Proximate and fatty acid composition of liver and fatty tissue of patincatfish (*Pangasianodon hypophthalmus*)

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Abstract

The visceral storage fat and liver of patin catfish (*Pangasianodon hypophthalmus*) are normally discarded, which incurs cost and can cause environmental pollution. However, these may be potential sources to extract fish oil. The proximate and fatty acid compositions of liver and fatty tissue of patin catfish were investigated to evaluate the suitability of these by-products for extracting fish oil. Fat was extracted using a low temperature solvent extraction method. The average fat content of fatty tissue and liver of females were 77.64 and 11.71%, respectively, whereas in males this was73.23 and 9.59%, respectively. Fatty acids found in the extracted oil of these byproducts were C12:0, C14:0, C14:1, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C18:4, C20:0, C20:1, C20:4, C20:5, and C22:6.The major fatty acids presented in these tissues were palmitic (C16:0), oleic (C18:1n-9), and linoleic acid (C18:2 n-6). The total amount of polyunsaturated fatty acids of liver from male and female patin catfish were 13.31 and 13.30%, respectively, whereas in the fatty tissue these were11.64 and 12.09%, respectively. The n-3 to n-6 ratios of liver and fatty tissue of females were 1.61and 0.95, respectively, whereas in male fish these were 1.31 and 1.05, respectively. Results of this study indicated that the liver and fatty tissues of patin catfish are suitable sources of fish oil specifically due to the presence of monounsaturated and n-3 polyunsaturated fatty acids.

Keywords: Catfish, Fatty acid, Liver, Fatty tissue

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Introduction

Interest in fish oil has been increasing mainly because of findings of Bang *et al*., (1971) who found that the Greenlandic Eskimos diet rich in polyunsaturated fatty acids was the cause of low cardiovascular diseases in that population. Fish oil plays an important role in the development and function of brain and reproductive system (Bourre, 2007), immune system (Calder, 2001), and treatment of inflammatory diseases such as arthritis (Heard *et al*., 2003). Moreover, n-3 and n-6 polyunsaturated fatty acids are two families of essential fatty acids that must be provided in food (Kaur *et al*., 2012). Fish oil contains high amounts of polyunsaturated n-3 fatty acid, which have 5 to 6 non-conjugated carbon-carbon double bonds per ester side chain with *cis*-stereochemistry (Ackman, 1982). Reyes and Sepúlveda (2006) reported that palmitic acid (C16:0), oleic acid (C18:1 n-9), linoleic acid (C18:2 n-6), and α-lino lenic acid (C18:3 n-3) are the main constituents of fish oil. The Fatty acid composition of fish oils are affected by various factors such as fish species (Rahman *et al*., 1995), diet (Shirai *et al*., 2002; Hafezieh *et al*., 2010), age (Nakamura *et al*., 2007) as well as water temperature and salinity (Cordier *et al*., 2002). The fatty acid composition of oil extracted from various fish species have been studied (Ackman, 1967; Rahman *et al*., 1995; Reyes and Sepúlveda, 2006; Nisa and Asadullah, 2011), but little attention has been paid to evaluate the fatty acid profile of fish byproducts. Choi *et al*., (1985) reported that the viscera of catfish (*Parasilurus asotus*) had a higher lipid and polyunsaturated fatty acid content as compared to the muscle. Sathivel *et al*., (2002) reported that oil extracted from channel catfish (*Ictalurus punctatus*) byproducts including liver, gall bladder, digestive tract, and visceral storage fat had high amounts of unsaturated fatty acids same as the fillet and nugget. Since the fatty tissue and liver of patin catfish (*Pangasianodon hypophthalmus*) accumulate fat, they are potential sources for oil extraction. The aim of this study was to evaluate the fatty acid composition and nutritional value of these by products which is fund a mentally important for the fish oil industry and production of value added products such as foods fortified with fish oil.

