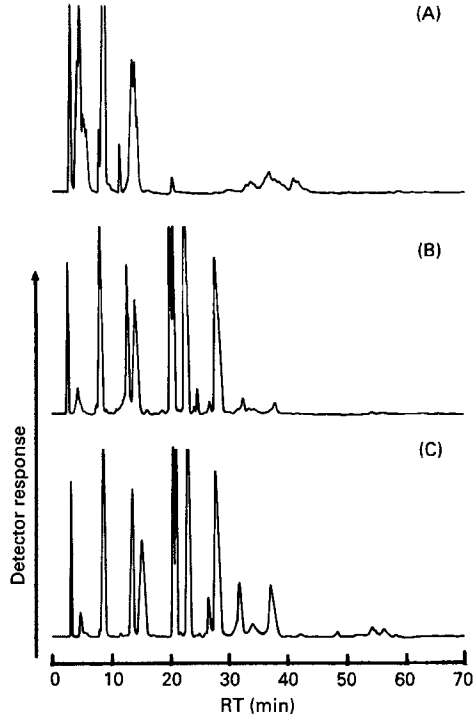

Fig. 6. Silver ion HPLC of halibut liver oil extracted from a single liver excised from a halibut (*Hippoglossus hippoglossus*) caught off Rockall in September 1992 (A) and commercial samples of encapsulated halibut liver oil (B and C). The components from the diluent used to achieve the designated concentration of vitamin A are evident from the sharp peaks in the profile with retention times (RT) of between 20 and 30 min.

acylglycerol based. Oil from orange roughy (*Hoplostethus atlanticus*), a deep-water species which is an important component of New Zealand's commercial fisheries and is also found in the North Atlantic, contains less than 100 mg triacylglycerol/g. The major lipid is wax ester composed of saturated and monounsaturated fatty acids and fatty alcohols (C. F. Moffat & A. S. McGill, unpublished results). Sterols are also present in most marine species and present in fish oils within the range 4·5–8·0 mg/g oil (Kinsella, 1987). During processing a proportion of the cholesterol undergoes dehydration to produce cholesta-3,5-diene. Other sterenes are also present in encapsulated fish oils together with *n*-alkanes including pentadecane and heptadecane which can give a combined concentration around 100 μg/g. Analysis of an encapsulated fish oil gave a substantial unresolved complex mixture on the GLC profile, composed of cyclic and branched hydrocarbons. Polyaromatic hydrocarbons and pesticides have also been found in commercial fish oil products although at very low levels (ng/g). Processing of fish oils for edible use does reduce the levels of these contaminants to make oils acceptable for human consumption, but close quality control and monitoring is required to ensure consumer safety. Pb, Hg and Cd are present in the marine environment but again processing of fish oils greatly reduces the concentrations of heavy metals to a level which meets the Food and Agricultural Organization/World Health Organization Codex standards (Elson *et al.* 1981).

Not all minor components are necessarily contaminants. Fish oils are good sources of

vitamins A, D and E. There is substantial inter- and intraspecies variation in vitamin A content which, for liver oils, can vary between 10 and 50 000 μg retinol equivalent/g oil (Kinsella, 1987). The intra- and interspecies variation of vitamin D content is less than observed for vitamin A. Most fish oils contain only moderate amounts of vitamin D (<125 μg/g oil), although halibut liver oil and oil from several tuna species contain higher concentrations with up to 6250 μg/g liver oil.

Fish cannot synthesize vitamin E. As a consequence, the concentration of this vitamin, mainly α-tocopherol, is related to diet (Ackman & Cormier, 1967; Watanabe *et al.* 1981). The concentration in liver oil is higher than that in body oil where concentrations range from less than 10 μg/g oil to 750 μg/g oil (Kinsella, 1987). Vitamin E is an important antioxidant and, thus, helps to inhibit lipid oxidation and the associated production of hydroperoxides, aldehydes, short-chain alkanes, ketones, lactones and polymeric material (Frankel, 1980, 1982, 1984; Miyashita *et al.* 1991; Shukla & Perkins, 1991). Lipid peroxides and their aldehydic breakdown products, which are responsible for the off flavours associated with rancid fat (Terao & Matsushita, 1986), are important cytotoxic components associated with oxidative stress and damage (Halliwell, 1993; Parke, 1993). Lipid peroxidation proceeds by a free-radical chain reaction and lipid radicals can cause cell membrane damage and could promote oxidative DNA damage, which may contribute to the aetiology of inflammatory autoimmune diseases such as rheumatoid arthritis as well as cancer. The contrasting physiological influences of PUFA pose interesting questions which have to be fully investigated so that the outcome of clinical trials are not compromised. In conclusion, therefore, although there is now considerable evidence from biological studies to support the view that fish oils can play a major role in promoting human health (British Nutrition Foundation, 1992) the reasons are not understood. Before the factors which influence the activity associated with fish oils can be determined, it is imperative that researchers are made more aware of the chemical composition of the oils they utilize for their studies.

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