

Fatty Acids Composition of Selected Malaysian Fishes (Komposisi Asid Lemak Ikan Terpilih Malaysia)

NUR AIRINA MUHAMAD & JAMALUDIN MOHAMAD*

ABSTRACT

A comparative study on the fatty acids contents of three Malaysian marine fishes Tenualosa toli (Terubok), Rastrelliger kanagurta (Kembong), Stolephorus baganensis (Bilis) and three freshwater fishes Channa striatus (Haruan), Pangasius hypothalamus (Patin), Clarias macrocephalus (Keli) were carried out. Marine fishes showed more unsaturated fatty acids with 3, 4, 5 and 6 double bonds than in freshwater fishes. Two n-3 polyunsaturated fatty acids (PUFAs); eicosapentaenoic acid (EPA) and docosahaexaenoic acid (DHA) were found more in marine fishes PUFAs than in freshwater fishes. Based on the fatty acids content of DHA, EPA and arachidonic acid (AA), Stolephorus baganensis provides the best source of it (4.68%, 10.5%, 4.68%) followed by Rastrelliger kanagurta (10.62%, 4.85%, 3.17%) and Tenualosa toli (9.93%, 2.50%, 0.16%). However, freshwater fishes showed small range of DHA (0.63% - 1.41%), EPA (0.11% - 0.25%) and AA (1.41% - 4.46%). Saturated fatty acids palmitic was the major fatty acid found in all fishes studied.

Keywords: Docosahaexaenoic acid; eicosapentaenoic acid; fresh water fish; marine fish; polyunsaturated fatty acid

ABSTRAK

Kajian perbandingan kandungan asid lemak tiga jenis ikan marin Malaysia Tenualosa toli (Terubok), Rastrelliger kanagurta (Kembong), Stolephorus baganensis (Bilis) dan tiga jenis ikan air tawar Channa striatus (Haruan), Pangasius hypothalamus (Patin), Clarius macrocephalus (Keli) telah dijalankan. Ikan marin menunjukkan lebih kandungan asid lemak tak tepu terutamanya dengan ikatan dubel 3, 4, 5, dan 6 berbanding ikan air tawar. Dua asid lemak tepu n-3 (PUFA) iaitu asid eikosapentaenik (EPA) dan asid docosahaexaenik (DHA) terdapat lebih dalam PUFA ikan marin daripada ikan air tawar. Berdasarkan kandungan asid lemak DHA, EPA dan asid arachidonik (AA) ikan marin Stolephorus baganensis adalah sumber terbaik (4.68%, 10.5%, 4.68%) diikuti dengan Rastrelliger kanagurta (10.62%, 4.85%, 3.17%) dan Tenualosa toli (9.93%, 2.50%, 0.16%). Walaupun demikian ikan air tawar menunjukkan julat yang kecil DHA (0.63% - 1.41%), EPA (0.11% - 0.25%) dan AA (1.41% - 4.46%). Asid lemak tepu palmitik didapati adalah asid lemak utama yang terdapat didalam semua ikan yang telah dikajikan.

Kata kunci: Asid docosahaexaenik; asid eikosapentaenik; asid lemak tepu; ikan air tawar; ikan marin

INTRODUCTION

Fish is consumed as food all over the world. It is generally recognized that polyunsaturated fatty acids (PUFA) composition might vary among species of fish. However, little attention has been paid to the PUFA composition of different species when selecting fish for diet (Hearn & Sgoutas 1987). When fish is suggested as a means of improving health, both the lipid content and the PUFA distribution must be considered.

The fishery department in Malaysia is encouraging the expansion of fresh water industry to alleviate income among the agriculturist and fishermen. Karim (1990) proposed a study to evaluate the fatty acid composition especially the omega-3 and omega 6 of selected species of freshwater and marine fishes in Malaysia. However, little work has been done on this subject in Malaysia. The nutritional importance of fish consumption is associated largely with the n-3 polyunsaturated fatty acid content.

These findings have created a new market for fish oil as food and dietary supplement. Fatty acid composition

data are essential for food scientist and nutritionist to help them in dietary formulation, processing and product development.

In this research, three species of marine fishes (*Tenualosa toli*, *Stolephorus baganensis* and *Kanagurta rastrelliger*) and three species of freshwater fishes (*Pangasius hypothalamus*, *Channa striatus* and *Clarias macrocephalus*) were studied.

Tenualosa toli comes from the Clupeidae family that includes herrings, shads, sardines and menhadens. It is classed as Actinopterygii which are the ray finned-fishes and can reach to a maximum length of 60 cm. *Tenualosa toli* or commonly known by the locals as terubok can be found in brackish environment in a depth range up to 10 meters (Nurasyikin et al. 2006). *Channa striata* comes from the family Channidae that has a snake headed features. The locals commonly call it as the snakehead or haruan. The length of this fish can reach up to a maximum length of 100 cm and has maximum weigh of 3000 g. It lives in a tropical climate in an environment of brackish freshwater with a

pH range of 7 to 8 and in a depth range of 1 to 10 m (Qin 2000). These fishes are easily found in Asian countries.

Clarias macrocephalus is from Clariidae family or the air breathing catfish. It is commonly called the broad head catfish and can be found in benthopelagic or potamodromous freshwater in a depth range of 1 meter. It lives in tropical environment and easily found in Asia.

Anchovies or its scientific name *Stolephorus baganensis* comes from the Engraulidae family. It is a marine fish mainly found in the brackish marine environment in the depth range of 1 to 50 m. It is easily found in tropical countries like the Indo-pacific Ocean and Western Pacific Ocean such as the gulf of Thailand, Singapore, Sumatra and Malaysia.

Pangasius hypothalamus or commonly called patin by the locals is from the pangasiidae family, which is known to have shark headed features. The common length of this fish is 120 cm long and usually found in tropical countries such as in Asia. It lives in potamodromous freshwater.

Indian mackerel or known by the locals as *kembung* are from the Scombridae family, which are same family for tunas, mackerel and bonitos. This fish can reach up to a maximum size of 35 cm and has been reported to live up to 4 years. This marine fish are always associated with the ocean reef and usually found in a depth range of 20-90 m in tropical climates. It is mainly distributed in Indo-West Pacific that is from the Red Sea and East Africa to Indonesia, north to the Ryukyu Islands and China, South to Australia and Malaysia. The objective of this study was to investigate the comparative composition fatty acid between Malaysian marine fishes and freshwater fishes and fatty acid profile. Therefore, the present paper reports the lipid contents and fatty acid compositions of these fishes.

MATERIALS AND METHODS

MARINE AND FRESHWATER FISH SAMPLE

This study was carried out on six selected Malaysian fishes. The fishes chosen for this study are not only selected from the marine species but also from the freshwater species. Three of the freshwater fishes selected were, *Channa striatus* (haruan), *Pangasius hypothalamus* (patin) and *Clarias macrocephalus* (keli), meanwhile, the marine fishes selected were *Tenualosa toli* (terubok), *Rastrelliger kanagurta* (kembong) and *Stolephorus baganensis* (bilis). The fish were randomly chosen according to their availability as a dietary source. Five specimens of similar body weight and length for all analyzed fish species were collected. Biological characteristics of whole fresh fish were determined. Species, body weight (g) and length (cm) were noted and fish were dissected immediately. Prior to analysis, about 25 g of fish muscle tissue was separated for the determination of fatty acid composition and as well as for other tests.

CHEMICALS

All solvents used (analytical and GCMS grades) were obtained from Fisher Scientific (Pittsburgh, PA, USA). Anhydrous sodium sulphate, iodine, sodium hydroxide were purchased from E. Merck Damstadt, F.R. Germany. Trichloroacetic acid (TCA), linoleic acid, FAME standards 1809-01 and other chemicals were purchased from Sigma Chemicals (USA). All other materials otherwise specified, were of reagent grade or the highest available grade from usual commercial sources.

INSTRUMENTATION

The extracts were concentrated using a rotary evaporator from Buchi Instruments, Germany. The ultraviolet (UV) spectra were obtained using Shimadzu model 160/A spectrophotometer. The gas chromatograph analysis was performed on a Shimadzu QP 5000 M/S and GC-17A equipped with a Shimadzu capillary column.

EXTRACTION OF TOTAL LIPIDS

Total lipids were extracted from muscle tissue according to Folch et al. (1957). The dorsal muscle tissue of each fish was taken after they have been partially thawed. A chloroform/methanol solvent mixture (2:1 v/v) was added to fish muscle tissue sample in the ratio solvent: tissue of 20:1. The samples were homogenized 3 times for 10 min with 3000 rpm - 4000 rpm.

Cooling of the sample for 1 hour at 4°C followed was done after each homogenization step. 4 mL of 0.034% MgCl₂ was added to the extracts for each 1 g of tissue. The chloroform/methanol extracts were incubated overnight at 4°C, allowing the separation of the organic (containing the extracts of total lipids) and aqueous layers completely. Upper (aqueous) layer was removed and lower (organic) layer was rinsed with mixture of chloroform: methanol (2:1), and placed in glass tubes. Evaporation of the lower phase furnished the total lipid fraction. The solvent was removed in rotary evaporator under vacuum at 40°C.

