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Extraction and Characterization of Fish Oil from Monopterus Albus

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Abstract. Fish oils have been recognized as good sources of polyunsaturated fatty acids (PUFA) which are widely used for pharmaceutical purposes and as food supplements. It has been reported that tropical fishes are rich in arachidonic acid (AA) and docosahexaenoic acid (DHA). These fatty acids have been recommended as infant food supplements by health agencies. In this study fish oil from *Monopterus albus* (a tropical freshwater fish) were extracted using a solvent system. The oil was extracted separately from the head and body of the fish.The fatty acid composition of the oil was analyzed and quantified using gas chromatography after being converted into methyl ester derivative. Results showed that the lipid content of *Monopterus albus* is between 0.50 and 1.06 g/100 g tissue of the fillet and between 0.40 and 0.78 g/100 g tissue of the head. In the fatty acid analysis it was discovered that the major fatty acids in the oil from the body and head were palmitic, oleic, arachidonic and docosahexaenoic acid. Arachidonic acid and docosahexaenoic acid content of the body oil were 8.25 and 6.21 g/100 g lipid respectively. While in the head oil the content of these acids were 8.77 and 6.11 g/100 g lipid respectively. In the saponified body oil the percentage of arachidonic acid was 10.17% and DHA 7.16%.

Abstrak. Minyak ikan telah diketahui sebagai suatu sumber asid-asid lemak politaktepu yang digunakan secara meluas untuk tujuan-tujuan farmaseutikal dan sebagai makanan tambahan. Ikan-ikan tropika telah dilaporkan sebagai kaya dengan asid arakidonik dan asid dokosaheksaenoik (DHA). Asid-asid lemak ini telah disyorkan sebagai makanan bayi oleh beberapa agensi kesihatan. Di dalam kajian ini minyak ikan daripada *Monopterus albus* (sejenis ikan air tawar tropika) telah diekstrak dengan menggunakan sistem pelarut. Pengekstrakan minyak telah dijalankan secara berasingan daripada kepala dan badan ikan. Kandungan asid lemak daripada minyak tersebut telah ditentukan dengan menggunakan kromatografi gas setelah ditukarkan kepada terbitan metil ester. Keputusan menunjukkan bahawa kandungan lipid di dalam *Monopterus albus* adalah di antara 0.50 dan 1.06 g/100 g tisu bagi isi dan di antara 0.40 dan 0.78 g/100 g tisu bagi kepala. Di dalam analisis asid lemak didapati bahawa asid-asid lemak yang utama di dalam minyak isi dan kepala adalah asid palmitik, oleik, arakidonik dan dokosaheksaenoik. Kandungan asid arakidonik dan dokosaheksaenoik di dalam minyak isi adalah 8.25 dan 6.21 g/100 g lipid masing-masing. Manakala di dalam minyak kepala kandungan asid-asid ini adalah 8.77 dan 6.11 g/100 g lipid masing-masing. Di dalam minyak isi terhidrolisis, peratus asid arakidonik adalah 10.17% dan bagi DHA 7.16%.

Key words : Fish oil, monopterus albus, eel, arachidonic acid, docosahexaenoic acid.

Introduction

During the last two decades polyunsaturated fatty acids (PUFA) have attracted great interest among scientists for their medicinal and nutritional properties. Among the common sources of these PUFAs are fish oils. The PUFA composition in fish oils are affected by several factors, such as geographical location, temperature and water salinity [1,2]. The oils extracted from the northern hemisphere coldwater fishes are rich in n-3 PUFA, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). An increase in environmental temperature and decrease in water salinity will bring about a higher content of arachidonic acid, which is an n-6 PUFA, in replacement of EPA. Thus, tropical freshwater fishes are expected to contain high levels of arachidonic acid (AA) and DHA. These fatty acids have been approved as essential fatty acids. Studies have shown that AA and DHA are present in human milk and health agencies have recommended that infant formula are supplemented with AA and DHA [3].

At present, arachidonic acid is produced from certain marine fish, mammals and microorganisms while DHA is obtained from certain coldwater fish. Little has been done to study the PUFA content of Malaysian freshwater fish. Endinkeau and Tan Kim Kiew studied the lipid and fatty acid content of several Malaysian freshwater fishes [4]. They discovered that these fishes contain low level of EPA and DHA. However, the eel, *Monopterus albus* contain a high level of DHA. The study was based on the oil extracted from the fillet of the fishes.

