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Study on ω-fatty acids from Malaysian giant mudskipper (*Periophthalmodon schlosseri*) fish oil

MUHAMMAD NOR OMAR, C.O. SITI-FAIRUZ, M.T. HASAN, M.N. NOR-NAZUHA[1], M.N. NOR-DALILAH[1] and Y. KAMARUZZAMAN

Kulliyyah of Science, International Islamic University Malaysia,
Jalan Sultan Ahmad Shah, Bandar Indera Mahkota - 252 00 (Malaysia).
[1]NikkyDylla Corporation, Bandar Bukit Mahkota, 43000 Kajang, Selangor (Malaysia).

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ABSTRACT

The main aim of the present study is to look into the composition of Malaysian giant mudskipper lipid which might be useful to human health by looking into the ratio of ω-fatty acids present in *P. schlosseri* oil. Lipid from the fish flesh was extracted using the Folch method and the fatty acid composition was analysed using GC and GC-MS. It was found that the major constituents of *P. schlosseri* lipid were palmitic acid (C16:0) (29.2%), oleic acid (C18:1 ω-9) (23.0%) and stearic acid (C18:0) (12.6%). Besides that, the essential ω-fatty acids eicosapentaenoic acid (EPA, C20:5, ω-3) and docosahexaenoic acid (DHA, C22:6, ω-3) contents were 3.3 and 4.3% respectively. The result shows that the total ω-fatty acid (ω-3 and ω-6) content of *P. schlosseri* was 15.4% and is nutritionally significant due to its ω-3:ω-6 ratio of 1:1.

Key words: *Periophthalmodon schlosseri*, giant mudskipper, ω-fatty acids.

INTRODUCTION

The giant mudskipper (*P. schlosseri*) is an amphibious fish belongs to the *Periophthalmin spp.* within the family *Gobiidae*. The fish lives in brackish water near mangrove swamps and intertidal habitats which are found only in tropical and subtropical regions, including Malaysia, Indonesia, India, Australia and Africa[1]. The mudskipper is also a valuable source of protein in some countries throughout the Asian and African region, including Japan, Korea and India[2,3]. Besides protein, it contains a high amount of lipid substances, which act as the sources of fatty acids, including polyunsaturated fatty acids (PUFA). Most fish lipids contain PUFA categorized as ω-fatty acids including arachidonic acid (AA, C20:4 ω-6), eicosapentaenoic acid (EPA, C20:5 ω-3) and docosahexaenoic acid (DHA, C22:6 ω-3)[4,5,6]. These ω-fatty acids have been recognized to exhibit special physiological and pharmacological effects on human health. Many studies have been carried out to determine the effect of ω-3 fatty acid on lipid profiles. Rivellese *et al.*[7] found that a significant decrease in LDL-cholesterol of healthy subjects when they are fed with ω-3 fish oil supplements. The results of an *in vivo* study by Eitsuka *et al* [8] conclude that ω-3 fatty acids inhibit proliferative colorectal cancer cell by downregulating the reversed transcriptase via protein kinase C inhibition. Meanwhile, a report by Helland *et al* [9] show that the intake of ω-3 fatty acid supplement during pregnancy has favorable effect in the cognitive development of the children.

Besides pharmacological effects, ω fatty acids also play important roles in human nutrition. Since the human body cannot synthesize ω-fatty acids *de novo*, it can form 20- and 22-carbon PUFAs from the eighteen-carbon ω-fatty acid, such as α-linolenic acid. Both ω-3 α-linolenic acid and ω-6

linoleic acid are essential nutrients which must be obtained from food. In addition, in order to include fish oil as a dietary food supplement, the ratio of ω-3 to ω-6 could be used as an indicator in evaluating its nutritive significance. It is suggested that an ω-3: ω-6 ratio of 1:1-1.5 would constitute a healthy human diet[10]. Previous studies on Malaysian freshwater fish found that most of them had an ω-3: ω-6 ratio of less than 1[11] while in marine fish the ratio was between 1:2 and 1:8[12]. Although data on ω fatty acid profiles of Malaysian giant mudskipper (*P. schlosseri*) are not reported elsewhere, other results can be used as references in present study. For example, Benerjee *et al* [2] report that the Indian mudskipper (*Boleophthalmus boddaerti*) contains PUFA *ca.* 24% of the total lipid with the ω-3:ω-6 ratio of 1:1.8 while the Nigerian mudskipper (*P. koelreuteri*) contains PUFA ca. 22% and the ω-3: ω-6 ratio is 1:2.2 [13]. The main aim of the present study is to look into the composition of Malaysian giant mudskipper lipid which might be useful to human health by looking into the ratio of ω-fatty acids present in *P. schlosseri* oil.

EXPERIMENTAL

Sample preparation

The mudskipper fish was collected from mangrove areas in Kuantan, Malaysia in September 2009. The fish flesh was homogenized using a homogenizer (DIAX 900, Heidolph Elektro GmbH, Kelheim, Germany) and dried to release excess moisture by using an Alpha 1-4 freeze dryer (Christ GmbH, Osterode, Germany).

Fat extraction

The freeze-dried homogenate of fish flesh was weighed and subjected to fat extraction using a modified Folch method[14] utilizing chloroform: methanol in the ratio of 2:1 according to Zuraini *et al.*[15]. In brief, 200 g of sample was transferred into a flask containing 300 ml of chloroform: methanol solvent system (2:1) and the flask was shaken for 5 days at room temperature using a Unimax 2010 platform shaker (Heidolph Elektro GmbH, Kelheim, Germany). The solvent extract was concentrated *in vacuo* using a rotary evaporator (Rotavapor R-200, *Buchi* Labortechnik AG, Switzerland).

