



International Journal of Fisheries and Aquatic Studies

ISSN: 2347-5129

(ICV-Poland) Impact Value: 5.62

(GIF) Impact Factor: 0.352

IJFAS 2016; 4(4): 312-314

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www.fisheriesjournal.com

Received: 09-05-2016

Accepted: 10-06-2016

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Extraction and identification of PUFA from African Catfish (*Clarias gariepinus*) Skin

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Abstract

Fish and fish oil is the major source of polyunsaturated fatty acids (PUFA). These PUFA are variously important for human health. In this experiment, the most cheap and fatty fish species was used for the extraction and identification of PUFA using soxhlet extraction method and gas chromatography (GC) analysis revealed that 0.80%, 2.48% EPA and DHA were present in the test sample, respectively. The level of different fatty acid content range between 0.03% to 22.6%. Therefore, the objective of this study was to extraction and identification of PUFA from the cheaply available catfish waste part (skin) for the rural poor Bangladeshi.

Keywords: Fish oil, polyunsaturated fatty acid (PUFA), EPA and DHA

Introduction

Fish is the cheaply reachable protein content diet in Bangladesh. There are 260 species of fresh water fish, 12 species of exotic fish, 475 species of marine fish and 60 species of prawn and shrimps are available in Bangladesh throughout of the year (Chandra 2006) [5]. Fisheries sector contributes 5.24% in GDP and 4.76% in foreign exchange respectively (Chandra 2006) [5]. Moreover, a notable promotion of fish consumption has been increased in recent years. It provides 17% of the total animal protein and 6% of all protein consumed by humans. The nutritional benefits of fish are mainly due to the content of high-quality protein, vitamins and many other essential nutrients. Furthermore, compared with fatty meat products, fish are not high in saturated fat.

Bangladeshi people are used to consume fresh water fish and usually avoid marine water fish due to have their smell and exceptional taste except Ilesha. Among the fresh water fish species, African catfish (*Clarias gariepinus*) which is known as *African Magur* by the common people of Bangladesh occupy a large area in aquaculture in Africa and once in Bangladesh. Among the fishes it also belongs to the high protein and high lipid content species (Kleimenov, 1971) [11]. Due to its improper cultural management practices especially their feed and omnivorous feeding habit most people have discarded this species from their daily diet list. Now, it has spread in Europe and southern Asia for its great economic interest: rapid growth rate, omnivorous feeding habit, and high resistance to environmental stress (Kris *et al.*, 2002; Graaf *et al.*, 1996; Bureau *et al.*, 1995) [10, 6, 4]. It is also considered one of the most important tropical catfish species for aquaculture (Bureau *et al.*, 1995) [4]. In Indian sub-continent people usually throw out catfish viscera, skin and bone as worthless garbage and consume only muscle and head. Annually 25% of total fish production or equivalents to 20 million tones are being disposed off as waste materials (Sarker *et al.*, 2012) [15]. However, these materials could be a reliable source of bioactive components of lipid, fat and enzymes. Marine fish like Salmon, mackerel, and red mullet are the species have maximum content of poly unsaturated fatty acid (Domingo *et al.*, 2007) [7]. Contrary with marine water fish species fresh water fish have fewer amounts of PUFA i.e. eicosapentaenoic acids (EPA) and docosahexaenoic acids (DHA). For the proper growth and development PUFA is very important for human. However, it cannot be synthesized by the human body itself. Therefore, it must be taken via diet. Among the different sources of PUFA in the nature, the fish and purified fish oils are the major source of omega-3 PUFA especially DHA and EPA (Bourre *et al.*, 1997) [3] rather than seed oils (Mataix *et al.*, 2003) [12] or microalgae. National Academy of Science 2002, recommended that the optimum level of DHA and EPA for pregnant and lactating women is 0.14 and 0.13 g/d respectively.

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Noteworthy, it is not only necessary for proper development of vision and nervous system of the baby but also reduce the risk of preterm delivery.