These extracts, representing the total lipids, were weighted and dissolved once again in a small volume (1-2 mL) of chloroform/methanol (2:1). The resulting extract of total lipids was stored at 4°C for further analysis. The total lipid was expressed as g/100 g tissue.

FATTY ACID ANALYSIS

Total fatty acid content and fatty acid composition were determined simultaneously in the fish oil samples. Fatty acid analysis was performed in triplicate consisted two consecutive steps, preparation of fatty acid methyl ester (FAME) and chromatographic analysis. In this work, preparation of fatty acid methyl ester (FAME) was prepared by the two following procedures.

DIRECT FATTY ACID METHYL ESTER (FAME) SYNTHESIS

Fatty acid methyl ester (FAME) was synthesized by one step extraction-transesterification method as described by Majid et al. (2003), after suitable modifications. Fish oil (± 20 mg) was weighed into clean, 10 mL screw-top glass bottles, to which 4 mL fresh solution of a mixture of methanol, concentrated sulphuric acid, and chloroform (1.7:0.3:2.0 v/v/v) was added. The bottles were closed tightly with Teflon cap to avoid leakage and the weighed.

Transesterification was done at different combination of temperature and reaction time. Bottles were placed inside a water bath at 90°C for 30 min. On completion of the reaction, the bottles were cooled down to room temperature and weighed again to dismiss leaking samples.

Then, 1 mL distilled water was added into the mixture and thoroughly vortexed for 1 min. After the formation of two phases, the lower phase containing fatty acid methyl ester (FAME) was transferred to a clean, 10 mL bottle and dried with anhydrous sodium sulphate (Na_2SO_4).

FATTY ACID METHYLESTER (FAME) ANALYSIS BY GAS CHROMATOGRAPHY MASS SPECTROMETER (GCMS)

Fatty acid compositions of lipid extracts were determined by gas chromatography of the corresponding methyl esters. Fatty acid methyl esters were obtained by transesterification lipid extracts as described.

The gas chromatograph analysis of methylated fatty acids was performed on a Shimadzu QP 5000 M/S and GC-17A equipped with a SHIMADZU capillary column. The column was made up of fused silica with non-polar bounded phase.

One microliter of sample was injected into the capillary column CBP-M25-025 with a diameter of 0.25 mm and a length of 25 m. The carrier gas used was helium, regulated for column head pressure of 43.0 kPa with 1 mL/min flow rate.

The gas chromatograph was developed by temperature programming with the initial temperature of 60°C. The temperature was increased with a rate of 15°C/min to 230°C. The injection and detector temperature were set 230°C.

The mass spectrum from the chromatogram was compared with NBS and NIST Mass Spectra Library. Besides that, the fatty acid methyl esters were identified also by comparing with their retention times with those of the commercial fatty acid methyl ester standards (GC 18-91).

STATISTICAL ANALYSIS

The result reported as mean standard. Student's t-test was employed for calculation of statistical value of less than 0.05 as a significant criterion.

RESULTS AND DISCUSSIONS

The lipid contents of these Malaysian fish species are listed in Figure 1. *Tenualosa toli* (ikan terubok) which is a species belonging to the category of "fatty fishes" produces the highest oil content which is 13.82 g/100 g. This was followed by *Pangasius hypothalamus* (patin), 6.23 g/100 g; *Rastreligger kanagurta* (kembung), 4.54 g/100 g; and *Clarias macrocephalus* (keli) 4.25 g/100 g while other species showed low to moderate oil content which ranged from 2.42 g/100 g – 3.25 g/100 g.

The oil content of local marine and freshwater fishes is generally low if compared to temperate marine fishes (Stansby 1962). This probably is due to the different storage sites of fat in the different species. If the fish is "lean fish" its oils and fats are stored in the liver or other parts of the body. The *Tenualosa toli* is a member of the Clupe group of fishes, which is under "fatty fish" category, where the oils and fats are stored mainly in muscle tissues. For instance, cod has low oil content when compared to trout and pilchard because of its fat is store in the liver.

FATTY ACID COMPOSITION OF MARINE AND FRESHWATER FISH OILS

The mass spectrums and structure of fatty acid methyl esters were obtained by using the mass spectrometer (MS). In this fatty acid analysis study, fish oils give a total of 10 to 16 fatty acid peaks with a slight variation of fatty acid concentration (%). Detailed fatty acid compositions are listed in Table 1 to Table 6 which is based on GC chromatogram obtained from GCMS analysis Figure 2 to Figure 7 and total fatty acid content of these fish oils are summarized in Table 7. In addition, Table 8 shows the ratios of PUFA to SFA ratio and ω -6 to ω -3 ratios in oils extracted from the fish studied. These ratios are useful to determine which fish gives out a better index in comparing relative nutritional value of fish oils in different species. A typical cod oil GC profile is used as a standard in this study.

Generally, more peaks are seen in marine fish oil samples than in freshwater fish oils samples. The chromatograms also showed that all the local fish oils are made up of long chain fatty acid, with a minimum carbon chain length of 12-to carbon 24 which is considered as typical characteristic of fish oil.

Generally the PUFA content in marine fishes was much higher (32.25-65.31%) than the saturated fatty acids (27.76-36.74%) and MUFAs are lowest (9.30-30.54%). The trend is different when compared to freshwater fish, where the concentration of MUFA (15.51-41.86%) is higher than saturated (26.24-32.90%) and PUFA (18.05-32.39%) (Rahman et al. 1995). Other researches have also shown that freshwater fish have lower content of PUFAs (Vlieg & Body 1988). The differences can be attributed to the fact that freshwater fishes feed largely on vegetation and plant materials, whereas marine fish staple diets are mainly zooplanktons, rich in PUFAs.

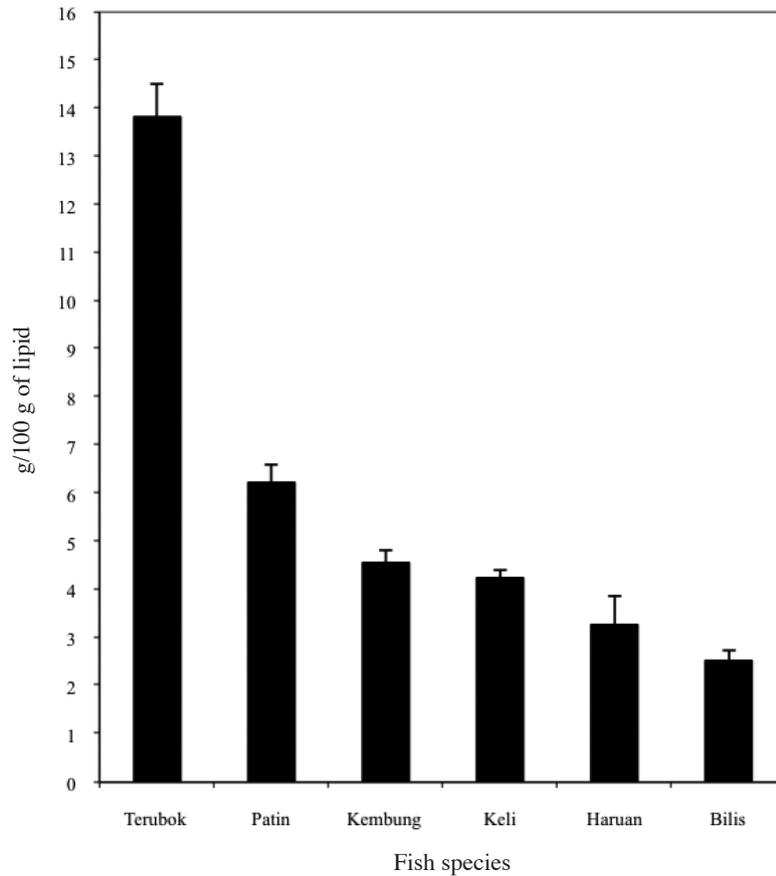


FIGURE 1. Lipid content of 6 different fish species in Malaysia

TABLE 1. Fatty acid composition of *Rastrelliger kanagurta* (Kembung) oil based on chromatogram Figure 2

Peak no	Compound Name	Symbol	Molecular Weight	Molecular Formula
1	Dodecanoic	12:0	214	$C_{13}H_{26}O_2$
2	Pentadecanoic acid, methyl ester	15:0	256	$C_{17}H_{34}O_2$
3	9-hexadecenoic acid, methyl ester	16:1 (n-7)	268	$C_{17}H_{32}O_2$
4	Hexadecanoic acid, methyl ester (palmitic acid)	16:0	256	$C_{16}H_{32}O_2$
5	7-hexadecenoic acid, methyl ester	16:1	268	$C_{17}H_{32}O_2$
6	Heptadecanoic acid, methyl ester	17:0	284	$C_{18}H_{36}O_2$
7	9,12,15- octadecatrienoic acid, methyl ester (alpha-linolenic)	18:3 (n-3)	292	$C_{19}H_{32}O_2$
8	9,12- octadecatrienoic acid, methyl ester (linoleic acid)	18:2 (n-6)	268	$C_{17}H_{32}O_2$
9	Cis-9-octadecenoic acid, methyl ester (Oleic acid)	18:1 (n-6)	296	$C_{19}H_{36}O_2$
10	Octadecanoic acid, methyl ester	18:0	298	$C_{19}H_{38}O_2$
11	5,8,11,14,17-eicosapentaenoic acid, methyl ester (EPA)	20:5 (n-3)	316	$C_{21}H_{32}O_2$
12	Cis-13-docosenoic acid, methyl ester (erucic acid)	22:1 (n-9)	352	$C_{23}H_{44}O_2$
13	Eicosanoic acid, methyl ester	20:0	326	$C_{21}H_{42}O_2$
14	4,7,10,13,16,19 - Docosahexaenoic acid (DHA)	22:6 (n-3)	419	$C_{23}H_{36}O_2$