In this study, fish oil was extracted separately from the body and head of *Monopterus albus* and the

fatty acid composition was determined by gas chromatography. Subsequently, the oil was saponified to isolate the free fatty acids (FFA) and the fatty acid composition of the FFA was determined.

Experimental

Materials.

Chloroform and hexanes were obtained from from JT Baker. Butylated hydroxytoluene (BHT) used was from Sigma Chemical Company. Sodium sulphate anhydrous was bought from BDH Limited. Fatty acid methyl ester standards were obtained from Sigma Chemical Company.

Sample preparation.

The fish (eels or *Monopterus albus*) were bought fresh from the market. Prior to analysis, the internal organs were removed and the fish was washed to remove the residual blood. Fish fillet was obtained by cutting the fish lengthwise along the backbone to obtain maximum amount of flesh without including the backbone. The fillet was cut into small pieces. The 10% in length of the anterior part of the fish is considered as the head.

Extraction of Lipid.

Extraction of the fish lipids were done according to the method of Bligh and Dyer [5] with some modification by Kinsella *et al* [6]. Representative samples of fish tissue (50g) were homogenized in a blender for 2 minutes with a mixture of methanol (100 ml) and chloroform (50 ml). Then 50 ml of chloroform was added to the mixture. After blending for an additional 30 seconds, distilled water (50 ml) was added. (Whenever 50 g of fish tissue was not available, the solvent volumes used were adjusted to the same ratio.) The homogenate was stirred with a glass rod and filtered through a Whatman no.1 filter paper on a Buchner funnel under vacuum suction. 20 ml chloroform was used to rinse the remainder. The filtrate was allowed to settle to separate into the organic and aqueous layers. The chloroform layer containing the lipids was transferred into another beaker and ca. 3 g of anhydrous sodium sulphate was added to remove any remaining water. The mixture was filtered through a Whatman no. 1 filter paper and chloroform was used to rinse the remainder. Finally, a known amount of BHT (of about 0.02 g) was added to the lipid solution as an antioxidant [6]. The solution was then evaporated to a constant weight in a tared 100 ml round-bottom flask with a rotary evaporator at 40 ºC. The determination of lipid content was done separately for each fish. Six fish were used in this experiment. The extracted oil was pooled into body oil and head oil for further analysis.

Saponification.

Saponification was carried out on the body lipid. Eel lipid (1.1 g) was mixed with 20 ml of NaOH solution in ethanol/water and heated, with stirring, for 30 min at 50 ºC. The NaOH solution was prepared by dissolving 6 g of NaOH in 10 ml water and 50 ml of 96% ethanol. After saponification, 50 ml hexanes was added and the mixture was stirred. The lower aqueous layer which contains fatty acid soaps was separated and acidified to pH 1 with concentrated hydrochloric acid. The FFA formed was extracted with 30 ml hexanes. BHT (0.01g) was added to the FFA solution and the solvent was evaporated with a rotary evaporator at 40 ºC.

Preparation of Methyl Esters.

The samples (lipid or FFA) were converted to their constituent fatty acid methyl esters (FAME) according to the method used by Hammond [7]. 50 mg of the sample was refluxed in 5 ml of reagent consisting of concentrated sulphuric acid-toluene-methanol (1:10:20 v/v/v) for one hour at 90 - 100 ºC. Then water (3 ml), hexanes (2 ml) and a mixture of internal standards (1 ml) were added. The internal standards used were the methyl esters of C15 (pentadecanoic acid methyl ester) and C19 (nonadecanoic acid methyl ester). The hexanes layer was recovered, dried over anhydrous sodium sulphate and was ready for injection. A triplicate methylation was done on each sample.

Analysis of Methyl Esters.

Analysis of methyl esters were performed by a Hitachi D-2500 gas chromatograph, equipped with a Stabilwax® column, 30 m x 0.32 mm id (Restek Corp., USA) and an FID detector. The injector and detector temperatures were 250 and 280 ºC respectively. The column temperature was held at 108 ºC for 2 mins and then programmed to 240 ºC at 6 ºC/min. The FAMEs were identified by comparing their retention time against those of authentic standards. Quantification of the FAMEs was done using internal standards method. Pentadecanoic acid methyl ester was used to calculate the amount of myristic and palmitic acid methyl esters. While nonadecanoic acid methyl ester was used to calculate the rest of the FAMEs.

Results and Discussion

The lipid content of the eels obtained were between 0.50 and 1.06 g/100 g wet tissue in the body and between 0.40 and 0.78 g/100 g wet tissue in the head. Figure 1 shows the chromatogram of fatty acid methyl esters derived from the body oil of eels.

