Pertanika J. Trop. Agric. Sci. 16(3): 215-221(1993)

ISSN:0126-6128

Profile of Fatty Acid Contents in Malaysian Freshwater Fish

K. ENDINKEAU and TAN KIM KIEW
Department of Chemistry
Faculty of Science and Environmental Studies
Universiti Pertanian Malaysia
43400 UPM Serdang, Selangor Darul Ehsan
Malaysia.

ABSTRAK

Sembilan spesies ikan air tawar Malaysia telah dianalisis kandungan lipid dan asid lemaknya. Empat spesies ikan yang biasa dimakan oleh penduduk tempatan didapati mengandungi lemak sangat tinggi (julatnya 11-17% berat basah). Dalam semua ikan air tawar yang dikaji kandungan asid lemak tak tepunya melebihi asid lemak tepu. Nisbah asid lemak tak tepu/tepu adalah di antara 1.2 hingga 2.3. Asid kumpulan omega-3, pada amnya rendah dalam semua spesies dikaji, kecuali belut sawah yang mengandungi asid C22:6w3 sangat tinggi (9.4 g/100g minyak). Jumlah ini setanding dengan nilai-nilai yang terdapat pada ikan salmon, cod dan herring. Oleh itu penternakan belut sawah dan pengekstrakan minyaknya berpotensi dieksploit secara komersial. Pecahan fosfolipid bagi semua spesies yang dianalisis, kecuali jelawat mempunyai nisbah politak tepu/tepu lebih daripada 1.0.

ABSTRACT

Nine species of Malaysian freshwater fish were analysed for their lipid and fatty acid contents. The results show that 4 species of fish commonly consumed by local people contained significantly high levels of fat (range 11-17% of wet weight). Malaysian freshwater fish analysed also contained high levels of unsaturated acids compared to saturated acids. The ratio of unsaturates/saturates ranged from 1.2 to 2.3. The omega-3 acids were generally low in most species analysed except for the belut sawah which contained significantly high levels in C22:6w3 (9.4/100g oil). This quantity is comparable to that of salmon, cod and herring and thus warrants consideration for commercial exploitation. Phospholipid fractions of all fish analysed (except jelawat) had polyunsaturates/saturates ratios greater than 1.0.

Keywords: freshwater fish, fatty acid, phospholipid, fish lipid

INTRODUCTION

Fish is a major source of protein in the Malaysian diet. The freshwater fish industry in this country is emerging rapidly due to technology advancement and government support. However, for some reason, marine fish is preferred to freshwater fish among Malaysians. Demand for freshwater fish has remained low in the past few years. Lack of information on the nutritional value of freshwater fish could be one of the main reasons for the above situation.

The importance of fish in maintaining health was realised after studies were conducted on Greenland Eskimos and several Japanese populations (Dyerberg *et al.* 1975; Dyerberg and Bang 1979; Dyerberg 1982; Kagawa *et al.* 1982). The studies showed very little incidence of coronary heart disease among the subjects studied. These observations have been correlated with high intake of fish and marine organisms in their diet. The marine products consumed by these people are rich in polyunsaturated fatty acids (PUFA), particularly in eicosapentaenoic (EPA, C22:5ω3) and docosahexaenoic (DHA, C22:6ω3) acids. The effects of these acids on chronic diseases are well documented (Dyerberg *et al.* 1978; Jones and Davies, 1982; Kenneth 1986; Simopoulos *et al.* 1986; Kinsella 1988). Recent research has shown that polyunsaturated fish oils could lower serum triglyceride and cholesterol levels, and also help to prevent blood clotting (Dyerberg 1986; Herold and Kinsella 1986).

Most studies in the past have been carried out on northern hemisphere cold water fish (Ackman 1982) such as mackerel, herring, sardine and cod. These species are known to be excellent sources of PUFA, especially the omega-3 fatty acids. Recently, fish obtained from tropical waters were also found to be rich in polyunsaturated fatty acids (O'Dea and Sinclair 1982).

A similar result has been reported by Gibson (1983) for the fish caught in temperate waters of southern Australia. Analysis of Malaysian freshwater fish, however, has never been published before, although there is a report on marine fish (Gibson *et al.* 1984). The present study was conducted to quantify PUFA of some commonly reared freshwater fish such as tilapia, lampam jawa, siakap, etc. in Malaysia to determine the nutritional value of these fishes.