Materials and methods

Sample preparation

Two batches of fresh female *P. hypophthalmus* catfish (each batch containing five fish) and two batches of fresh males (each batch containing five fish) were obtained from a wholesale market (Pasar Borong, Selangor, Malaysia). Each fish were individually weighed. Liver and fatty tissues were separated from the whole fish and weighed. Each tissue was individually ground and stored at -18°C until analyzed.

Proximate analysis

Lipid content of the liver and fatty tissue of male and female patin catfish was extracted according to the method of Bligh and Dyer (1959). Rotary evaporator was used to remove solvents from oil. The total lipid content was determined

gravimetrically (n=2, a=3). The moisture content was determined according to AOCS (1998) by measuring the sample weight loss. Samples were weighed before and after drying in an oven at 105°C, when a constant weight was achieved (n=2, a=3). The protein content of the liver and fatty tissue was determined. The total nitrogen was determined by Kjeldahl method according to AOCS (1998). Percentage of protein was calculated as N × 6.25 (n=2, a=3).

Fat extraction

Each sample (10 g) was placed in a screw capped test tube and 37.5 ml 1:2 (v/v) $CHCl_3$: Me OH was added and vortexed, followed by adding 12.5 ml and vortexed. Finally, 12.5 ml distilled water was added and vortexed well. Centrifuging the mixture was done at 1000 rpm for 5 min at room temperature which gave two phase system including aqueous phase at the top and organic phase at the bottom of the tube. The organic phase was withdrawn through a pasture pipette, carefully. Fat extraction was done twice for each batch.

Esterification of fatty acids

Fatty acid methyl esters (FAMEs) were prepared according to the AOCS (1998). Extracted oil from each sample was placed into a 50 mL reaction flask, separately. Four ml of methanolic sodium hydroxide (2 g of NaOH dissolved in 100 ml of methanol), and 10 boiling chips were added to the flask. The condenser was attached to the flask. Five ml of boron trifluoride was added to the mixture and refluxed for 12 min. The esterified fatty acids were removed from the mixture by addition of 5 ml of heptane and refluxing for 1 min. Then, the mixture was cooled to room temperature. A saturated solution of NaCl was added steadily and mixed well until the heptane solution containing FAMEs reached the neck of the flask. The heptane containing FAMEs was recovered and anhydrous sodium sulfate (1.5 g) used for dehydration. Dry heptane solution was then used for analysis by Gas Chromatography (GC). GC injection was done twice for each sample.

Fatty acid analysis by GC

The FAMEs were analyzed with a GC Varian; model 3400. The column was DB-23 with the following dimensions: 60 m long, 0.32 mm ie with 0.25 µm phase thickness (J and W). One micro liter of esterified fatty acids was injected. The injector temperature was 220°C. The head pressure was set at 2 psi. The carrier gas was nitrogen, and the makeup gas was helium. The GC was equipped with flame ionization detector (FID). The detector temperature was 260°C. The temperature program was regulated at 100°C for 2 min, then 180°C at 10°C/min for 5 min, and at last 220°C at 3°C/min for 10 min. The fatty acids were identified by retention times obtained from the FAMEs standards (Sigma Company, St. Louis, MO). GC injections were conducted twice for each sample.

Statistical analysis

All data were statistically analyzed using a SPSS program (version 16.0). Homogeneity of data was tested using Levene's test. Fatty acid composition data were arcsine transformed before statistical

analysis. A Tukey's post-hoc test was done together with an ANOVA to find the significant difference ($p<0.05$) between the means of the results.

Results

The total weight and weight of liver and fatty tissue of female and male *P. hypophthalmus* are shown in Table 1. The total weight of the liver and fatty tissue of male and female patin catfish was 119.04 and 144.88 g, respectively, which was approximately 4.21 and 4.45% by weight of a whole male and female fish, respectively.