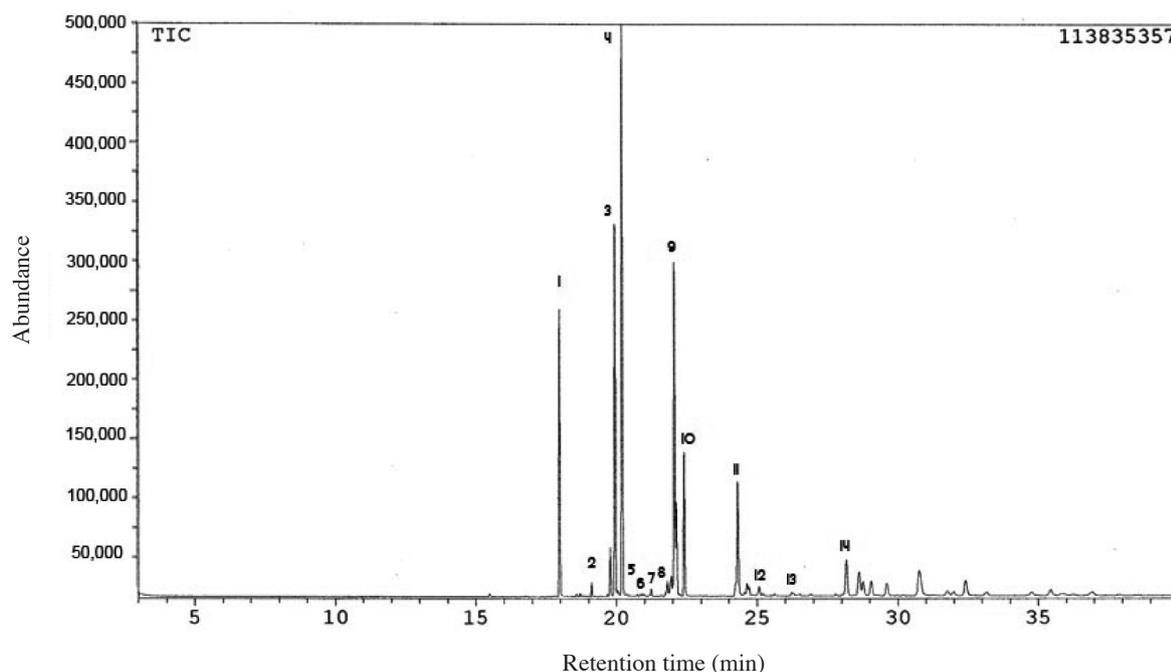


FIGURE 2. GC separation of fatty acid methyl ester of *Rastreligger kanagurta* (kembung) mixture on a CBP-M25-025 non-polar phase capillary column. Column temperature: Initial temperature of 60°C with an increase rate of 15°C/min to 230°C. Helium flow rate : 1 mL/min

TABLE 2. Fatty acid composition of *Tenuialosa toli* (terubok) based on chromatogram Figure 3

Peak no	Compound Name	Symbol	Molecular Weight	Molecular Formula
1	Dodecanoic	12:0	214	C ₁₃ H ₂₆ O ₂
2	Pentadecanoic acid, methyl ester	15:0	270	C ₁₇ H ₃₄ O ₂
3	9-hexadecenoic acid, methyl ester	16:1 (n-7)	268	C ₁₇ H ₃₂ O ₂
4	Hexadecanoic acid, methyl ester (palmitic acid)	16:0	256	C ₁₆ H ₃₂ O ₂
5	7-hexadecenoic acid, methyl ester	16:1	268	C ₁₇ H ₃₂ O ₂
6	Heptadecanoic acid, methyl ester	17:0	284	C ₁₈ H ₃₆ O ₂
7	9,12,15- octadecatrienoic acid, methyl ester (alpha-linolenic)	18:3 (n-3)	292	C ₁₉ H ₃₂ O ₂
8	Cis-9-octadecenoic acid, methyl ester (Oleic acid)	18:1 (n-6)	296	C ₁₉ H ₃₆ O ₂
9	Octadecanoic acid	18:0	298	C ₁₉ H ₃₈ O ₂
10	5,8,11,14,17-eicosapentaenoic acid, methyl ester (EPA)	20:5 (n-3)	316	C ₂₁ H ₃₂ O ₂
11	Cis-13-docosenoic acid, methyl ester (erucic acid)	22:1 (n-9)	352	C ₂₃ H ₄₄ O ₂
12	Eicosanoic acid, methyl ester	20:0	326	C ₂₁ H ₄₂ O ₂
13	4,7,10,13,16,19 - Docosahexaenoic acid (DHA)	22:6 (n-3)	419	C ₂₃ H ₃₆ O ₂

Majority of PUFAs, in fish oils ranged between C18 to C22 in chain length and are the omega-3 type. They are the linoleic acid family rather than of the alpha-linolenic acid family (omega-6 type) which occurs predominantly in other oils and fats. However, the fresh water fishes usually contained higher amounts of omega-6 fatty acids than the marine fish oils, (Stansby 1969). This was found to be the same in Malaysia freshwater fishes where the freshwater

fishes are rich in 18:2 (n-6) but low in PUFAs content than the marine fishes. The MUFAs, however, appeared to be the major fatty acid in freshwater fishes.

The local freshwater fishes such as the snakehead, catfish and patin, contained higher proportions of saturated fatty acids and C18 PUFAs but lower levels of C20 and C22 compared to marine fishes. The major monoene in these three local freshwater fishes is 18:1.

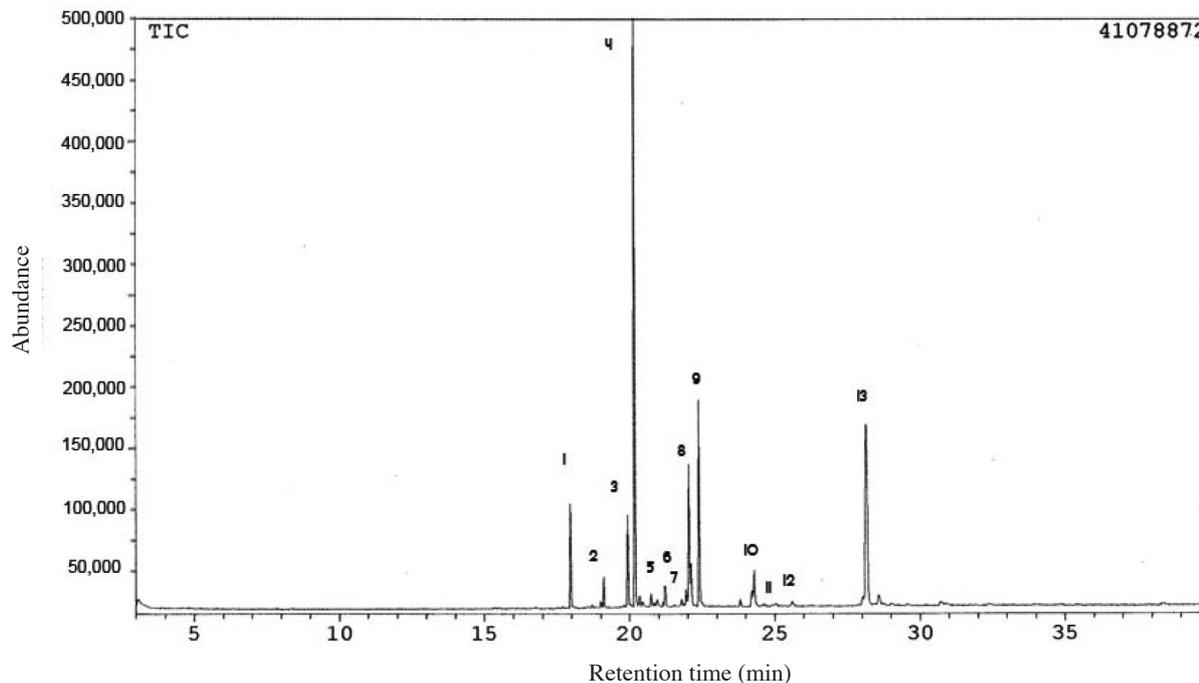


FIGURE 3. GC separation of fatty acid methyl ester of *Tenuulosa toli* mixture on a CBP-M25-025 non-polar phase capillary column. Column temperature: Initial temperature of 60°C with an increase rate of 15°C/min to 230°C. Helium flow rate : 1 mL/min

TABLE 3. Fatty acid composition of *Stolephorus baganensis (bilis)* based on chromatogram Figure 4