MATERIALS AND METHODS

All fish were bought fresh from the local market at separate times during the course of this study. Fish species were identified by an officer from the State Department of Fisheries. Prior to analysis, fish fillets were obtained by carefully cutting the fish lengthwise along the backbone to obtain maximum flesh without traces of backbone. Two to three fish were used each time, and fillets were mixed and cut into small portions before analysis.

Samples of fish fillets (50 g) were homogenized in a blender for 2 min with a mixture of chloroform-methanol (150 ml, 1:2 v/v), according to the method of Bligh and Dyer (1959). Butylated hydroxytoluene (BHT) at a concentration of 0.2% (of the fillet) was added at the beginning of extraction to prevent oxidation. The extract was filtered and evaporated to dryness *in vacuo,* on a rotary evaporator at 40°C. The resulting lipid fraction was weighed and stored at -18°C for further analysis.

Lipid samples were converted to constituent fatty acid methyl esters (FAME) by refluxing the lipid (50 mg) in 5 ml of reagent consisting of concentrated sulphuric acid-toluene-methanol (1:10:20 v/v) for one hour at 90°C, according to the method of Hammond (1987). After cooling, water (3 ml), hexane (3 ml) and internal standards (6µl) (C15 and C19 of Sigma Chem. Co.) were added. The hexane layer was recovered, dried over anhydrous Na_2SO_4 and the FAME were ready for injection.

Routine analyses of FAME were performed by gas chromatography. The esters were analysed using a gas chromatograph (Shimadzu GC-9A provided with an FID and coupled with a Shimadzu C-R3A computerised integrator). A fused silica capillary column (30 m x 0.53 mm id) of Supelcowax-10 with 0.50 µm film thickness (Supelco, Inc.) was used. The oven temperature was programmed from 100°C to 240°C at a ramp rate of 5°C/min, after an initial isothermal period of 2 min and was held for 10 min after final temperature. The detector and injector port temperatures were 280°C and 250°C respectively. The carrier gas was helium, set at a flow rate of 50 ml/min. Identification of FAME was based on comparison of retention times between unknown peaks and those of authentic standards (Sigma Chem. Co). Individual esters were quantified by the internal standard method. Duplicate injections were carried out on each FAME sample.

Phospholipid fraction of extracted fish lipid was separated by column chromatography on silica gel (70-230 mesh). The lipid (3 g) was dissolved in hexane and added to the column, using chloroform, acetone and methanol as eluents respectively (Carroll 1976). The phospholipid obtained was concentrated with a rotary evaporator, weighed and esterified as before.

All organic solvents used in this study were reagent grade and used without further purification.

RESULTS AND DISCUSSION

Nine species of commercially reared Malaysian freshwater fish were analysed. The fish are usually reared in captivity and fed with commercial feed. The species chosen are either commonly eaten by Malaysians or are very popular among seafood lovers. All fish samples used were mature and at normal harvesting size.

The lipid contents of all selected Malaysian freshwater fish are shown in Table 1 and ranged from 1.8 to 17.8 g/100g wet weight. Four species known locally as keli *(Clarius* sp.*)*, lampam jawa *(Puntius gonionotus)*, lee koh *(Cyprinus carpio innaeus)* and tilapia *(Oreochromis* sp.*)* contained relatively high levels of lipid, 13.0%, 14.8%, 17.8% and 11.0% of wet fillet respectively. In contrast, siakap *(Lates calcarifer)* had a much lower fat content (2.0%). Haruan *(Channa striatus)* which is believed to have healing properties (Mohsin and Ambak 1983) and in high demand was also low in fat (2.0%). The most expensive local fish which is noted for its delicate flavour, jelawat *(Leptobarbus hoevenii)*, was found to contain 7.9% fat. The lipid content obtained from this study is generally high compared to those reported for Malaysian marine fish (Gibson *et al.* 1984) and Australian tropical water fish (O'Dea and Sinclair 1982). The previous highest values reported for Malaysian and Australian fish were 3.9 and 7.8 g/100 g wet weight respectively. The high fat levels observed in this study suggest that the freshwater fish are fed the right diet whereas marine fish rely mainly on limited food available from the surroundings. The observed variables in

TABLE 1
Local, common and scientific names and lipid content of fish (g/100g fillet)