Table 1: Total length, total weight, and weight of liver and fatty tissue of female and male *P. hypophthalmus.*

Catfish	Total Weight (g)	Liver Weight (g)	Fatty tissue Weight (g)
Female	3250±1.55	26.79±1.53	118.09±1.32
Male	2825±1.05	20.43±1.60	98.61±1.16

The protein, fat, and moisture content of fatty tissue and liver of male and female catfish are shown in Table 2. Total fat contents of the fatty tissue and liver of male fish were 73.23 and 9.59%, respectively, whereas in female fish were 77.64 and 11.71%, respectively. The total fat content of fatty tissue and liver of female fish was significantly ($p<0.05$) higher than that of similar tissues in male fish. The protein and moisture content of the liver was significantly ($p<0.05$) higher than that of the fatty tissue.

Table 2: Protein, fat, and moisture content of fatty tissue and liver of male and female *P. hypophthalmus.*

Patin catfish body constituent (%)	Fatty tissue		Liver	
	Male	Female	Male	Female
Protein	2.14 ± 0.02^{c}	1.75 ± 0.02^{d}	13.49 ± 0.01^{a}	12.70 ± 0.01^{b}
Fat	73.23 ± 0.01^{b}	77.64 ± 0.01^{a}	9.59 ± 0.02^{d}	11.71 ± 0.03^{c}
Moisture	24.61 ± 0.01^{c}	18.81 ± 0.02^{d}	72.53 ± 0.02^{b}	73.69 ± 0.01^{a}

Note: Values with the same superscripts within a row are not significantly different at $p>0.05$.

The fatty acid compositions of the fatty tissue and liver of male and female *P. hypophthalmus* are shown in Table 3.The major fatty acids presented in these tissues were palmitic (C16:0), oleic (C18:1n-9), and linoleic acid (C18:2 n-6). The total amount of unsaturated fatty acids (USFA) of liver of female and male patin catfish were 51.02 and 50.55 %, respectively, whereas the total amounts of USFA of fatty tissues were 50.46 and 50.31%, respectively. The total proportions of USFA were not significantly different between various tissues (p>0.05)and two sexes as well as the total amounts of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). The total amounts of polyunsaturated fatty acids (PUFA) of liver were significantly higher than that of the fatty tissue (p<0.05).In *P. hypophthalmus* the amount of eicosapentaenoic acid (EPA, C20:5 n-3) were not significantly different between various tissues (p>0.05)and two sexes, whereas the amount of docosahexaenoic acid (DHA, C22:6 n-3) of the liver from male and female catfish were significantly higher than that of fatty tissue (p<0.05).

Table 3: Fatty acid composition of fatty tissue and liver of male and female *P. hypophthalmus*.

Fatty acid (%)	Fatty tissue		Liver	
	Male	Female	Male	Female
C12:0	0.51±0.02^{b}	0.72±0.09^{a}	0.56±0.04^{b}	0.51±0.01^{b}
C14:0	4.57±0.15^{b}	4.57±0.10^{b}	3.88±0.05^{c}	5.69±0.20^{a}
C16:0	31.96±0.13^{a}	31.79±0.59^{a}	31.45±0.58^{a}	32.10±0.38^{a}
C18:0	8.52±0.11^{ab}	8.73±0.55^{a}	7.78±0.57^{b}	5.49±0.13^{c}
C20:0	0.79±0.02^{b}	0.87±0.05^{b}	0.80±0.05^{b}	1.07±0.01^{a}
Total SFA	46.36±0.09^{a}	46.67±0.78^{a}	44.44±0.90^{a}	44.86±0.56^{a}
C14:1 n-7	1.03±0.10^{a}	1.04±0.11^{a}	1.19±0.31^{a}	1.34±0.02^{a}
C16:1 n-7	3.72±0.10^{bc}	3.54±0.15^{c}	4.10±0.40^{b}	5.44±0.05^{a}
C18:1 n-9	29.42±0.65^{a}	30.11±0.12^{a}	27.42±0.15^{b}	21.98±0.14^{c}
C18:1 n-7	2.82±0.07^{b}	2.41±0.22^{b}	3.19±0.73^{b}	7.74±0.16^{a}
C20:1 n-9	1.43±0.01^{a}	1.27±0.03^{c}	1.35±0.06^{b}	1.23±0.01^{c}
Total MUFA	38.67±0.11^{a}	38.37±0.23^{a}	37.24±0.95^{a}	37.73±0.12^{a}
C18:2 n-6	4.71±0.07^{ab}	5.16±0.15^{a}	4.31±0.45^{b}	4.23±0.05^{b}
C18:3 n-3	0.71±0.03^{a}	0.63±0.04^{b}	0.44±0.01^{c}	0.72±0.02^{a}
C18:4 n-3	1.64±0.06^{b}	1.80±0.02^{a}	1.51±0.03^{c}	0.96±0.01^{d}
C20:4 n-6	0.96±0.01^{b}	1.03±0.14^{b}	1.45±0.26^{a}	0.85±0.02^{b}
C20:4 n-3	1.83±0.05^{c}	1.68±0.07^{c}	3.20±0.25^{b}	4.07±0.04^{a}
C20:5 n-3	0.93±0.06^{a}	0.96±0.02^{a}	0.92±0.03^{a}	0.97±0.01^{a}