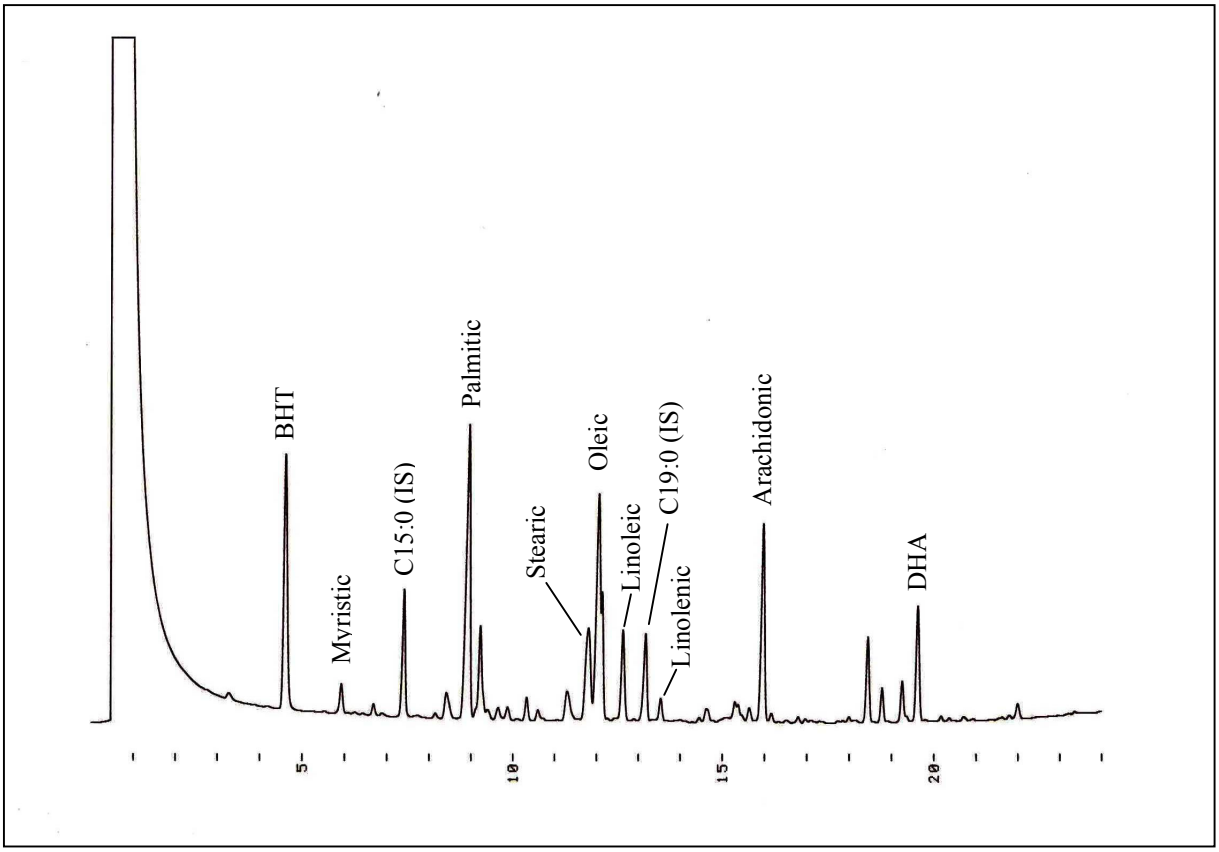

Figure 1 : GC Chromatogram of the FAME derived from the body oil of eel. Attenuation = 7

Table 1 : The fatty acid composition of oil from eel.[a]

Fatty Acid	Body Oil (g/100 g lipid)	Head Oil (g/100 g lipid)
C14:0 (Myristic)	0.80	0.21
C16:0 (Palmitic)	10.75	7.31
C18:0 (Stearic)	4.42	4.60
C18:1n-9 (Oleic)	8.54	6.32
C18:2n-6 (Linoleic)	2.51	1.51
C18:3n-3 (Linolenic)	0.75	0.22
C18:4n-3	0.24	0.33
C20:0 (Arachidic)	nd	nd
C20:4n-6 (Arachidonic)	8.25	8.77
C20:5n-3 (EPA)	0.26	0.26
C22:6n-3 (DHA)	6.21	6.11

a - mean of 3 separate determinations
nd = not detectable

Table 2 : Fatty acid composition of saponified oil from the body of eel.[a]