It has been estimated that consumption of approximately 900 mg of omega-3 fatty acid (i.e., EPA and DHA) per day beneficially affect coronary heart disease (CHD) mortality rates in patients with coronary disease (Kris-Etherton *et al.*, 2002) [10]. Moreover, it could be the potential protective reduction of cancer risk (Hooper *et al.*, 2006; MacLean *et al.*, 2006) [9, 13]. DHA helps in brain memory and performance in early stage and adults to maintain normal functioning of brain and also improves the learning ability. Clinical trials have shown fish oil enrich with EPA and DHA act as a anti carcinogenic (Von *et al.*, 1999; FAO 1998), ulcerative colitis, multiple sclerosis and migraine headaches (Simopoulos, 2002) agents as well as a good heart health promoter by increasing the HDL (good cholesterol) and decreasing triglycerides (fats in the blood).

The waste materials from the African Catfish, mainly the skin, can be a reliable source of raw material for industrial scale the extraction of lipid at as it has minimal/negligible seasonal variation regarding chemical composition throughout the year (Osibona *et al.*, 2009) [14]. Therefore, the objective of this study was to extraction and identification of PUFA from cheaply available catfish for the rural poor Bangladeshi.

Materials and methods

Experimental site

The experiment was conducted on June 2010 to August 2010 in Centre of Excellence for Food Safety Research (CEFSR) Laboratory, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia.

Materials

Fresh African Catfish (*Clarias gariepinus*) samples were collected from the local market of Malaysia. All chemicals and solvents used in this experiment were analytical grade and obtained in Malaysia.

Sample Preparation for Experiments

The fresh samples were immediately de-headed and washed with copious amounts of fresh cool water to separate the skin. Then the skin were separated by using a de-boner and immediately the experimental sample were stored overnight in a freezer at -18 °C, and then freeze dried (Model: Labconco, USA) at a constant drying temperature of -47 °C and vacuumed at 0.133 bar. The dried samples were ground using a blender and stored in an airtight glass bottle in a cold room at 6 °C pending laboratory use.

Moisture Content Determination

The moisture content of the dried sample was determined by the oven dry method (AOAC, 1994) [2]. Five grams of finely ground sample was place in pre-weighted ceramic crucibles before being put into the oven. The temperature of the oven was set at 105 °C, and the heating process continued until constant weights of the samples were achieved. Then, the crucibles were transferred to a desecrator to cool before reweighing. The difference between the two weights (initial and final) indicated the moisture content (3.10 ± 0.1%) of skin.

Soxhlet Extraction

Total lipid content from the catfish skin was determined by soxhlet extraction method. Five grams of finely ground dried samples were extracted with 200 mL of petroleum ether with three replications over an extraction period of 8 h. Extra water and petroleum ether residue in the extracted oils were evaporated using a rotary evaporator (Heidolph, Germany) at a temperature of 45 °C. The evaporated sample was then kept in an oven at 45 °C for 1 h for the confirmation to totally disappear moisture and petroleum ether. Total lipid content of the catfish skin was 48 ± 0.4%/100g sample based on the dry weight basis. After each extraction, the extraction yield was calculated by the following formula

$$\text{Oil Yield} = \frac{\text{Mass of extracted oil}}{\text{Mass of dried material}} \times 100 \dots\dots\dots(1)$$