Local Name	Common Name	Scientific Name	Lipid Content*
Belut sawah	Rice-field eel	*Monopterus alba* (Zuiew)	1.77
Jelawat	Sultan fish	*Leptobarbus hoevenii* (Bleeker)	7.90
Kap rumput	Grass carp	*Ctenopharyngodan idellus* (C&V)	7.52
Keli	Walking fish	*Clarius* spp.	12.96
Lampam jawa	Javanese carp	*Pluntius gonionotus* (Bleeker)	14.82
Lee koh	Common carp	*Cyprinus carpio* Linnaeus	17.76
Siakap	Sea bass, Sea perch	*Lates calcarifer* (Bloch)	1.97
Tilapia	African bream	*Oreochromis* spp.	11.01
Haruan	Snake head	*Channa striatus* (Bloch)	2.10

* mean of two determinations

lipid content among the species studied (ranging from 1.8 - 17.8%) could be attributed to differences in type of diets, age of fish, the habitat, the activity pattern and the species (Kinsella 1987).

Normal separation of fatty acid methyl esters (FAME) obtained from fish lipid samples is illustrated in *Fig. 1*. The chromatograms show some major peaks derived from FAME of kap rumput and its phospholipid fraction. The peaks are well separated on Supelcowax-10 and thus give a better area integration. The percentage of the major fatty acids composition in fish lipids is given in Table 2. Palmitic acid (C16:0) was the major component of the saturated fatty acids followed by stearic acid (C18:0) in all species analysed. Keli (*Clarius* sp.) displayed the highest palmitic acid content (15.2 g/100 g fish oil). The other saturated fatty acids, including C12:0 and C14:0, were only minor components. In most species examined, oleic acid (the only monounsaturated acid quantified) was found to be a major constituent of freshwater fish (more than 17%). In contrast, haruan, siakap and belut sawah only contained 4.8, 7.3 and 4.8% oleic acid respectively.

Linoleic acid (C18:2ω6), was the predominant fatty acid in the polyunsaturated components or ω6 group in most of the species studied. Its concentration ranged from 0.6 to 11.5 g/100 g fish oil. Lampam jawa was the highest in C18:2 (11.5 g) followed by tilapia (11.4 g) and lee koh (10.0 g), whilst belut sawah was the lowest (0.6 g). Another ω6 acid, arachidonic acid (C20:4ω6) was also present at significant levels in the fishes studied. Siakap and haruan were among the highest in arachidonic acid content, which were 2.0 g (ca:6.1% of total fatty acids) and 2.3 g/100 g oil (ca:11.0%) respectively. The value obtained from haruan was comparable to that of Gibson *et al.* (1984) report on Malaysian shrimp (11.8%) (the highest arachidonic content in Malaysian marine fish analysed). The values obtained both from this study and by Gibson were higher than those from cold-water fish of the northern hemisphere (Ackman 1982). In this aspect, haruan may be considered as a potential source of arachidonic acid. This acid plays a major role in fat metabolism in the human body. It is the most important precursor of eicosanoids which have a physiological effect on the vascular system (Kinsella 1986).

An interesting result was observed in belut sawah. Although the fat content was low (1.8%), belut sawah contained a high concentration of docosahexaenoic acid (DHA) (C22:6ω3). The value obtained in this study (9.4 g/100 g oil)or ca. 0.17 g/100 g fillet was comparable to that of sockeye salmon (0.71 g/100 g tissue), Pacific herring (0.75 g/100 g) and cod (0.19 g/100 g) (Kinsella 1987). These species have won wide recognition as good sources of ω3 acids. A further analysis of belut of different sizes was carried out. The results show that the level of DHA increased according to size of fish. A similar result was also obtained for fat contents (Table 3). This result may lead to the exploitation of ω3 acid in belut sawah commercially. *Fig. 2* shows a significant peak of DHA derived from FAME of

