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Bioconversion of ω-Fatty Acid from Giant Snakehead (*Channa micropeltes*) Fish Oil

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ABSTRACT

Study on bioconversion of ω-fatty acids extracted from giant snakehead (*Channa micropeltes*) fish oil was carried out using guinea pig lung homogenate. The fish oil, after hydrolysis to their fatty acids, was incubated aerobically at 37°C in phosphate buffer solution with the addition of guinea pig lung homogenate. After incubation and chemoreduction, it was found that ca. 76% arachidonic acid has been converted to the prostaglandin, PGE_1. The fatty acid constituents in fish oil were analysed by GC-MS after methylation.

Key words: Channa micropeltes, , fish oil, ω-fatty acids, bioconversion.

INTRODUCTION

ω-fatty acids play important roles in human nutrition. Their functions in our daily health include lowering of triglyceride level, controlling of blood pressure and antiatherogenic activity[1]. The human body cannot synthesize ω-fatty acids *de novo*, but it can form 20- and 22-carbon unsaturated ω-fatty acids from the eighteen-carbon ω-fatty acid, ω-linolenic acid. Both the ω-3 α-linolenic acid and ω-6 linoleic acid are essential nutrients which must be obtained from food.

Channa micropeltes is a giant snakehead fish belongs to *Channidae* family. The fish is indigenous to many tropical countries such as Malaysia. It is a freshwater, air breathing as well as carnivorous fish, which is a valuable source of protein throughout the Asia Pacific region. It is reported that *Channa* spp. fish possesses anti-inflammatory properties. Besides that, fish is known to have certain PUFAs that can regulate prostaglandin synthesis and also induce wound healing. For cardiovascular diseases and cancers, the ω-3 and ω-6 PUFAs have been shown to have positive effects on those diseases. The composition of PUFA may vary between species of fish, even from marine and fresh water fish. Zuraini *et al.*[2] reported that they have compared fatty acid and amino acid composition on all three *Channa* spp. such as *C. striatus, C.*

micropeltes and *C. lucius.* It is stated that all three *Channa* spp. fish contained AA (C20:4) which is a precursor for prostaglandin and thromboxane biosynthesis. This will obstruct the process of blood clotting and its attachment to endothelial cells during wound healing.

Molecular modifications, such as enzymatic transformations of fatty acids, particularly at the double bonds of mono-, di- or poly-unsaturated acids, have been carried out for many years in the oils and fats industry. The addition of functional groups to the fatty acids may enhance the reactivity of the molecule[3]. Chemical reactions often produce random or mixed products while through molecular modifications carried out by cells or by enzymes can frequently be achieved with better selectivity and under mild conditions. In this way the conversion of fatty acids may provide products with potential industrial application[4]. Previous study by Bergstrom *et al.*[5] found that arachidonic acid was converted in good yield into prostaglandin E_2 by homogenates of sheep vesicular glands. This study reported that prostaglandins E_1 and E_2 was formed in an analogous manner from homo-γ-linolenic acid and allciseicosa-5,8,11,14,17-pentaenoic acid, respectively.

The main aim of the present study is to look into the bioconversion metabolite of C*. micropeltes* fatty acid which might be beneficial to human health. Besides that, the fatty acid profiles of the giant snakehead fish oil before and after bioconversion will be established.

EXPERIMENTAL

Sample preparation

The giant snakehead fish was collected from Tasik Kenyir, Malaysia. 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[6] utilizing chloroform: methanol in the ratio of 2:1 according to the methods previously reported[7,8]. 200 g of sample was transferred briefly into a flask containing 300 ml of solvent system of chloroform:methanol (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* until dry using a rotary evaporator (Rotavapor R-200, *Buchi* Labortechnik AG, Switzerland).

Fractionation of ω-fatty acid from *C. micropeltes* fish oil

The fish oil was hydrolysed to their corresponding fatty acids by reacting with sulphuric acid under reflux for 24 hrs according to the method previously reported[9]. 100 g of fish oil was added 200 ml of 20% sulphuric acid in aqueous solution and reflux for 24 hr. The reactant was extracted three times with 100 ml diethyl ether. The extract was then dehydrated using anhydrous sodium sulphate and evaporated to dryness using a rotary evaporator. The residue containing fatty acids (including ω-fatty acids) was kept at -18°C prior further analysis.

Bioconversion of ω–fatty acids

The triacylglycerols of fish oil were converted into fatty acids by hydrolysis according to the reported method[9]. The fatty acids (including ω–fatty acids) were incubated at 37°C in phosphate buffer containing guinea pig lung homogenate under stream of oxygen. After inhibition, the suspensions were extracted using diethyl ether. The residual extract was reduced with $NaBH_4$ and extracted with diethyl ether. The ethereal extract was concentrated and methylated prior to GC, and GC-MS analysis.

Analysis of fish oil

The fish oil was methylated using a boron trifluoride methanolic sodium hydroxide solution according to the method previously reported[10]. 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 x 0.12 x 0.5 µm, SGE, Australia) was used and the temperature was initially kept at 50°C for 2 min 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 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 are similar with GC analysis. The fatty acid constituents were recognized by comparing the MS spectrum with standard library (Wiley Registry of Mass spectral data).