Results and Discussion

Fatty acids profiles were analyzed by the preparation of Fatty acid methyl esters (FAMES), assisted with a gas chromatography (GC) (model Shimadzu GC-14B, Tokyo, Japan) equipped with a flame ionization detector and integrator. A 50 µl test sample was taken in a 2 ml vial with 1 ml n-hexane, and 50 µl of 1 M sodium methoxide (30% methanol in sodium methoxide). Then the mixture was shaken vigorously for 30s using an auto-vortexer (Stuart, UK) and then it was stored for another 2 min at an uninterrupted condition in order to form a bi-layer. The clear upper layer of the mixture containing the FAME (1.0 µl) was pipetted off and injected into the gas chromatography immediately to avoid the reverse reaction using an external standard method, the PORIM test method no. P^{3,4}, 1995 (AOCS, 1994). A capillary column (30m×0.25mm, BP-21 SGE Analytical science, Australia) was used. GC system was calibrated using external standard technique. The calibration was done at three points (25ppb, 50ppb and 100ppb) by composite stock standard solution. GC injector temperature was fixed at 250 °C; column at 280 °C, oven at 180 °C, detector was FID set at 280 °C and total run time was 30 min. A representation of a typical chromatogram is presented in Fig. 1.

Table 1: The fatty acid composition of African catfish skin oil

Fatty acid	(%)
C14:0	3.2
C16:0	20
C16:1n7	3.9
C16:2n4	-
C16:3n4	0.03
C18:0	0.1
C18:1n9	22.6
C18:3n4	12
C18:3n3	0.04
C18:4n3	0.06
C20:1n9	0.03
C20:4n6	0.1
C20:4n3	1.3
C20:5n3 (EPA)	0.8
C22:5n3	-
C22:6n3 (DHA)	2.48

Notes: Values are % of eluted methyl esters; -: not detected

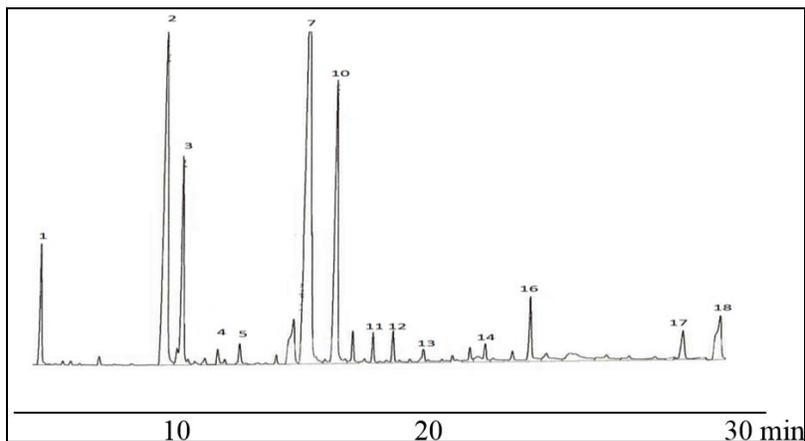


Fig 1: Gas chromatograph showing the fatty acid in African Catfish skin oil.
Pick number 17= EPA, Pick number 18= DHA

In general, the fatty acid composition of the fish part analyzed is in agreement with the data available on the fatty acid composition of the similar fish species as reported in previous work (Ackman, 1980) [1]. Moreover, the obtained result was also partially similar with Osibona *et al.*, 2009 [14] who has used the same species muscle to know the proximate composition and fatty acid profile. According to Osibona *et al.*, 2009 obtained 1.0% EPA and 3.0% DHA where as in my experiment I have got 0.80%, 2.48% EPA and DHA, respectively. Though, the PUFA composition may vary among fish species but emphasis has yet to pay to the PUFA composition of different species as selecting fish for the diet. This study indicates that the waste part (Skin) from the *C. gariepinus* had significant quantities of EPA and DHA and was therefore an average source of polyunsaturated fatty acids (PUFA). Moreover, this method is easier, convenient and as well as required less chemical solvents than other solvent extraction method.

Conclusion

From the findings of this study, I would like to suggest the poor rural Bangladeshi to consume *C. gariepinus* fish species with their skin very cheaply to avoid excessive and excessive consumption of saturated fat rich poultry and beef protein. Moreover, due to having EPA and DHA could be a good health promoter. Therefore, the studied fresh water fish species found to be important sources of nutrients.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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