Refined oil production from patin catfish (*Pangasianodon hypophthalmus*) by-products

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Received: July 2014 Accepted: December 2015

Abstract

In this study, oil was extracted from the liver and visceral fat of Patin (*Pangasianodon hypophthalmus*) and refined. The yield of oil after refining was 49.98%. The major yield loss (34.20%) happened during the degumming procedure. Fatty acids found in the crude and refined oil 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 dominant saturated, monounsaturated and polyunsaturated fatty acids were C16:0, C18:1 n-9, and C18:2 n-6, respectively. The total amounts of monounsaturated fatty acids did not change significantly during refining procedure ($p>0.05$), whereas the total amount of saturated and polyunsaturated fatty acids changed significantly ($p<0.05$). The n-3 to n-6 ratios of crude, degummed, neutralized, bleached, and deodorized oils were 1.11, 1.06, 1.05, 1.02, and 1.01, respectively.

Keywords: Byproduct, Fish oil refining, Fatty acid composition, n-3 fatty acids

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Introduction

An increased interest in consumption of fish oil was initiated mainly by findings of Bang *et al*. (1971), who found that the Eskimos diet rich in polyunsaturated fatty acids was the cause of low amounts of pre β-lipoprotein, plasma triglycerides, cardiovascular diseases, and absence of diabetes mellitus in that population. Today, fish oil is used in different industries. In food industry, consumer acceptance of foods fortified with fish oil (Hughes *et al*., 2012) and n-3 fatty acids have been investigated (Borneo *et al*., 2007). Various examples of foods which are enriched with n-3 fatty acids are available in the market such as bread and bakery products, milk and dairy products, spreadable fats, eggs and egg products, meat and poultry products, juices, and soft drinks (Kolanowski and Laufenberg, 2006). Fish oil is also used for the production of lubricants, surfactants, protective coatings, sealants, and inks (Gruger, 1967). Useful polymers can be produced from fish oil (Li *et al*., 2000). Lin and Li (2009) produced biodiesel using transesterified refined fish oil, which was extracted from discarded marine byproducts.

Byproducts from fish processing may reach to more than half of the total weight of a live fish (Sathivel *et al*., 2002). Sathivel *et al*. (2002) reported that channel catfish viscera including fatty tissue, liver, gallbladder and digestive tract contained health promoting fatty acids. Abdi *et al*. (2011) reported that the liver of Asian redtail catfish (*Hemibagrus nemurus*) and African catfish (*Clarias gariepinus*) were good sources of protein and unsaturated fatty acids.

Patin catfish (*P. hypophthalmus*) is one of the most common catfish species that contribute to Malaysian diet. The muscle and ovaries of Patin catfish are consumed while the visceral fat and liver are discarded. Disposal of these wastes incur cost and environmental pollution. These tissues accumulate fat; therefore, they are potential sources of extractable fish oil. Crude fish oil is a mixture of several compounds such as glycerides, sterols, phospholipids, pigments, tocopherols, free fatty acids, and lipid oxidation products thus refining needs to be done to decrease impurities (Young, 1982). Refining steps include degumming, neutralizing, bleaching, and deodorizing. The soluble and insoluble impurities are removed during degumming. Neutralization eliminates free fatty acids (FFA) and bleaching removes pigments, aldehydes, ketones, trace metals, sulfurous compounds, and soap. The remaining aldehydes, ketones, and FFA, which are responsible for unacceptable odor and flavor, are removed during deodorization (Young, 1982).

The fatty acid composition of fish byproducts have been studied (Kim and Mendis, 2006; Effiong and Fakunele, 2013), but little attention has been paid to catfish wastes and the application of refining procedure for the production of edible oil. Refined Patin catfish oil is a new product that has not yet been produced. Therefore, the objective of this study was to produce refined oil from the liver and visceral fat of *P. hypophthalmus* and to evaluate the quality of oil at various refining steps.