GC and GC-MS Analyses of fish oil

The fish oil was methylated using a boron trifluoride methanolic sodium hydroxide solution according to the method previously reported[16]. The oil was then analysed via on-column GC technique using Agilent 6890N gas chromatograph (Agilent, Avondale, USA) equipped with a flame ionization detector (FID). An HP-5 non-polar capillary column (50m × 0.12 × 0.5 mm, SGE, Australia) was used and the temperature was initially kept at 50°C for 2 minutes and then programmed at 5°C min^{-1} to 250°C. The injector and detector temperatures were 220° and 250°C respectively and He gas was used as the carrier gas with a flow rate of 1.2 ml min^{-1}. For identification of fatty acids in fish oil, the GC-MS technique using Agilent 6890N gas chromatograph coupled with an Agilent 5973N mass selective detector (Agilent, Avondale, USA) was used. The column and temperature conditions set were similar to that of GC analysis. The fatty acid constituents were recognized by comparing the MS spectrum to a standard library (Wiley Registry of Mass spectral data).

RESULTS AND DISCUSSION

Lipid recovery

The oil recovery from fresh fish was 5.53 ± 0.15% (% wet basis) of total body weight (200 g). The recovery was slightly higher compared to the result obtained by Banerjee *et al.*[2]. This might be due to the size of the fish used. The present study used bigger fish while previous data were obtained from smaller sources.

Composition of Giant Mudskipper Fish Oil

The fatty acid profile of the mudskipper fish oil was analysed after methylation with a BF_3-methoxide solution according to reported methods[16]. Table 1 shows the result of fatty acid compositions obtained by GC analysis on non-polar (HP-5, Agilent, Avondale, USA) capillary column. It was found that the major chemical constituents of *P. schlosseri* oil were palmitic acid (C16:0) (29.2%), oleic acid (C18:1, ω-9) (23.0%) and stearic acid (C18:0) (12.6%). The palmitic acid content was similar to previously reported results (28.4%)[2]. However, the oleic acid content was slightly higher compared to data obtained by Eboh *et al.*[13]. The total saturated fatty acid (SFA) was also found to be high (48.3%). The ratio of the total saturated (SFA) and unsaturated fatty acids (USFA) was 1:0.9

and it is considered that the mudskipper fish oil is good for human consumption according to Zuraini *et al.*[15] who found that the ratio of TSA:USFA was around 1:0.8-1.1.

Meanwhile, the level of arachidonic acid (C20:4 ω-6) was slightly lower (1.0%) compared to the results of a previous study (2.4%)[4]. Since arachidonic acid is a precursor for prostaglandin and thromboxan biosynthesis[5], it may interfere with the blood clotting process during wound healing [11,17]. Besides AA, eicosapentaenoic acid (EPA ω-6) as well as docosahexaenoic acid (DHA, ω-3) contents showed slight variations from previous results. The distribution of ω-3 and ω-6 fatty acids in the present research differed from results obtained by other researchers. In this study the total ω-3 and ω-6 were 7.6 and 7.8 % respectively compared to other results, which ranged from 12.2 to 21.1 %[2]. The variations in the ω-fatty acid content in *P. schlosseri* might be due to the difference in locations and feeding habitats. This is because *P. schlosseri* is classified as an amphibious fish that depends on other fish for its diet. Additionally, the Kuantan intertidal area is the largest natural mangrove forest in Malaysia, covering 340 ha of the state of Pahang (the largest state in Peninsular Malaysia), and contains a variety of major food objects comprising algae, crustaceans, insects, earthworms, molluscs, nematodes and fungi that are the feeding prey for *P. schlosseri* [3].

Table 1: Fatty acid profile of Malaysian Giant Mudskipper (*P. schlosseri*) fish oil

Fatty acid	Mean ± S.D
C14:0	3.3 ± 0.2
C15:0	1.1 ± 0.4
C16:0	29.2 ± 1.2
C17:0	2.1 ± 0.3
C18:0	12.6 ± 1.4
C20:0	tr
Total Saturated (SFA)	48.3
C16:1 ω-9	8.3 ± 1.3
C18:1 ω-9	23.0 ± 2.5
C18:1 ω-9, trans	4.8 ± 1.2
Total monounsaturated	36.1
C18:2 ω-6	6.8 ± 1.6
C18:3 ω-6	tr
C20:2 ω-6	tr
C20:3 ω-6	tr
C20:4 ω-6	1.0 ± 0.3
C20:5 ω-3 (EPA)	3.3 ± 1.1
C22:6 ω-3 (DHA)	4.3±1.3
Total polyunsaturated	15.4
Total unsaturates (USFA)	51.5
Total ω-3 fatty acids	7.6
Total ω-6 fatty acids	7.8
Ratio ω-3/ω-6	1:1
SFA: USFA ratio	1:0.9

tr: trace amount (<0.01%)

In terms of ω-fatty acid ratio, the ω-3: ω-6 ratio in *P. schlosseri* was *ca*. 1: 1. This is in accordance to the suggested ratio from previous reports which state that most freshwater and intertidal fish have an ω-3: ω-6 ratio of less than 1[11].

CONCLUSION

Lipid from the flesh of *P. schlosseri* can be extracted using Folch method and the fatty acid composition identified using GC and GC-MS. The major fatty acid constituents of *P. schlosseri* lipid were palmitic acid (C16:0) (29.2%) and oleic acid (C18:1 ω-9) (23.0%) while the essential ω-fatty acid such as eicosapentaenoic acid (EPA, C20:5, ω-3) and docosahexaenoic acid (DHA, C22:6, ω-3) contents were small, i.e. 3.3 and 4.3% respectively. The result shows that the total ω-fatty acid (ω3 and ω6) content of *P. schlosseri* was 15.4%. This indicates nutritive significance due to the ω-3:ω-6 ratio of 1:1.

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