C22:6 n-3	0.88±0.05[b]	0.84±0.02[b]	1.49±0.09[a]	1.49±0.02[a]
Total PUFA	11.64±0.08[b]	12.09±0.11[b]	13.31±0.90[a]	13.30±0.08[a]

Note: Values with the same superscripts within a row are not significantly different at $p>0.05$.

The n-3 to n-6 ratios of fatty tissue and liver of male and female patin catfish are shown in Table 4. The total amount of n-3 fatty acids (that included C18:3 n-3,C18:4 n-3, C20:4 n-3, C20:5 n-3, and C22:6 n-3) were significantly higher in the liver of female fish followed by liver of male, fatty tissue of male, and fatty tissue of female fish ($p<0.05$). The total n-6 fatty acids (that included C18:2 n-6, and C20:4 n-6) were significantly ($p<0.05$) higher in female fatty tissue, whereas female liver contained the lowest amount. The n-3 to n-6 ratio of the liver of female fish was relatively higher than that of males, whereas the n-3 to n-6 ratios of the fatty tissue of male fish was higher than that of females.

Table 4: The total n-3 fatty acids, n-6 fatty acids, and n-3/ n-6 ratios of fatty tissue and liver of male and female *P. hypophthalmus.*

Catfish tissue	**Fatty tissue**		**Liver**	
	Male	**Female**	**Male**	**Female**
Total n-3 fatty acids (%)	5.99±0.04[c]	5.91±0.13[c]	7.55±0.25[b]	8.21±0.06[a]
Total n-6 fatty acids (%)	5.68±0.07[ab]	6.18±0.17[a]	5.76±0.66[ab]	5.09±0.07[b]
n-3/ n-6 ratio	1.05	0.95	1.31	1.61

Note: Values with the same superscripts within a row are not significantly different at $p>0.05$.

Discussion

Despite the higher lipid content of the fatty tissue, the liver contained more health beneficial due to greater amount of PUFA. The most desirable organ for oil extraction is the one with a high oil content (Zuta *et al*., 2003). Therefore, the fatty tissue of patin is a better organ for oil extracti because of its higher fat content as compared to the liver. Abdi *et al*.(2011) reported that the protein content of the liver of Asian red tail catfish (*Hemibagrus* (C16:0). Ackman (1967) pointed out that the total proportions of C16 fatty acids and polyunsaturated C18 fatty acids were higher in fresh water fish oil than in marine oil. Linoleic acid (C18:2 n-6) was *nemurus*) and African catfish (*Clarias gariepinus*) were higher than the total fat content which is in accordance with our findings. Sathivel *et al*. (2002) reported that an inverse relationship existed between the fat and moisture content of the fatty tissue and liver of channel catfish, which was in accordance with our findings on patin catfish fatty tissue.

The major saturated fatty acid found in the fatty tissue and liver of male and female patin catfish was palmitic acid the dominant polyunsaturated fatty acids of the liver and fatty tissue of patin catfish. Linoleic acid is an essential fatty acid and the predominance of it is closely related to catfish diet (Satoh *et al*., 1989). Ng et al.