Materials and methods

Sample preparation

Twenty fresh *P. hypophthalmus* catfish (including ten males and ten females) were obtained from a wholesale market (Pasar

Borong, Selangor, Malaysia). Liver and fatty tissues were separated from the whole fish. Each tissue was individually ground and stored at -18°C until analyzed.

Fatty acid analysis

Fat were extracted according to the method of Sathivel *et al*. (2003). For the production of 200 g mixed samples, 18 and 82 g of liver and fatty tissue of females and 17 and 83 g of liver and fatty tissue of males were taken, respectively, based on their weight ratio in the fish. Water (water/ground tissues, 5:1 v/w) was added to the mixture of samples and heated to 70°C for 15 min. Cheese cloth was used for separation of solid particles from liquid. The remaining solid particles and water were separated from oil by centrifuging at 5000 rpm for 30 min. Four batches of mixed samples were prepared and two crude oil extractions conducted for each batch. Fatty acid methyl esters (FAMEs) were prepared according to the American Oil Chemists' Society Official Method Ce 1b-89 (AOCS, 1998). Extracted oil from each sample was placed into 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 happened 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, 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). The FAMEs were analyzed with a GC Varian, model 3400. Column was DB-23 with the following dimensions: 60 m long, 0.32 mm i.d. with 0.25 µm phase thickness (J&W). One micro liter of esterified fatty acids was injected. Injector temperature was 220 °C. The head pressure was set at 2 psi. Carrier gas was nitrogen, and make up gas was helium. GC was equipped with flame ionization detector (FID). Detector temperature was 260 °C. 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, St. Louis, Mo, USA). GC injections were conducted twice for each sample.

Refining procedure

Four batches of crude catfish oil were prepared and refining procedure was done twice for each batch. Degumming was done following the modified method of Dijkstra and Opstal (1989). Around 100 g crude catfish oil was removed from frozen storage, and placed in a 600 mL beaker. Fish oil was heated to 70 °C in a temperature controlled water bath. Three mL of 3% aqueous citric acid solution was added, and thoroughly mixed with fish oil at 70 °C for 1 min. The oil was cooled at room temperature. Precipitated gum was removed by centrifuging at 5000 rpm for 10 min. Neutralizing was done following the AOCS Official Method Ca 9a-52 (AOCS, 1998). Sodium hydroxide (12.6 g of 9.5% NaOH solution) was added to the 100 g degummed fish oil and heated to 65 °C for 30 min with constant stirring with a magnetic stirrer. The mixture was cooled at room temperature

and kept undisturbed for 6 h. Then, after centrifuging at 5000 rpm for 10 min, the oil was decanted from the precipitated soap. Around 50 mL of demineralized water was added to wash out any remaining soap. Washing the sample was repeated three times. Impurities and water was removed by centrifuging at 5000 rpm for 10 min. Bleaching was done following the method of Scott and Latshaw (1991). The neutralized oil was heated in a water bath. At 70 °C, activated earth (CS Z1077, American Oil Chemists' Society, Champaign, IL, USA) with the amount of 4% (w/w) was added to the oil sample and stirred using a magnetic stirrer for 10 min. The activated earth with absorbed impurities was removed by centrifuging at 5000 rpm for 30 min. Deodorizing was done using a laboratory deodorization unit which was prepared according to the method of Baldwin (1948). The deodorization unit was made from two 500 mL round bottom flasks. The bleached catfish oil (100 mL) was added to the flask and heated in a water bath to 100°C for 30 min under vacuum. The flask had one outlet and one inlet. The outlet was connected to a distillation unit and a vacuum pump. The inlet was connected to the 500 mL round bottom flasks containing 400 mL water that was heated by a heating system. The temperature was manually controlled. The volatile products were condensed in a cooling system that was installed on the vacuum line and the deodorized oil was collected from the flask.