Peak no	Compound Name	Symbol	Molecular Weight	Molecular Formula
1	Dodecanoic acid, methyl ester	12:0	214	C ₁₃ H ₂₆ O ₂
2	Pentadecanoic acid, methyl ester	15:0	270	C ₁₇ H ₃₄ O ₂
3	9-hexadecenoic acid, methyl ester	16:1 (n-7)	268	C ₁₇ H ₃₂ O ₂
4	Hexadecanoic acid, methyl ester (palmitic acid)	16:0	256	C ₁₆ H ₃₂ O ₂
5	7-hexadecenoic acid, methyl ester	16:1	268	C ₁₇ H ₃₂ O ₂
6	9,12,15- octadecatrienoic acid, methyl ester (alpha-linolenic)	18:3 (n-3)	292	C ₁₉ H ₃₂ O ₂
7	Cis-9-octadecenoic acid, methyl ester (Oleic acid)	18:1 (n-6)	296	C ₁₉ H ₃₆ O ₂
8	Octadecanoic acid	18:0	298	C ₁₉ H ₃₈ O ₂
9	5,8,11,14,17-eicosapentaenoic acid, methyl ester (EPA)	20:5 (n-3)	316	C ₂₁ H ₃₂ O ₂
10	4,7,10,13,16,19 - Docosahexaenoic acid (DHA)	22:6 (n-3)	419	C ₂₃ H ₃₆ O ₂

This has become a feature for freshwater fish oil compared to marine fish oil. Without exception, 22:6 (n-3) is the principle hexaenoic fatty acid in these local freshwater and marine fishes. These similar results were obtained by Ackman et al. (2002).

Omega-3 fatty acids are long chain polyunsaturated fatty acids (18-22 carbon atoms in chain length) with the first of many double bonds beginning with the third carbon atom. It is concentrated throughout the food chain, but is most abundant in the oils of cold-water fish such as mackerel, salmon, sardines, herring and cod. Omega-3 fatty acids can be divided into three main categories that is eicosapentaenoic Acids (EPA), docosahexaenoic Acids (DHA) and Alpha-Linolenic Acids. EPA and DHA are

found mainly in fish oils while Alpha-Linolenic is usually derived from plant sources. However, there is an increase of awareness in the role of omega-3 fatty acids. Omega-3 fatty acids aids in the prevention and management of heart diseases. This can help in reducing one's risk of developing an abnormal heartbeat that can lead to heart problems and even sudden death. Omega-3 prevents asthma, hypertension, diabetes, cancer, and kidney dialysis and tends to inhibit the development or metabolism of these diseases in the body.

The values of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of local fishes studied are listed in Table 7. The amounts of EPA were found to be the highest in anchovies (4.68%) followed by kembong

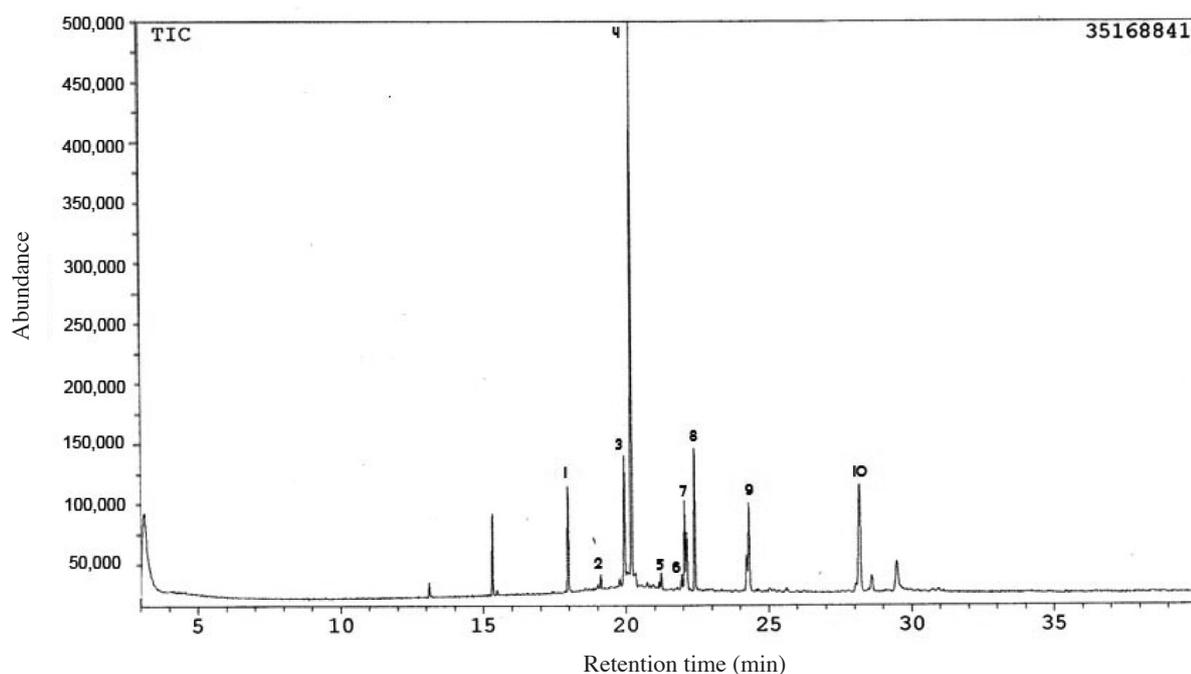


FIGURE 4. GC separation of fatty acid methyl ester of *Stolephorus baganensis* (bilis) mixture on a CBP-M25-025 non-polar phase capillary column. Column temperature: Initial temperature of 60°C with an increase rate of 15°C/min to 230°C. Helium flow rate : 1 mL/min

TABLE 4. Fatty acid composition of *Clarias macrocephalus* (keli) based on chromatogram Figure 5

Peak no	Compound Name	Symbol	Molecular Weight	Molecular Formula
1	Dodecanoic	12:0	214	C ₁₃ H ₂₆ O ₂
2	Pentadecanoic acid, methyl ester	15:0	270	C ₁₇ H ₃₄ O ₂
3	Hexadecanoic acid, methyl ester (palmitic acid)	16:0	256	C ₁₆ H ₃₂ O ₂
4	7-hexadecenoic acid, methyl ester	16:1	268	C ₁₇ H ₃₂ O ₂
5	Heptadecanoic acid, methyl ester	17:0	284	C ₁₈ H ₃₆ O ₂
6	9,12,15- octadecatrienoic acid, methyl ester (alpha-linolenic)	18:3 (n-3)	292	C ₁₉ H ₃₂ O ₂
7	9,12- octadecatrienoic acid, methyl ester (linoleic acid)	18:2 (n-6)	268	C ₁₇ H ₃₂ O ₂
8	Cis-9-octadecenoic acid, methyl ester (Oleic acid)	18:1 (n-6)	296	C ₁₉ H ₃₆ O ₂
9	Octadecanoic acid, methyl ester	18:0	298	C ₁₉ H ₃₈ O ₂
10	5,8,11,14-eicosatetraenoic acid, methyl ester (arachidonic acid)	20:4 (n-6)	318	C ₂₁ H ₃₄ O ₂
11	Cis-13-docosenoic acid, methyl ester (erucic acid)	22:1 (n-9)	352	C ₂₃ H ₄₄ O ₂
12	4,7,10,13,16,19 - Docosahexaenoic acid, methyl ester (DHA)	22:6 (n-3)	419	C ₂₃ H ₃₆ O ₂

(2.66%). Wang et al. (1990) found that the ω -3: ω -6 ratio of freshwater fish was lower than marine fish, whereby ratio ranged from 4.7 to 14.4. Freshwater fish usually consist of more ω -6 polyunsaturated fatty acid, i.e linoleic acid (C18:2) and arachidonic acid (C20:4), whereas the marine fishes are rich in ω -3, especially DHA and EPA (Wang et al. 1990). Cod oil that is marine fish oil has been proposed by the USFDA as a PUFA supplement.