Fatty Acid	Content (g/100 g total fatty acid)
C14:0 (Myristic)	1.07
C16:0 (Palmitic)	14.18
C18:0 (Stearic)	5.84
C18:1n-9 (Oleic)	10.61
C18:2n-6 (Linoleic)	3.46
C18:3n-3 (Linolenic)	0.95
C18:4n-3	0.26
C20:0 (Arachidic)	nd
C20:4n-6 (Arachidonic)	10.17
C20:5n-3 (EPA)	0.33
C22:6n-3 (DHA)	7.16

a - mean of 3 separate determinations
nd = not detectable

The fatty acid composition of the body oil, head oil and FFA of the body oil is tabulated in Table 1. The most abundant fatty acid in the body oil is palmitic acid and in the head oil is arachidonic acid (AA). The results also indicate high levels of DHA and AA in the body and head oils. The contents of these fatty acids in both areas are quite comparable, i.e in the body oil DHA is 6.21 and AA is 8.25 g/100 g lipid while in the head oil DHA is 6.11 and AA is 8.77 g/100 g lipid. These high values indicate that this species is a potential source of AA and DHA.

Saponification was done in order to determine the percentage of each fatty acid out of total fatty acids. The values are tabulated in Table 2. These values could be used as a comparison with the fatty acid composition of some fish which are reported as percentage of fatty acids. The fatty acid composition of some principal fish used in commercial fish oil production was summarized by Kinsella [8]. The fish are pink salmon, silver salmon, mackerel, menhaden, albacore tuna and bluefin tuna. The DHA content of these fish range between 10.8 and 18.9% which are higher than that of eel, i.e 7.16% (Table 2). However, they contain low percentage of AA. Mackerel which contains 10.8% DHA has only 3.9% of AA. The AA content in eel (10.17%) is much higher than this.

Another popular source of AA is microorganisms. Chaudhuri *et al* reported that *Mortierella elongata* SC-208, cultured under controlled mediums, gave high yields of AA [9]. The highest yield of AA was 33.2% out of total lipid. However, the DHA produced in that medium was only 1.1%. In another medium which gave high content of both fatty acids, the AA and DHA contents were 9.8 and 11.6% respectively. As shown in Table 2, the contents of AA and DHA in Malaysian eel are 10.17 and 7.16% respectively, which are more or less comparable to these values. Hence, The high content of DHA and AA in Malaysian eel is an advantage since both fatty acids are used as supplement in infant milk in some countries. The advantage is both essential fatty acids could be produced from a single source.

References

1. Diana H. Green, 1990, Lipid Metabolism in Fish, in *Fish Oils in Nutrition*, edited by Stansby, M.E., Van Nostrand Reinhold, New York, p. 226-241.
2. Zdzislaw E. Sikorski, Anna Kolakowska and Bonnie Sun Pan, 1990, The Nutritive Composition of the Major Groups of Marine Food Organisms, in *Seafood: Resources, Nutritional Composition, and Preservation*, edited by Zdzislaw E. Sikorski, CRC Press Inc, p. 29-54.
3. Iqbal Gill and Rao Valivety, 1997, Polyunsaturated fatty acids, part 1: Occurrence, biological activities and applications, *Trends in Biotechnology*, vol 15, 401-409.
4. K. Endinkeau and Tan Kim Kiew, 1993, Profile of Fatty Acid Contents in Malaysian Freshwater Fish, *Pertanika J. Trop. Agric. Sci*. 16(3): 215-221.
5. Bligh, E. G. and W. J. Dyer, 1959, A rapid method of total lipid extraction and purification, *Can. J. Biochem. Physiol*. 37: 911-917.
6. Kinsella, J.E., Shimp, J.L., Mai J. and Weihrauch, J., 1977, Fatty Acid Content and Composition of Freshwater Finfish, *J. Am. Oil Chem. Soc*. 54: 424-429.
7. Hammond, E.W., 1986, Packed-Column Gas Chromatography, in *Analysis of Oils And Fats*, edited by Hamilton, R.J. and Rossell, J.B., Elsevier App. Sci., p. 123-124.
8. Kinsella, J.E., 1987, *Seafoods and Fish Oils in Human Health and Diseases*, Marcel Dekker Inc., p 211.
9. S. Chaudhuri, S. Ghosh, D.K. Bhattacharyya, and S. Bandyopadhyay, 1998, Effect of Mustard Meal on the Production of Arachidonic Acid by *Mortierella elongata* SC-208, *J. Am. Oil Chem. Soc*. 75: 1053-1055.