Statistical analysis

All data was analyzed using a SPSS program (version 16.0). Data sets of fatty acid composition were transformed to arcsine values before statistical analysis. Post hoc Tukey's test was done in conjunction with an ANOVA to find the significant difference (p<0.05) between the means of the results.

Results

The quantity of oil produced from each refining step is shown in Table 1. An average of 200 g of mixed sample of liver and visceral fat was required to produce 100 g of crude Patin catfish oil. About 49.98 g of refined oil was produced from 100 g of crude *P. hypophthalmus* oil.

Table 1: Quantity of oil produced from each refining step.

	Refining steps			
	Degummed	**Neutralized**	**Bleached**	**Deodorized**
Yield of oil (g)	65.8 ± 0.12	55.52 ± 0.09	51.23 ± 0.04	49.98 ± 0.03

Based on an initial 100 g of crude oil

Detailed fatty acid compositions of catfish oil from each refining step are shown in Table 2. The total amount of saturated fatty acids (SFA) of crude, degummed, neutralized, bleached, and deodorized Patin catfish oils were 46.89, 47.34, 47.47, 47.64, and 48.11%, respectively. The amount of saturated fatty acids in deodorized oil were significantly (p<0.05) higher than that of crude, degummed, neutralized, and bleached oils, respectively. Palmitic acid (C16:0) was dominant among saturated fatty acids and accounting for 69.13% of all saturated fatty acids in deodorized oil.

The total unsaturated fatty acids of patin catfish oil amounted to 50.12, 50.01, 49.89, 49.71, and 49.06% for crude, degummed, neutralized, bleached, and deodorized oils,

respectively. Oleic acid (C18:1 n-9) was dominant among monounsaturated fatty acids (MUFAs), whereas linoleic acid (C18:2 n-6) was dominant among polyunsaturated fatty acids (PUFAs). In deodorized catfish oil, oleic acid, linoleic acid, EPA, and DHA accounting for 58.68, 9.76, 1.69, and 1.61% of all unsaturated fatty acids, respectively, whereas in crude catfish oil accounting for 57.72 9.38, 1.90, and 1.96%, respectively. The total amounts of MUFAs of crude, degummed, neutralized, bleached, and deodorized Patin catfish oil were 38.13, 38.16, 38.12, 38.02, and 37.75%, respectively, whereas the total amounts of PUFAs were 11.99, 11.85, 11.78, 11.69, and 11.32%, respectively. The total amounts of MUFAs of oil at different refining steps was not significantly changed (p>0.05), whereas the total amount of PUFAs of deodorized oil were significantly (p<0.05) lower than that of crude, degummed, neutralized, and bleached oil, respectively.

Table 2: Fatty acid composition from each refining step.