A possible explanation to these variations in fatty acids composition between the marine and freshwater fish is the dietary source. The freshwater fishes available in the

market are mainly cultured type that is fed with artificial food rather than the natural food. This is probably why a higher content of odd chain fatty acids was found in the wild type snakehead than the cultured one. In general the lipids of freshwater algae contained higher levels of C18 than C20 or C22 polyunsaturated fatty acids and in this case would make the freshwater fishes have higher PUFA levels. (Wood 1974)

The omega-3 fatty acids were present in a higher amount in marine fish oil such as the *Tenuulosa toli*, *Stolephorus baganensis* and *Rastrelliger kanagurta*

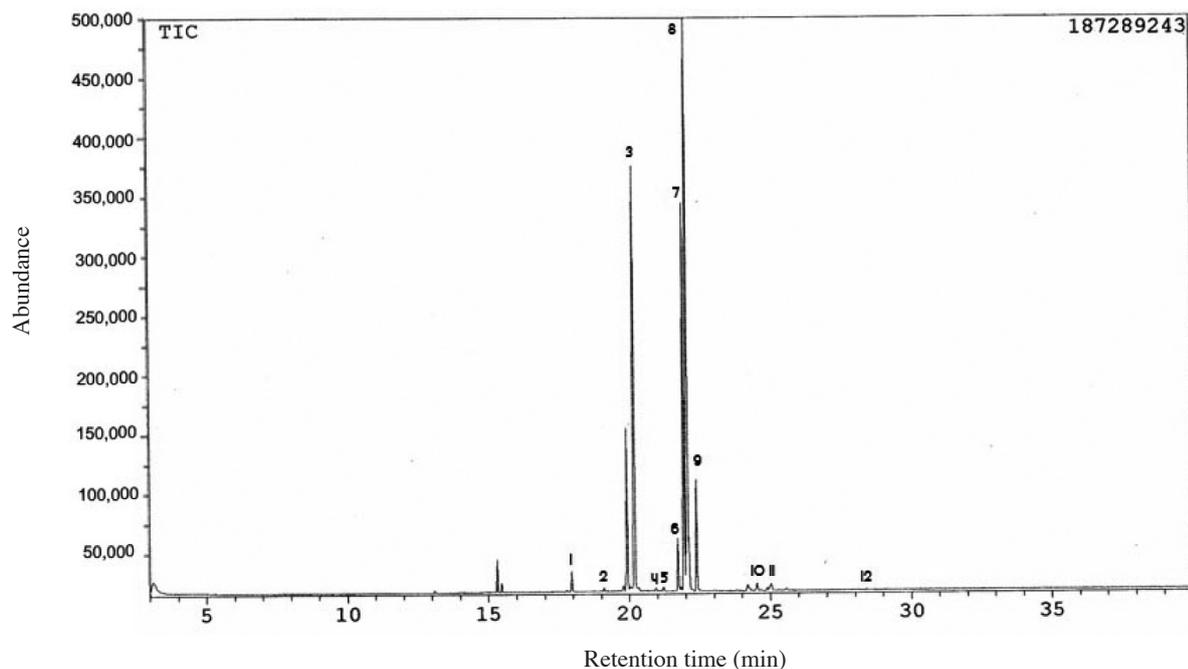


FIGURE 5. GC separation of fatty acid methyl ester of *Clarias macrocephalus* (keli) mixture on a CBP-M25-025 non-polar phase capillary column. Column temperature: Initial temperature of 60°C with an increase rate of 15°C/min to 230°C. Helium flow rate : 1mL/min

TABLE 5. Fatty acid composition of *Channa striatus* (haruan) based on chromatogram Figure 6

Peak no	Compound Name	Symbol	Molecular Weight	Molecular Formula
1	Dodecanoic	12:0	214	C ₁₃ H ₂₆ O ₂
2	Pentadecanoic acid, methyl ester	15:0	270	C ₁₇ H ₃₄ O ₂
3	9-hexadecenoic acid, methyl ester	16:1 (n-7)	268	C ₁₇ H ₃₂ O ₂
4	Hexadecanoic acid, methyl ester (palmitic acid)	16:0	256	C ₁₆ H ₃₂ O ₂
5	7-hexadecenoic acid, methyl ester	16:1	268	C ₁₇ H ₃₂ O ₂
6	Heptadecanoic acid, methyl ester	17:0	284	C ₁₈ H ₃₆ O ₂
7	9,12,15- octadecatrienoic acid, methyl ester (alpha-linolenic)	18:3 (n-3)	292	C ₁₉ H ₃₂ O ₂
8	9,12- octadecatrienoic acid, methyl ester (linoleic acid)	18:2 (n-6)	268	C ₁₇ H ₃₂ O ₂
9	Cis-9-octadecenoic acid, methyl ester (Oleic acid)	18:1 (n-6)	296	C ₁₉ H ₃₆ O ₂
10	Octadecanoic acid, methyl ester	18:0	298	C ₁₉ H ₃₈ O ₂
11	5,8,11,14-eicosatetraenoic acid, methyl ester (arachidonic acid)	20:4 (n-6)	318	C ₂₁ H ₃₄ O ₂
12	8,11,14-eicosatetraenoic acid, methyl ester (dihommo-gamma-linolenic)	20:3 (n-6)	306	C ₂₀ H ₃₄ O ₂
13	11,14-eicosadienoic acid, methyl ester	20:2 (n-6)	322	C ₂₁ H ₃₈ O ₂
14	Cis-13-docosenoic acid, methyl ester (erucic acid)	22:1 (n-9)	352	C ₂₃ H ₄₄ O ₂
15	Eicosanoic acid, methyl ester	20:0	326	C ₂₁ H ₄₂ O ₂
16	4,7,10,13,16,19 - Docosahexaenoic acid (DHA)	22:6 (n-3)	419	C ₂₃ H ₃₆ O ₂

compared to the freshwater fish oils. These local marine fishes are very rich in DHA (22:6 n-3) while EPA (20:5 n-3) content was much lesser. This was agreed with the report prepared by Sinclair (1990) that the omega-6 PUFA in fish oils increased in the warmer tropical waters. As

for the Australian, fish are classified into three categories, firstly rich in DHA and EPA, secondly rich in DHA and AA and lastly, rich in DHA only. Based on this classification and from the results obtained, most of the Malaysian fishes could be classified in the third group, with a few species

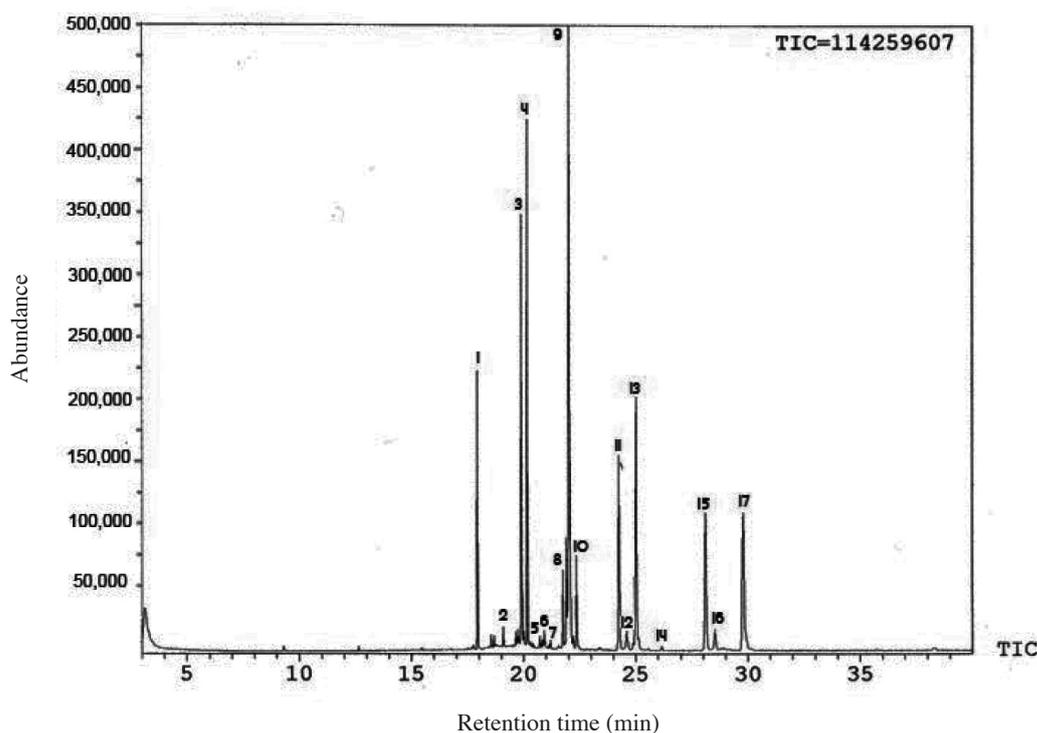


FIGURE 6. GC separation of fatty acid methyl ester of *Channa striatus* (haruan) mixture on a CBP-M25-025 non-polar phase capillary column. Column temperature: Initial temperature of 60°C with an increase rate of 15°C/min to 230°C. Helium flow rate : 1 mL/min

TABLE 6. Fatty acid composition of *Pangasius hypothalamus* (patin) based on chromatogram Figure 7

Peak no	Compound Name	Symbol	Molecular Weight	Molecular Formula
1	Dodecanoic acid, methyl ester	12:0	214	C ₁₃ H ₂₆ O ₂
2	9-hexadecenoic acid, methyl ester	14:0	242	C ₁₅ H ₃₀ O ₂
3	Hexadecanoic acid, methyl ester (palmitic acid)	16:2 (n-6)	268	C ₁₇ H ₃₂ O ₂
4	9,12,15- octadecatrienoic acid, methyl ester (alpha-linolenic)	18:3 (n-3)	292	C ₁₉ H ₃₂ O ₂
5	Cis-9-octadecenoic acid, methyl ester (Oleic acid)	18:1	296	C ₁₉ H ₃₆ O ₂
6	Octadecanoic acid, methyl ester	18:0	298	C ₁₉ H ₃₈ O ₂
7	5,8,11,14-eicosatetraenoic acid, methyl ester (arachidonic acid)	20:4 (n-3)	318	C ₂₁ H ₃₄ O ₂
8	8,11,14-eicosatetraenoic acid, methyl ester (dihommo-gamma-linolenic)	20:3 (n-6)	306	C ₂₀ H ₃₄ O ₂
9	11,14-eicosadienoic acid, methyl ester	20:2 (n-6)	322	C ₂₁ H ₃₈ O ₂
10	Cis-13-docosenoic acid, methyl ester (erucic acid)	22:1 (n-9)	352	C ₂₃ H ₄₄ O ₂
11	4,7,10,13,16,19 - Docosahexaenoic acid (DHA)	22:6 (n-3)	419	C ₂₃ H ₃₆ O ₂

grouped under the second category such as *Tenulosa toli*, *Stolephorus baganensis* and *Rastrelliger kanagurta*.