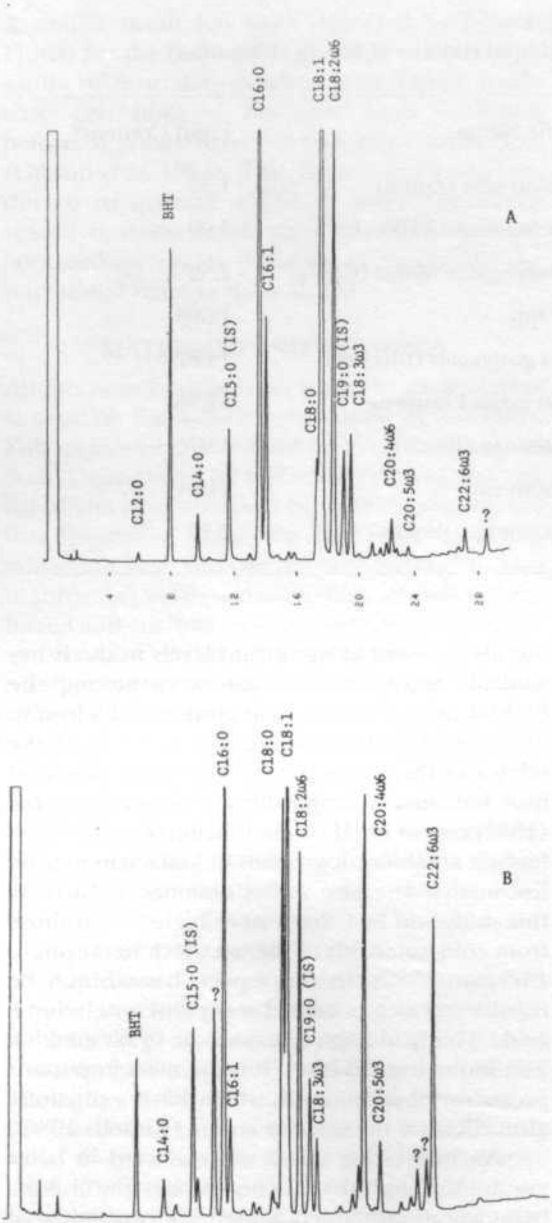

Fig. 1: Separation of fatty acid methyl esters derived from (A): total lipid, and (B): phospholipid fraction of kap rumput on a Supelcowax-10 capillary column (30 m x 0.53 mm id, Atten. = 1). Note the peak heights of C20:4ω6 and C22:6ω3 in phospholipid fraction.

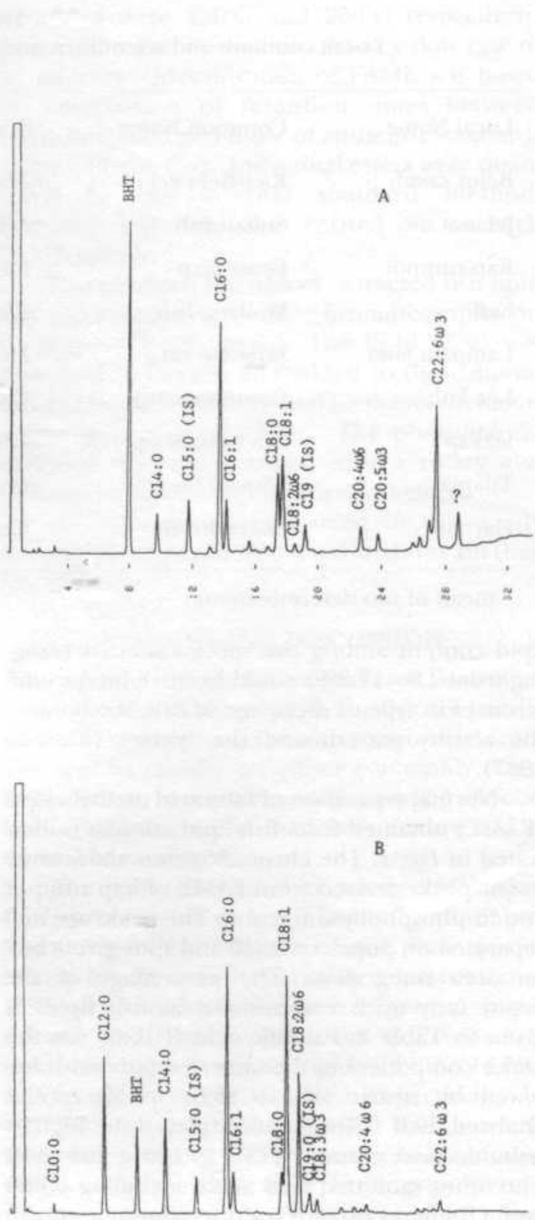

Fig. 2: Separation of fatty acid methyl esters obtained from lipids of (A): belut sawah, amd (B): tilapia on a Supelcowax-10 (Atten. =3). Note the peak heights of C22:6ω3 in (A) and (B), and the appearance of more saturated acid peaks at the lower molecular weight region (B).

belut (A) compared to tilapia (B). Additionally, siakap (6.1 g) and jelawat (3.0 g) were also found to contain considerable amounts of DHA. In all cases, the content of eicosapentaenoic acid (EPA) (C20:5ω3) was much lower compared to DHA.