	Refining steps				
Fatty acid (%)	**Crude**	**Degummed**	**Neutralized**	**Bleached**	**Deodorized**
C12:0	0.56 ± 0.17^{a}	0.51 ± 0.01^{ab}	0.49 ± 0.01^{b}	0.53 ± 0.01^{ab}	0.52 ± 0.04^{ab}
C14:0	4.56 ± 0.04^{a}	4.49 ± 0.02^{ab}	4.39 ± 0.01^{c}	4.42 ± 0.03^{bc}	4.55 ± 0.04^{a}
C14:1n-7	0.95 ± 0.02^{a}	0.94 ± 0.01^{ab}	0.92 ± 0.01^{ab}	0.90 ± 0.01^{b}	0.84 ± 0.03^{c}
C16:0	32.63 ± 0.45^{b}	32.93 ± 0.06^{ab}	33.04 ± 0.03^{ab}	33.14 ± 0.11^{a}	33.26 ± 0.19^{a}
C16:1n-7	3.79 ± 0.12^{a}	3.93 ± 0.01^{a}	3.86 ± 0.01^{a}	3.90 ± 0.02^{a}	3.87 ± 0.10^{a}
C18:0	8.27 ± 0.04^{c}	8.56 ± 0.03^{b}	8.56 ± 0.02^{b}	8.56 ± 0.05^{b}	8.67 ± 0.06^{a}
C18:1n-9	28.93 ± 0.02^{a}	28.94 ± 0.02^{a}	28.92 ± 0.01^{a}	28.90 ± 0.13^{a}	28.79 ± 0.31^{a}
C18:1n-7	3.13 ± 0.02^{ab}	3.11 ± 0.01^{b}	3.16 ± 0.02^{a}	3.10 ± 0.01^{b}	3.06 ± 0.02^{c}
C18:2n-6	4.70 ± 0.07^{c}	4.78 ± 0.03^{bc}	4.80 ± 0.01^{b}	4.92 ± 0.01^{a}	4.79 ± 0.05^{bc}
C18:3n-3	0.67 ± 0.01^{a}	0.65 ± 0.01^{ab}	0.63 ± 0.02^{bc}	0.64 ± 0.02^{abc}	0.61 ± 0.01^{c}
C18:4n-3	1.65 ± 0.03^{a}	1.56 ± 0.01^{b}	1.54 ± 0.02^{bc}	1.51 ± 0.01^{cd}	1.47 ± 0.01^{d}
C20:0	0.88 ± 0.02^{c}	0.86 ± 0.02^{c}	0.99 ± 0.02^{b}	1.00 ± 0.03^{b}	1.11 ± 0.02^{a}
C20:1n-9	1.33 ± 0.02^{a}	1.25 ± 0.03^{b}	1.26 ± 0.02^{b}	1.23 ± 0.01^{bc}	1.19 ± 0.01^{c}
C20:4n-6	0.99 ± 0.01^{a}	0.96 ± 0.03^{a}	0.95 ± 0.02^{a}	0.88 ± 0.01^{b}	0.84 ± 0.01^{b}
C20:4n-3	2.06 ± 0.08^{a}	2.08 ± 0.02^{a}	2.08 ± 0.02^{a}	2.03 ± 0.04^{a}	1.98 ± 0.05^{a}
C20:5n-3	0.95 ± 0.02^{a}	0.90 ± 0.02^{ab}	0.88 ± 0.01^{bc}	0.86 ± 0.02^{bc}	0.83 ± 0.02^{c}
C22:6n-3	0.98 ± 0.02^{a}	0.92 ± 0.02^{b}	0.90 ± 0.01^{b}	0.88 ± 0.01^{b}	0.79 ± 0.01^{c}

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

The n-3 to n-6 ratios of catfish oils are shown in Table 3. The n-3 to n-6 ratios of Patin catfish oil at different refining step was higher than 1.

Table 3: The total n-3 fatty acids, n-6 fatty acids, and n-3/ n-6 ratios of *P. hypophthalmus* oil from each refining step

Fish oil	**Crude**	**Degummed**	**Neutralized**	**Bleached**	**Deodorized**
Total n-3 fatty acids (%)	6.31 ± 0.05^{a}	6.11 ± 0.06^{b}	6.03 ± 0.07^{bc}	5.91 ± 0.11^{c}	5.68 ± 0.10^{d}
Total n-6 fatty acids (%)	5.69 ± 0.07^{ab}	5.74 ± 0.06^{ab}	5.75 ± 0.03^{ab}	5.79 ± 0.01^{a}	5.64 ± 0.06^{b}
n-3/ n-6 ratio	1.11	1.06	1.05	1.02	1.01

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

The total amount of n-3 fatty acids (combined C18:3 n-3, C18:4 n-3, C20:4 n-3, C20:5 n-3, and C22:6 n-3) of deodorized Patin catfish oil accounted for 5.68%, which was significantly (p<0.05) lower than that of crude oil. The total n-6 fatty acids

(combined C18:2 n-6, and C20:4 n-6) in the deodorized Patin catfish oil was relatively lower than those in bleached, neutralized, degummed, and crude catfish oil, respectively.