The old adage that ‘fish is brain food’ has been recognized through the observation of the relationship between docosahexaenoic acid (DHA) and its requirement in the brain. Many experimental studies are related to the effects of omega-3 fatty acids (particularly EPA and DHA) to various chronic diseases such as coronary heart disease

(CHD), arteriosclerosis, high blood pressure, cancer and immunological and inflammatory processes. The summary of pharmaceutical activity of fish oil in relation to the fatty acid components has been reported. They possessed many healing effects ranging from curing of wounds and burn to tuberculosis alleviating actions. Furthermore, there is also research on reticuloendothelial stimulation with application to phagocytosis (Stansby 1969).

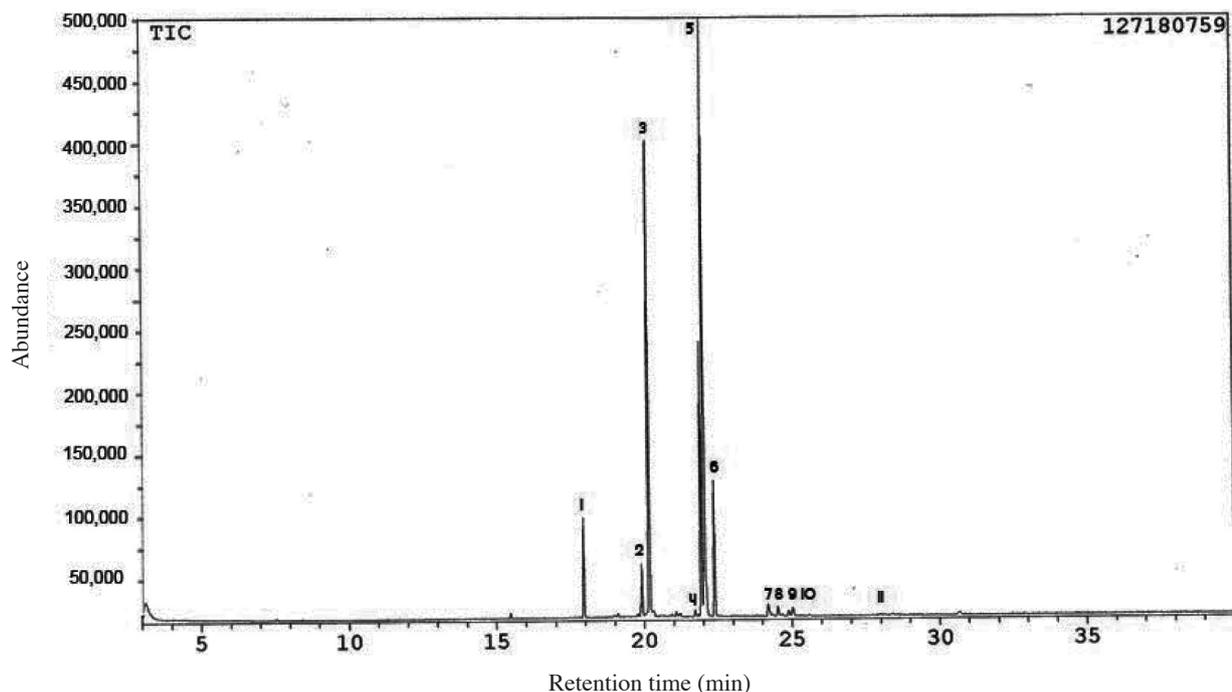


FIGURE 7. GC separation of fatty acid methyl ester of *Pangasius hypothalamus* (patin) mixture on a CBP-M25-025 non-polar phase capillary column. Column temperature: Initial temperature of 60°C with an increase rate of 15°C/min to 230°C. Helium flow rate: 1 mL/min

TABLE 7. Fatty acid composition between Malaysia freshwater and marine fishes presented as mean \pm Standard deviation (n=5)

Fatty acids composition (%)	Fresh water fishes			Marine fishes		
	Patin	Keli	Haruan	Terubuk	Bilis	Kembong
a) Saturated Fatty acid						
C16:0	24.83 \pm 0.15	20.19 \pm 0.12	21.94 \pm 0.13	21.22 \pm 0.11	20.64 \pm 0.12	25.06 \pm 0.11
C18:0	7.14 \pm 0.01	5.47 \pm 0.02	6.26 \pm 0.04	5.51 \pm 0.01	6.21 \pm 0.01	11.42 \pm 0.04
C20:0	0.19 \pm 0.02	0.20 \pm 0.01	4.57 \pm 0.01	6.74 \pm 0.03	0.57 \pm 0.02	4.68 \pm 0.01
C22:0	0.37 \pm 0.01	0.07 \pm 0.02	1.26 \pm 0.02	3.67 \pm 0.01	0.22 \pm 0.01	5.58 \pm 0.02
C24:0	0.37 \pm 0.02	0.30 \pm 0.01	0.08 \pm 0.01	1.02 \pm 0.01	0.12 \pm 0.01	trace
Σ STAD	32.90 \pm 0.15	26.24 \pm 0.12	34.11 \pm 0.14	38.16 \pm 0.12	27.76 \pm 0.03	46.74 \pm 0.12
b) Monounsaturated Fatty Acid (MUFA)						
C16:1	2.97 \pm 0.01	1.83 \pm 0.01	1.94 \pm 0.01	12.57 \pm 0.19	2.50 \pm 0.01	2.37 \pm 0.01
C18:1	33.94 \pm 0.16	7.15 \pm 0.03	4.57 \pm 0.02	16.81 \pm 0.11	2.77 \pm 0.02	2.92 \pm 0.02
C20:1	3.94 \pm 0.02	3.76 \pm 0.01	5.31 \pm 0.01	0.48 \pm 0.01	3.58 \pm 0.01	1.99 \pm 0.01
C22:1	0.83 \pm 0.01	2.77 \pm 0.03	4.2 \pm 0.03	0.68 \pm 0.02	2.24 \pm 0.03	2.02 \pm 0.02
Σ MUFA	41.68 \pm 0.16	15.51 \pm 0.04	16.20 \pm 0.04	30.54 \pm 0.22	11.09 \pm 0.04	9.30 \pm 0.03
c) ω -6 PUFA						
C18:2	0.21 \pm 0.01	5.23 \pm 0.01	2.22 \pm 0.01	2.14 \pm 0.01	0.33 \pm 0.01	2.67 \pm 0.01
C18:3	2.38 \pm 0.02	2.35 \pm 0.02	trace	4.17 \pm 0.03	1.28 \pm 0.01	4.25 \pm 0.05
C20:2 ω 6	3.76 \pm 0.01	0.08 \pm 0.01	0.19 \pm 0.01	0.51 \pm 0.01	6.21 \pm 0.04	2.42 \pm 0.01
C20:3 ω 6	3.04 \pm 0.03	0.07 \pm 0.03	0.57 \pm 0.02	1.5 \pm 0.02	7.36 \pm 0.01	trace
C20:4 ω 6	1.41 \pm 0.01	1.60 \pm 0.01	4.46 \pm 0.01	0.16 \pm 0.01	7.37 \pm 0.02	3.17 \pm 0.02
C22:5 ω 6	0.74 \pm 0.01	0.20 \pm 0.01	1.27 \pm 0.02	5.14 \pm 0.04	4.2 \pm 0.01	0.74 \pm 0.01
Σ PUFA ω 6	11.54 \pm 0.04	9.53 \pm 0.04	8.71 \pm 0.03	13.62 \pm 0.06	26.75 \pm 0.05	13.25 \pm 0.06

(continue)

Continued (TABLE 7)