(2003) evaluated the effect of diet on the fatty acid profile of African catfish (*C.gariepinus*). They found that palmitic acid (C16:0), which was presented in high amount in muscle, was not related to the fish diet, where as the amount of n-3 fatty acids were affected by dietary lipids as well as n-3 to n-6 ratios of fatty acids. The extracted oil from the liver and fatty tissue of male and female patin catfish was characterized by more than 50% of unsaturated fatty acids.

The total amounts of polyunsaturated fatty acids and n-3 fatty acids of the liver were significantly (p<0.05) higher than that of fatty tissue.N-3 fatty acids exert beneficial effects on human health. N-3 fatty acids are the precursors of eicosanoids (prostacyclins, prostaglandins, leukotrienes, and thromboxanes) with lower activity than the ones constructed from omega-6 fatty acids (Simopoulos, 2002). Polyunsaturated n-3 fatty acids compete with n-6 fatty acids in the metabolic pathway and consolidating in the cell membrane which influences cellular function and improves the profile of plasma lipids. This reduces the risk of inflammation, high blood pressure, and blood clotting (Simopoulos, 1991).The n-3 to n-6 ratios of oils extracted from the liver and fatty tissue of male and female patin catfish were relatively higher than that reported on the oil extracted from the muscle of some Malaysian freshwater fish such as baung, rohu, and big head carp that were 0.11, 0.18, and 0.43, respectively (Rahman *et al*., 1995).

The n-3 to n-6 ratios of various tissues of two sexes were in this order: liver of female>liver of male>fatty tissue of male>fatty tissue of female. The ratio of n-3 to n-6 fatty acids has been suggested as an important determinant of human health. Recommended adequate intakes (AI) ratio of dietary n-3 to n-6 fatty acids is between 0.5 to 1 (Simopoulos, 2002).The n-3 to n-6 ratios of all extracted oil from the liver and fatty tissue of female and male *P. hypophthalmus* were higher than 1.00. Therefore these byproducts can be considered as valuable sources of fish oil extraction.

References

Abdi, H., Christianus, A., Ramezani-Fard, E., Saad, C.R. and Hosseini, S.A., 2011. Proximate and fatty acid composition of the liver of cultured Asian redtail catfish (*Hemibagru snemurus*) and African catfish (*Clarias gariepinus*). *Journal of Fisheries and Aquatic Science*,6(**7**), 840-845.

Ackman, R.G., 1967. Characteristics of the fatty acid composition and biochemistry of some fresh-water fish oils and lipids in comparison with marine oils and lipids. *Comparative Biochemistry and Physiology*, 22, 907-922.

Ackman, R. G., 1982. Fatty acid composition of fish oil.In Nutritional evaluation of long-chain fatty acids in fish oil. Barlow, S. M., and Stans by, M. E., pp. 25-88. New York: Academic Press, USA.

AOCS, 1998. Official methods and recommended practices of the American Oil Chemists' Society.5th ed. Illinois, Champaign, USA.

Bang, H. O., Dyerberg, J. and Nielsen, A. B., 1971. Plasma lipid and lipoprotein pattern in Greenlandic West-coast Eskimos. *Lancet*, 297(**7710**), 1143-1145.

Bligh, E.G., and Dyer, W.J., 1959. A rapid method of total lipid extraction and purification.*Canadian Journal of Biochemistry and Physiology*, 37, 911-917.

Bourre, J. M., 2007. Dietary omega-3 fatty acids for women. *Biomedicine and Pharmacotherapy*, 61(**2-3**), 105-112.

Calder, P. C., 2001. Polyunsaturated fatty acids, inflammation and immunity. *Lipids*, 36(**9**),1007-1024.

Choi, J. H., Park, S. H., Ro, J. I. and Pyeun, J. H., 1985. Distribution of lipid components in various tissues of catfish *Parasilurus asotus*. *Korean Journal of Food Science and Technology*, 17(**1**), 15-21.