Generally, the fatty acids of the fish examined in this study showed a higher unsaturated, rather than saturated acid content. The ratio of unsaturates/saturates ranged from 1.2 to 2.3 (Table 2). Three species, namely kap rumput (2.3), lampam jawa (2.1) and lee koh (2.3) had a

TABLE 2
The average fatty acid composition of total lipids (g/100 g fish oil)

Fatty acid	Belut sawah	Jelawat	Kap rumput	Keli	Lampam jawa	Lee koh	Siakap	Tilapia*	Haruan
Saturates									
12:0	nd	0.08	0.10	nd	0.06	0.17	0.09	4.61	nd
14:0	0.98	1.20	0.65	0.42	0.52	0.68	1.23	3.89	0.34
16:0	6.76	11.82	11.47	15.19	12.39	13.74	9.14	11.70	6.55
18:0	3.35	4.69	1.39	2.41	2.54	1.96	4.17	2.40	2.24
20:0	nd	nd	nd	nd	nd	nd	0.08	0.09	nd
Monounsaturates									
18:1	4.75	17.19	18.56	18.15	19.98	26.94	7.20	15.06	4.73
Polyunsaturates									
18:2 ω6	0.55	6.76	9.22	9.65	11.54	9.96	1.00	11.44	2.05
18:3 ω3	0.14	1.30	2.25	0.24	0.65	0.31	nd	0.93	0.42
18:4 ω3	0.12	0.22	0.08	0.07	0.10	0.16	0.08	0.10	0.04
18:4 ω6	1.23	1.38	0.58	0.36	0.44	0.68	2.00	0.46	2.28
18:5 ω3	0.84	0.85	0.19	0.06	nd	0.20	1.56	0.11	0.09
18:6 ω3	9.37	3.03	0.55	0.49	0.31	0.34	6.13	0.77	2.11
Σ saturates	11.09	17.79	13.61	18.02	15.51	16.55	14.71	22.99	9.13
Σ unsaturates	17.00	30.73	31.34	29.02	33.02	38.59	17.97	28.87	11.72
ω3 acids (C20: 5 + C22:6)	10.21	3.88	0.74	0.55	0.31	0.54	7.69	0.88	2.20
ratio $\frac{\Sigma \text{ unsaturates}}{\Sigma \text{ saturates}}$	1.53	1.73	2.31	1.61	2.13	2.33	1.22	1.26	1.28

*Tilapia consists C10:0 = 0.30 g/100g fish oil nd = not detectable

TABLE 3
Comparative fatty acid (g/100 g fish oil) composition of belut in different sizes

Fatty Acid	Sizes of Belut 420 g	280 g	100 g
Saturates			
14:0	0.98	0.16	nd
16:0	6.76	3.71	1.67
18:0	3.35	1.94	1.34
20:0	nd	nd	nd
Monounsaturates			
18:1	4.75	2.04	0.89
Polyunsaturates			
18:2 ω6	0.55	0.37	0.43
18:3 ω3	0.14	nd	nd
18:4 ω3	0.12	nd	nd
20:4 ω6	1.23	3.06	1.92
20:5 ω3	0.84	0.39	0.21
20:6 ω3	9.37	4.10	1.92
Total lipid* (g/100g fillet)	1.77	1.14	0.90

*mean of two determinations

ratio of above 2; thus the fats of these fish can be classified as unsaturated. *Fig. 1(A)* shows a typical FAME chromatogram of kap rumput where more peaks appeared in the region of higher molecular weight (unsaturates). On the other hand, the FAME of tilapia *(Fig.2B)* contained more peaks on the lower molecular weight region (saturates).