Fatty acids composition (%)	Fresh water fishes			Marine fishes		
	Patin	Keli	Haruan	Terubuk	Bilis	Kembong
d) ω -3 PUFA						
C18:3 ω 3	0.26 \pm 0.01	0.23 \pm 0.01	2.36 \pm 0.04	0.77 \pm 0.01	0.83 \pm 0.01	2.39 \pm 0.02
C18:4 ω 3	2.52 \pm 0.02	4.43 \pm 0.04	0.17 \pm 0.01	0.27 \pm 0.01	7.98 \pm 0.04	6.60 \pm 0.07
C20:4 ω 3	0.2 \pm 0.01	0.11 \pm 0.01	3.3 \pm 0.03	0.51 \pm 0.02	3.21 \pm 0.01	3.10 \pm 0.01
C20:5 ω 3	1.41 \pm 0.03	0.63 \pm 0.03	0.98 \pm 0.01	9.93 \pm 0.01	4.68 \pm 0.03	10.62 \pm 0.13
C22:5 ω 3	3.92 \pm 0.01	0.06 \pm 0.01	1.26 \pm 0.02	1.50 \pm 0.03	1.88 \pm 0.01	4.12 \pm 0.05
C22:6 ω 3	0.14 \pm 0.01	0.11 \pm 0.01	0.25 \pm 0.01	2.50 \pm 0.01	10.5 \pm 0.11	4.85 \pm 0.01
Σ PUFA ω 3	8.45 \pm 0.04	5.57 \pm 0.05	8.32 \pm 0.06	15.48 \pm 0.04	29.08 \pm 0.12	31.68 \pm 0.16
e) Other types of PUFA						
C16:2 w-4	1.97 \pm 0.02	0.05 \pm 0.01	0.03 \pm 0.01	0.04 \pm 0.01	3.58 \pm 0.03	0.2 \pm 0.01
C16:3 w-4	0.05 \pm 0.01	0.83 \pm 0.03	1.93 \pm 0.05	0.1 \pm 0.01	0.29 \pm 0.01	0.1 \pm 0.01
C16:4 w-1	0.04 \pm 0.01	2.06 \pm 0.01	1.47 \pm 0.01	1.97 \pm 0.06	1.12 \pm 0.02	0.18 \pm 0.01
C18:3 w-4	0.24 \pm 0.02	2.38 \pm 0.05	2.93 \pm 0.02	0.04 \pm 0.01	0.89 \pm 0.01	0.25 \pm 0.02
Σ Other PUFA	2.3 \pm 0.03	5.32 \pm 0.06	6.36 \pm 0.06	2.15 \pm 0.06	5.88 \pm 0.04	0.73 \pm 0.03

The recent study regarding the nutritional properties in these local fish oils suggested that the *Tenuialosa toli*, *Stolephorus baganensis* and *Rastrelliger kanagurta*, have a higher nutritional value due to the unsaturated fatty acids, particularly the PUFAs are higher than in the freshwater fishes and with a lower saturated fatty acid content. However, the anchovies exhibited a significant high content of cholesterol and cholesterol esters compared to the other local species. The palmitic, stearic and myristic are the major types of saturated fatty acids but the content of these saturated fatty acids is considered low when compared to other animal fats. The high content of mono and polyunsaturated fatty acids in fish oil with a relatively low content of saturated fatty acids keeps the oil in liquid form. Fish oil is the only animal fat, which is in liquid form at room temperature.

In descending order, fish with the highest quantity of arachidonic acid were *Stolephorus baganensis* (7.37%), *Channa striatus* (4.46%), *Rastrelliger kanagurta* (3.17%), *Clarias macrocephalus* (1.60%), *Pangasius hypophthalmus* (1.41%) and *Tenuialosa toli* (0.16%). The level of arachidonic acid in *Channa striatus* (haruan) was the highest among the freshwater fish samples. In a study conducted by Mat Jais in 1994, *Channa striatus* showed similar results and it is also reported that this fish has been used for centuries for reducing pain, inflammation and promote wound healing in Malaysia. The levels of DHA in this fish also would explain the use of *C. striatus* for muscle pain and inflammation (Mohsin & Ambak 1983). The high level of polyunsaturated fatty acid, especially C20: 4 ω -6, in fish is most probably due to lower oxygen solubility in warmer waters. On the other hand, marine fish samples showed overall to have higher amount of arachidonic acid, EPA and DHA compared to freshwater fishes. Anchovies are among the fishes that showed high AA and DHA content.

In Figures 8 to 12, types of the fatty acid content of fish oil samples are summarized. These results are extracted from the detailed fatty acid content tabulated in Table 7. Generally, fatty acids are divided into saturated and unsaturated and this is further divided into more distinctive groups. Basically through the bar graph showed below, comparison can easily be done among the fatty acid groups and helps to determine which fish species has the potential to be commercialized.

The lipid is extracted from the flesh for the analysis of lipid composition because it is the part of the fish, which people eat and is considered one of the important sources of protein. By studying the lipid composition of these local fishes, some important data or information regarding the biochemistry of fish lipids and their nutritional properties can be obtained. The nutritional value of fish and fish oil has attracted a lot of public attention. It is believed to have many protective effects to various chronic diseases such as arteriosclerosis and coronary heart diseases (CHD).

CONCLUSION

In conclusion, *Tenuialosa toli* (Terubok), *Rastrelliger kanagurta* (Kembung) *Stolephorus baganensis* (Bilis), *Pangasius hypophthalmus* (Patin), *Clarias macrocephalus* (Keli) and *Channa striatus* (Haruan) showed a considerable amount of saturated and unsaturated fatty acids in both freshwater and marine fishes. About 10 to 15 major compounds are detected from each fish sample. Although not typical, the result of these study are in agreement with many other published results about the fatty acid content in different fishes species originating from different locations in Malaysia. Generally, the fatty acid composition of freshwater and marine fishes showed similar patterns, although marine fishes showed more favourable fatty acid composition, containing more unsaturated fatty

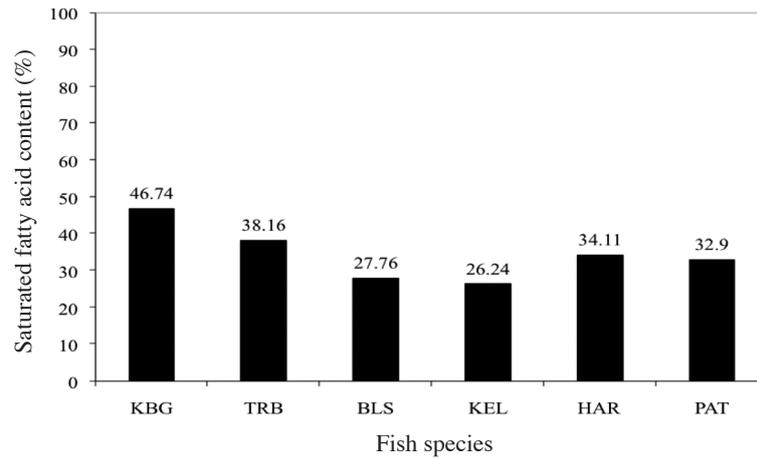


FIGURE 8. Total of saturated fatty acid present in fish oil samples. KBG – Kembong, TRB – Terubuk, BLS – Bilis, KEL – Keli, HAR – Haruan, PAT - Patin

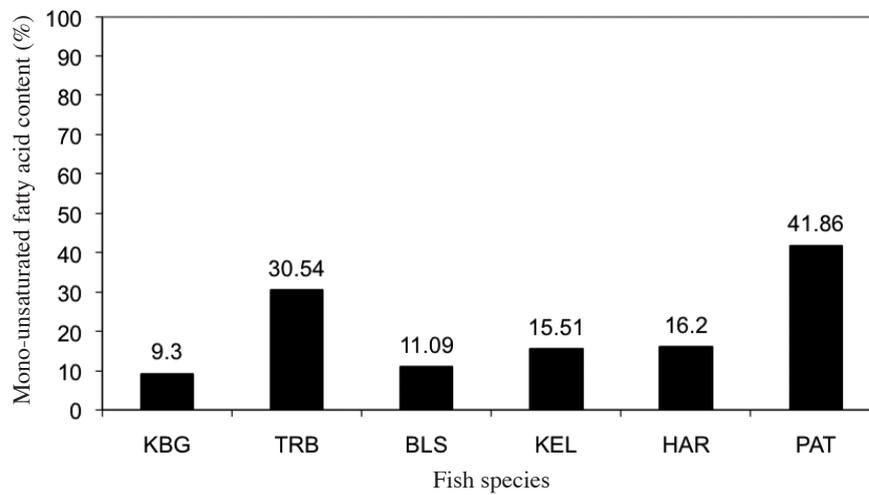


FIGURE 9. Total of monounsaturated fatty acid (MUFA) present in fish samples. KBG – Kembong, TRB – Terubuk, BLS – Bilis, KEL – Keli, HAR – Haruan, PAT - Patin

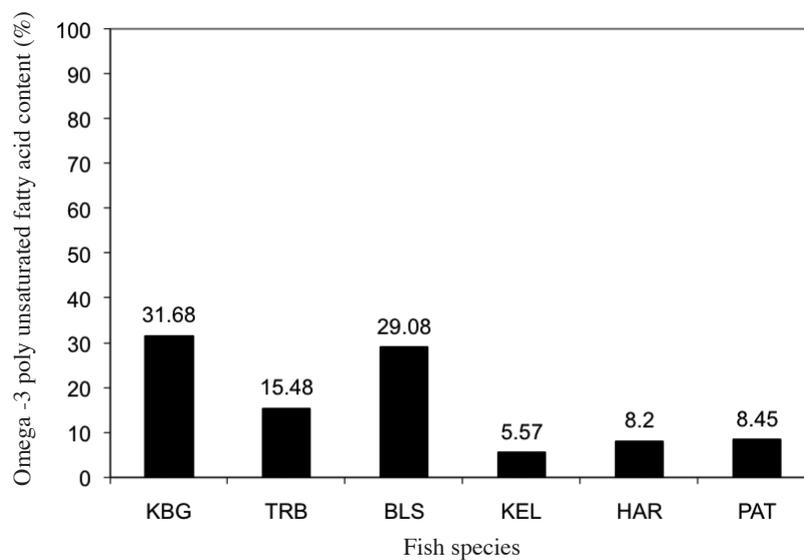


FIGURE 10. Total of omega-3 polyunsaturated fatty acid present in fish samples. KBG – Kembong, TRB – Terubuk, BLS – Bilis, KEL – Keli, HAR – Haruan, PAT - Patin