Cordier, M., Brichon, G., Weber, J.M. and Zwingelstein, G., 2002. Changes in the fatty acid composition of phospholipids in tissues of farmed sea bass (*Dicentrarchus labrax*) during an annual cycle.Roles of environmental temperature and salinity.*Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology*, 133(**3**), 281-288.

Hafezieh, M., Mohd Salah Kamarudin, S., Che Rose Bin Saad.,Mostafa Kamal AbdSattar., Agh, N., Valinassab, T., Sharifian, M. and Hosseinpour, H., 2010. Effects of enriched *Artemiaurmiana*with HUFA on growth, survival, and fatty acids composition of the Persian sturgeon larvae (*Acipenser persicus*).*Iranian Journal of Fisheries Sciences*, 9(**1**), 61-72.

Heard, C.M., Gallagher, S.J., Harwood, J. and Maguire, P.B., 2003. The in vitro delivery of NSAIDs across skin was in proportion to the delivery of essential fatty acids in the vehicle-evidence that solutes permeate skin associated with their solvation cages? *International Journal of Pharmaceutics*, 261(**1-2**), 165-169.

Kaur, N., Chugh, V. and Gupta, A. K., 2012. Essential fatty acids as functional components of foods- a review. *Journal of Food Science and Technology*, DOI 10.1007/s13197-012-0677-0.

Nakamura, Y.N., Ando, M., Seoka, M., Kawasaki, K. and Tsukamasa, Y., 2007. Changes of proximate and fatty acid compositions of the dorsal and ventral ordinary muscles of the full-cycle cultured Pacific bluefin tuna

Thunnus orientalis with the growth. *Food Chemistry*, 103(**1**), 234-241.

Ng, W.K., Lim, P.K. and Boey, P.L., 2003. Dietary lipid and palm oil source affects growth, fatty acid composition and muscle α-tocopherol concentration of African catfish, *Clarias gariepinus*. *Aquaculture*, 215(**1-4**), 229-243.

Nisa, K. and Asadullah, K., 2011. Seasonal variation in chemical composition of the Indian mackerel (*Rastrelliger kanagurta*) from Karachi Coast. *Iranian Journal of Fisheries Sciences*, 10(**1**), 67-74.

Rahman, S.A., Huah, T.S., Nassan, O. and Daud, N.M., 1995. Fatty acid composition of some Malaysian freshwater fish. *Food Chemistry*, 54(**1**), 45-49.

Reyes, J. F. and Sepúlveda, M. A., 2006. PM-10 emissions and power of a diesel engine fueled with crude and refined biodiesel from salmon oil. *Fuel*, 85(**12-13**), 1714-1719.

Sathivel, S., Prinyawiwatkul, W., Grimm, C.C., King, J.M. and Lioyd, S.,2002. FA composition of crude oil recovered from catfish viscera. *Journal of the American Oil Chemists' Society*, 79(**10**), 989-992.

Satoh, S., Poe, W.E. and Wilson, R.P. ,1989. Effect of dietary n-3 fatty acids on weight gain and liver polar fatty acid composition of fingerling channel catfish. *The Journal of Nutrition*, 119(**1**), 23-28.

Shirai, N., Suzuki, H., Tokairin, S., Ehara, H. and Wada, S., 2002. Dietary and seasonal effects on the dorsal meat lipid composition of Japanese (*Silurus asotus*) and Thai catfish (*Clarias macrocephalus* and hybrid *Clarias macrocephalus* and *Clarias galipinus*). *Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology*, 132(**3**), 609-619.

Simopoulos, A.P., 1991.Omega-3 fatty acids in health and disease and in growth and development. *The American Journal of Clinical Nutrition*, 54(**3**), 438-463.

Simopoulos, A.P., 2002.The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine and Pharmacotherapy*, 56(**8**), 365-379.

Zuta, C.P., Simpson, B.K., Chan, H.M. and Phillips, L., 2003. Concentrating PUFA from mackerel processing waste. *Journal of the American Oil Chemists' Society*, 80(**9**), 933-936.