The composition of fatty acids in the phospholipid fraction of five species analysed showed that this fraction was high in PUFA (Table 4). In some cases (siakap, tilapia and kap rumput) the total percentage of arachidonic acid and DHA was almost equal to those of saturated components, i.e. palmitic and stearic acids. Again, phospholipid of belut sawah was the highest in DHA (40.1%), followed by siakap (30.7% of total fatty acid). *Fig. 1(B)* shows a FAME chromatogram of phospholipid of kap rumput. Peaks correspond to major PUFA, especially the DHA and EPA which are relatively higher compared to those of saturated acids. When compared to total lipid fractions the phospholipid fractions contain higher percentages of PUFA (not including monounsaturated acid, C18:1), the percentages being in the range of 48.9 - 59.4%, while the corresponding levels in total lipid range from 36.7 - 45.0% of total fatty acids (Table 5). All ratios of polyunsaturated/saturated were above 1.3 except jelawat (0.7). In contrast, the total lipid fractions have a ratio less than 1 (Table 5). This

TABLE 4
Fatty acid composition of phospholipids in percentage of total fatty acids attained*

Fatty acid	Belut sawah	Jelawat	Kap rumput	Siakap	Tilapia#
Saturates					
12:0	0.12(0.001)	0.15(0.001)	nd(nd)	nd(nd)	0.98(0.005)
14:0	0.86(0.007)	3.13(0.021)	0.81(0.005)	1.16(0.005)	1.96(0.010)
16:0	11.59(0.094)	28.32(0.190)	15.19(0.094)	20.00(0.086)	15.32(0.078)
18:0	27.99(0.227)	9.54(0.064)	13.49(0.058)	13.49(0.058)	20.43(0.104)
20:0	nd(nd)	0.15(0.001)	nd(nd)	nd(nd)	0.39(0.002)
Monounsaturates					
18:1	nd(nd)	29.06(0.195)	19.39(0.195)	16.74(0.072)	11.79(0.060)
Polyunsaturates					
18:2 ω6	1.23(0.010)	14.61(0.098)	16.32(0.101)	3.49(0.015)	12.97(0.066)
18:3 ω3	nd(nd)	3.58(0.024)	2.75(0.017)	nd(nd)	0.59(0.003)
18:4 ω3	nd(nd)	0.60(0.004)	nd(nd)	0.23(0.001)	nd(nd)
20:4 ω6	15.17(0.123)	2.83(0.004)	15.51(0.096)	9.07(0.039)	20.04(0.102)
20:5 ω3	2.96(0.024)	2.68(0.018)	2.75(0.017)	5.12(0.022)	0.59(0.003)
22:6 ω3	40.07(0.325)	5.37(0.035)	13.73(0.085)	30.70(0.132)	14.73(0.075)

*Values in brackets are g/100 g fish oil
# Phospholipid of tilapia consists C10:0 = 0.20% (0.001g/100 g fish oil)
nd = not detectable

TABLE 5
Comparative analysis of fatty acid composition in total lipid and phospholipid
(results are expressed as percentage of total fatty acids attained)

	Total Lipid			Phospholipid		
Fish	Polyunsaturates*	Saturates	ratio*	Polyunsaturates*	Saturates	ratio
Belut sawah	43.62	39.49	1.10	59.43	40.56	1.47
Jelawat	27.89	36.66	0.76	29.67	41.29	0.72
Kap rumput	28.58	30.22	0.95	51.06	29.57	1.73
Siakap	32.95	45.01	0.73	48.61	34.65	1.40
Tilapia	26.62	44.33	0.60	48.92	39.08	1.25

*Polyunsaturates stated above did not include C18:1

$$\# \text{ Ratio} = \frac{\Sigma\% \text{ Polyunsaturates}}{\Sigma\% \text{ Saturates}}$$

observation is in accordance with the proposed high PUFA content in the phospholipid fraction of fish tissues (Henderson and Toche 1987) and marine animals (Goodnight *et al.* 1982).

CONCLUSION

This study suggests that the high fat content found in Malaysian freshwater fish makes it a good dietary item because of the significant amount of polyunsaturated fatty acids. Another interesting finding of this study was the high amount of DHA found in belut sawah. The results of this study should encourage the Malaysian public to eat more freshwater fish, particularly belut sawah, heeding the advice that "comsumption of as little as one or two fish dishes per week may be of preventive value in relation to coronary heart disease" (Kromhout *et al.* 1985).

REFERENCES

ACKMAN, R.G. 1982. Fatty acid composition of fish oils. In *Nutritional Evaluation of Long Chain Fatty Acids in Fish Oil.* ed. S.M. Barlow and M.E. Stansby, p. 25-88. London: Academic Press:

BLIGH, E.G. and W.J. DYER. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911-917.

CARROLL, K.K. 1976. Column chromatography of neutral glycerides and fatty acids. In *Lipid Chromatographic Analysis 1,* (2nd ed.) ed. G.V. Marinetti, p. 173-214. New York: Dekker.