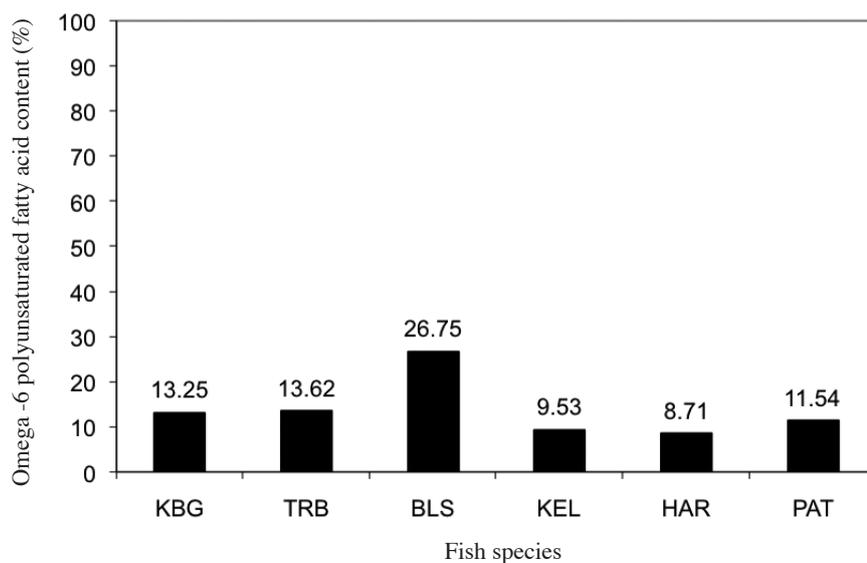


FIGURE 11. Total of omega-6 poly-unsaturated fatty acid present in fish samples. KBG – Kembong, TRB – Terubuk, BLS – Bilis, KEL – Keli, HAR – Haruan, PAT - Patin

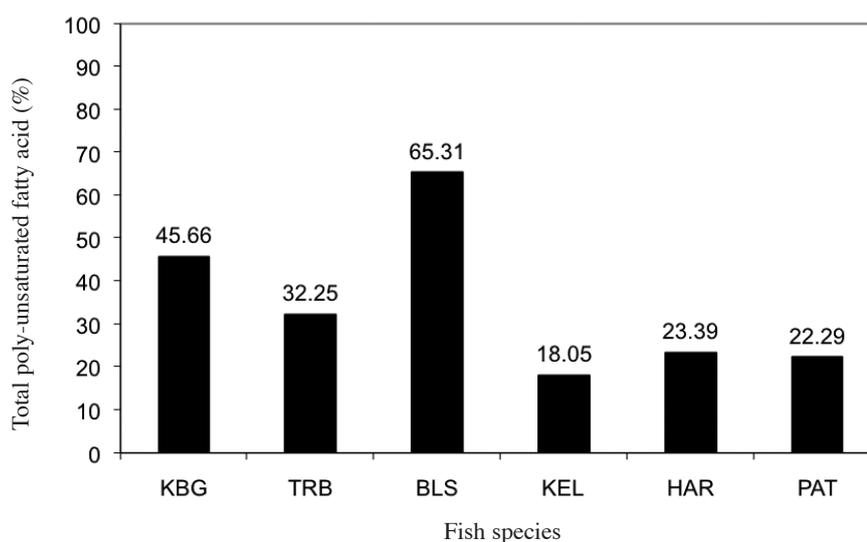


FIGURE 12. Total of polyunsaturated fatty acid present in fish samples. KBG – Kembong, TRB – Terubuk, BLS – Bilis, KEL – Keli, HAR – Haruan, PAT - Patin

TABLE 8. The polyunsaturated fatty acids/saturated (P/S) and ω -3/ ω -6 ratios of fatty acids from fish oils

Fish Species	Ratio of Polyunsaturated / Saturated Fatty Acid	Ratio of ω -3/ ω -6
Patin (<i>Pangasius hypothalamus</i>)	0.67	0.73
Catfish (<i>Clarias macrocephalus</i>)	1.84	0.25
Snakehead (<i>Channa Striatus</i>)	0.69	0.96
Terubok (<i>Tenualosa toli</i>)	0.85	1.14
Anchovies (<i>Stolephorus baganensis</i>)	0.96	2.35
Kembung (<i>Kanagurta rastrelliger</i>)	0.98	2.39

acid. Polyunsaturated fatty acids (PUFA) are found to be one of the highest compounds found in these fishes and n-3 PUFA is studied extensively in both types of fishes. The n-3 polyunsaturated fatty acid which is primarily docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) was found in appreciable amounts and would be suitable for inclusion in the formulation of highly unsaturated low-fat diets and to help prevent diseases.

Although the amount of fish lipids are generally low when compared to fishes from temperate countries, terubok and patin which are local fish contains high amount of lipid in their tissue muscle which might be a potential in commercializing it as a local oil capsule production. Presently, the production of fish oil is becoming more demanding, as there is a sizeable and growing world market demand for high quality fish oils. In order to meet the demand of the society there is the need to locate new oil fish and further research to know their characteristics and usefulness.

From this study, Malaysia fishes have the potential to be commercialized. Results indicate that all of the fishes has favourable amount of polyunsaturated fatty acid which are known to prevent all sorts of diseases. This signifies that the Malaysian fishes has good oil value and is suitable for applications in pharmaceutical and food industries.

ACKNOWLEDGEMENT

The authors thank University of Malaya for the research grant.

REFERENCES

- Ackman, R.G., Mcleod C.K., Misra, K. & Rakshit, S. 2002. Lipids and fatty acids of five freshwater fishes of India. *Journal of Food Lipid* 9: 127-145.
- Folch, J., Lees, M.S. & Stanley, G.H.A. 1957. A simple guide for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 226: 497-509.
- Hearn, T.L. & Sgoutas, D.S. 1987. Polyunsaturated fatty acids and fat in fish flesh for selecting species for health benefits. *Journal of Food Science* 52: 1209.
- Karim, G. 1990. *Information Malaysia 1990-1991 Year Book*. Berita Publishing Sdn. Bhd, Kuala Lumpur, Malaysia.
- Majid, M.I.A., Endarti, E., Hashim, R. & Chong, A. 2003. Direct FAME Synthesis for Rapid Total Lipid Analysis from Fish Oil and Cod Liver Oil. *Journal of Food Composition and Analysis* 18: 161-171.

- Mat Jais, A.M., Zuraini, A., Somchit, M.N., Solihah, M.H., Goh, Y.M., Arifah, A.K., Zakaria, M.S., Somchit, N., Rajion, M.A., & Zakaria, Z.A. 1994. Fatty acid and amino acid composition of three local Malaysian *Channa* spp. *Fish. Food Chemistry* 97: 674-678.
- Mohsin, A.K.M. & Ambak, M.A. 1983. *Freshwater Fishes of Peninsular Malaysia*. Serdang, Selangor: Universiti Pertanian Malaysia Publication.
- Nurasyikin Abdul Rahman, & Jumat, Salimon. 2006. Physicochemical characteristics of Terubok, *Tenualosa toli* fish oil. *Malaysian Journal of Analytical Sciences* 10(1): 115-120.
- Qin, J. 2000. Intensive culture of an air-breathing fish, snakehead (*Channa striatus*) at larval, fingerling and growout stages. In: *The Third World Fisheries Congress Abstracts Book*. Beijing, P.R. China. p. 83.
- Rahman, S.A., Teh, S.H., Hassan, O., & Daud, N.M. 1995. Fatty acid composition of some Malaysian freshwater fish. *Food Chemistry* 54: 45-49.
- Sinclair, H.M. 1990. *History of Essential Fatty Acids: Omega-6 Essential Fatty Acids: Pathophysiology and Roles in Clinical Medicine*. Horrobin, D.F.(ed.) New York: Alan Liss.
- Stansby, M.E. 1962. Proximate composition of fish. In: E. Heen & R. Kreuzer (eds.) *Fish in Nutrition*. London: Fishing news books Ltd. p. 55-60.
- Stansby, M.E. 1969. Nutritional properties of fish oils. *World review of Nutrition Dietary* 11: 46.
- Vlieg P. & Body D.R. 1988. *New Zealand Journal of Marine and Freshwater Research* 22: 151-162.
- Wang, Y.J., Miller, L.A., Perren, M. & Addis, P.B. 1990. Omega-3 fatty acids in lake superior fish. *Journal of Food Science* 55: 71-76.
- Wood, C.M. 1974. A critical examination of the physical and adrenergic factors affecting blood flow through the gills of the rainbow trout. *Journal of Experimental Biology* 60: 241-265.

Institute of Biological Sciences
Faculty of Science
University of Malaya
50603 Kuala Lumpur
Malaysia

*Corresponding author; email: jamal@um.edu.my

Received: 4 January 2011

Accepted: 7 June 2011