RESULT AND DISCUSSION

% Fat recovery

The oil recovery from fresh fish was 5.8 ± 2.2% (% DW) of total body weight (1.4 kg). The recovery was slightly lower compared to the result obtained by Zuraini *et al*[2]. This might be due to the size of the fish used. The present study used smaller fish while previous data were obtained from larger sources (2.0-2.3 kg).

Fatty acid composition before and after bioconversion

Table 1 shows the comparison of GC chromatographic result between typical fish oil contents before and after bioconversion. Before bioconversion, it was found that the fatty acid constituents of *C. micropeltes* oil were palmitic acid (C16:0) (14.1%), docosahexaenoic acid (DHA, C22:6) (13.9%), arachidonic acid (C20:4) (11.4%), stearic acid (C18:0; 10.6%), eicosapentaenoic acid (EPA, C20:5, 5.1%) and linoleic acid (C18:2; 3.8%). The palmitic acid content was slightly lower compared to previously reported results (26.2%)[2] and the total saturated fatty acid (SFA) was also found to be less (30.7%). The low SFA content makes this fish good for human consumption in order to promote a healthy diet. The ratio of the total

Table 1: Fatty acid profile of Malaysian Giant snakehead fish oil

	Fresh Oil Mean ± S.D	After Bioconversion Mean ± S.D
C12:0	2.5 ± 1.1	2.5 ± 0.1
C14:0	1.1 ± 0.2	3.1 ± 0.1
C15:0	1.2 ± 0.4	0.7 ± 0.1
C16:0	14.1 ± 1.2	10.7 ± 0.5
C17:0	0.7 ± 0.3	0.7 ± 0.05
C18:0	10.6 ± 1.4	7.6 ± 1.4
C20:0	0.4 ± 0.2	0.3 ± 0.05
Total Saturated (SFA)	30.6	25.1
C16:1 ω 7	2.6 ± 1.3	0.2 ± 0.05
C18:1 ω 9	9.2 ± 2.5	8.7 ± 2.5
C18:1 ω 7	2.6 ± 1.5	1.4 ± 0.5
Total monounsaturated	14.4	10.3
C18:2 ω 6	3.8 ± 1.6	11.5 ± 1.6
C18:3 ω 6	0.8 ± 0.3	0.1 ± 0.05
C20:2 ω 6	0.6 ± 0.2	0.6 ± 0.2
C20:3 ω 6	0.8 ± 0.2	0.3 ± 0.2
C20:4 ω 6	11.4 ± 2.3	2.7 ± 0.1
C20:5 ω 3, EPA	5.1 ± 1.1	1.0 ± 1.1
C22:6 ω 3, DHA	13.9 ± 3.5	1.8 ± 3.5
PGE_1	10.7± 2.3	
Total polyunsaturated (PUFA)	36.4	18.0
Total unsaturates (USFA)	50.8	28.3
Total ω 3 fatty acids	19.0	
Total ω 6 fatty acids	17.4	
Ratio ω 3/ ω 6	1:0.9	
PUFA: SFA (P/S) ratio	1.2:1	
USFA: SFA ratio	1.7:	

saturated and polyunsaturated acids was 1:1.2 and it is considered that the giant snakehead fish oil is good for human consumption according to Zuraini *et al.*[2] who found that the ration of TSA:USA was around 1:0.8-1.1.

During enzymatic bioconversion, the guinea pig lung homogenate was used as suggested by Anggard and Samuelsson[11]. This is because the guinea pig lung contains the cyclooxygenase enzyme that is responsible for conversion of arachidonic acid (AA) to prostaglandins. Most of the fatty acids decreased in concentration after bioconversion. Arachidonic acid (C20:4) was decreased to 2.7% while eicosapentaenoic acid (EPA) and decosahexaenoic acid (DHA) were also decreased to 1.8 and 1.0% respectively. However, the percentage of *cis*linoleic acid (C18:2) was increased from 3.8 % to 11.5 %. Small amount of dihomo-γ-linolenic acid (C18: 3 ω6) and EPA (C20:5 ω3) were detected and probably these fatty acids were converted efficiently into new metabolites. The decrease of arachidonic acid from 11.4 to 2.7% (76% conversion) has affected the formation of prostaglandin, PGE_1 (10.7%). This was supported by GC/MS analysis which detected the presence of prostaglandin, PGE_1. Thus, it could be concluded that arachidonic acid is converted into prostaglandin, PGE_1 by the enzyme cyclooxygenase found in the guinea pig lung homogenate. The presence of PGE_1 was in compliance with the previous result obtained by Anggard and Samuelsson[11]. However, the biosynthetic pathway of this metabolite was not postulated.

CONCLUSION

The present study has successfully converted the arachidonic acid of *C. micropeltes* oil into prostaglandin, PGE_1 via enzymatic process using guinea pig lung homogenates. The presence of fatty acids and PGE_1 were identified using GC-MS technique. The finding showed that the concentration of prostaglandin, PGE_1 produced was moderate and could be important since PGE_1 has many functions such as control hormone regulation, control cell growth, sensitize spinal neurons to pain and many more. This study can benefit the medical field and as reference for future research.

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