DYERBERG, J. 1982. Nutritional evaluation of long-chain fatty acids in fish oils. In *Nutritional Evaluation of Long Chain Fatty Acids in Fish Oil.* ed. S.M. Barlow and M.E. Stansby, p. 245-261. London: Academic Press.

DYERBERG, J. 1986. Linoleinate derived polyunsaturated fatty acids and prevention of atherosclerosis. *Nutr. Rev.* **4:** 125-134.

DYERBERG, J. and H.Q. BANG. 1979. Hemostatic function and platelet polyunsaturated fatty acid in Eskimos. *Lancet* **ii:** 433-435.

DYERBERG, J., H.Q. BANG and N. HJORNE. 1975. Fatty acid composition of the plasma lipids in Greenland Eskimos. *Am. J. Clin. Nutr.* **28:** 958-966.

DYERBERG, J., H.Q. BANG, E. STOFFERSEN, S. MONCADA and J.R. VANE. 1978. Eicosapentaenoic acid and prevention of atherosclerosis? *Lancet* **i:** 117-119.

GIBSON, R.A. 1983. Australian fish - An excellent source of both arachidonic acid and ω3 polyunsaturated fatty acids. *Lipids* **18:** 743-752.

GIBSON, R.A., R. KNEEBONE and G.M. KNEEBONE. 1984. Comparative levels of arachionic acid and eicosapentaenoic acid in Malaysian fish. *Comp. Biochem. Physiol.* **78C:** 325-328.

GOODNIGHT, S.H., W.S. HARRIS, W.E. CONNOR and D.R. ILLINGWORTH. 1982. Polyunsaturated fatty acids, hyperlipidemia and thrombosis. *Atherosclerosis* **2:** 87-113.

HAMMOND, E.W. 1987. Packed-column gas chromatoghraphy. In *Analysis of Oils and Fats,* ed. R.J. Hamilton and J.B. Rossell, p. 123. New York: Elsevier.

HENDERSON, R.J. and D.R. TOCHER. 1987. The lipid composition and biochemistry of freshwater fish. *Prog. Lipid Res.* **26:** 281-347.

HEROLD, P.M. and J.E. KINSELLA. 1986. Fish oil consumption and decreased risk of cardiovascular disease: a comparison of findings from animal and human feeding trials. *Am. J. Clin. Nutr.* **43:** 566-598.

JONES, D.B. and T.M.E. DAVIES. 1982. Eicosapentaenoic acid and ischemic heart disease. *Lancet* **i:** 221-226.

KAGAWA, Y., M. NISHIZAWA, M. SUSUKI, T. MIYATAKE, T. HAMAMOTO, K. GOTO, E. MOTONAGA, H. IZUMIKAWA, H. HIRATA and A. EBIHARA. 1982. Eicosapolyenoic acids of serum lipids of Japanese islanders with low incidence of cardiovascular disease. *J. Nutr. Sci. Vitaminol.* **28:** 441-453.

KENNETH, K.C. 1986. Biological effects of fish oils in relation to chronic diseases. *Lipids* **21:** 731-732.

KINSELLA, J.E. 1986. Food components with potential therapeutic benefits: The n-3 polyunsaturated fatty acids of fish oils. *Food Technol.* **40(2):** 89-97.

KINSELLA, J.E. 1987. *Seafoods and Fish Oils in Human Health and Disease.* New York: Marcel Dekker.

KINSELLA, J.E. 1988. Food lipids and fatty acid: Importance in food quality, nutrition, and health. *Food Technol.* **42(10):** 124-142.

KROMHOUT, D., E.B. BOSCHIETER and D.C. KOULANDER. 1985. The inverse relation between fish consumption and twenty year mortality from coronary heart disease. *New Engl. J. Med.* **312:** 1205-1209.

MOHSIN, A.K.M. and M.A. AMBAK. 1983. *Freshwater Fishes of Peninsular Malaysia.* Serdang: Penerbit Universiti Pertanian Malaysia.

O'DEA, K. and A.J. SINCLAIR. 1982. Increased proportion of arachidonic acid in plasma lipids after two weeks on a diet of tropical seafood. *Am. J. Clin. Nutr.* **36:** 868-872.

SIMOPOULOS, A.P., A.R. KIFER and R.E. MARTIN. 1986. *Health Effects of Polyunsaturated Fatty Acids in Seafoods.* New York: Academic Press.

(Received 27 February 1993)