Discussion

Zuta *et al*. (2003) pointed out that the best fish material for extraction of oil is the one with high oil content. The yield of oil from the mixture of fatty tissue and liver of Patin catfish (50%) was higher than that reported from the skin (38.1%), muscle (9.2%), and viscera (9.18) of mackerel. Sathivel *et al*. (2003) reported that the yield of oil from the whole channel catfish viscera was 25.87%. They mentioned that the major refining weight loss of oil (11.98%) took place during the degumming process, which was similar to our findings (34.20%). Loss of oil and decreasing the amount of PUFAs are disadvantages of fish oil refining procedure (Rubio-Rodríguez *et al*., 2010). According to Aubourg *et al*. (1996) lowering the amount of PUFAs and increasing the amount of SFAs caused by heat treatment is due to the changes of fatty acid distribution in *sn*-1 and *sn*-2 positions. Palmitic, stearic, and oleic acids are found in *sn*-1 position, whereas linolenic acid, EPA, DHA, and arachidonic acid are found in *sn*-2 position. Aubourg *et al*. (1996) reported that during thermal treatment the composition of fatty acids was changed in both locations. They evaluated these changes in eight commercial species of tuna and found that there is an increase in the amount of SFAs in *sn*-1 position and a decrease in the amount of unsaturated fatty acids, especially PUFAs in *sn*-2 position.

The proportions of unsaturated fatty acids of Patin catfish oil were relatively higher than the amounts of SFAs which were in agreement with previous studies on the fatty acid profile of oil extracted from the giant catfish (Chaijan *et al*., 2010) and Asian catfish (Thammapat *et al*., 2010). Ng *et al*. (2003) reported that C12:0, C14:0, oleic acid (C18:1), linoleic acid (C18:2 n-6), and n-3 fatty acids such as linolenic acid (C18:3 n-3), EPA (20:5 n-3), DPA (C22:5 n-3), and Docosahexaenoic acid (DHA, 22:6 n-3) were influenced by dietary lipids, whereas palmitic acid (C16:0) of African catfish (*C. gariepinus*) muscle was irrespective of diet.

Fish can accumulate n-3 fatty acids from DHA and linolenic acid (C18:3 n-3) from diet (Satoh *et al*., 1989). According to FAO (2014) patin catfish is omnivorous; feeding on zooplankton, insects, algae, higher plants and large specimens can also take crustacean, fish, and fruit. Unsaturated fatty acids of fish oil exert beneficial effects on human health. MUFAs are effective for the prevention of metabolic syndrome (Garg, 1998; Ros, 2003). Madigan *et al*. (2005) reported that improvement in Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) in diabetic patients was observed by changing the diet containing polyunsaturated linoleic acid (C18:2 n-6) to monounsaturated oleic acid (C18:1 n-9) diet. PUFAs are effective against autoimmune diseases (Belch and Muir, 1998) and certain type of cancer (Gogos *et al*., 1998).

Total fatty acid content of crude and refined Patin catfish oil, cod liver oil, sardine oil, and menhaden oil are shown in Table 4. Cod liver oil, sardine oil, and menhaden oil are well-known commercial oils with marine origin. Differences between the Patin catfish oil and other commercial oils presented in Table 4 are due to the differences in diet (Osman *et al*.,

2001), fish habitat, water temperature (Cordier *et al*., 2002), and water salinity (Cordier *et al*., 2002). Sathivel *et al*. (2003) reported that the total n-3 fatty acids for deodorized channel catfish oil and menhaden oil were 4.5 and 20.8%, respectively.

Table 4: Total fatty acid content of Patin and three commercial fish oils.

Fatty acids (%)	Patin crude catfish oil	Patin refined catfish oil	Cod liver oil (Guil-Guerrero and Belarbi, 2001)	Sardine oil (Gámez-Meza *et al*., 1999)	Menhaden oil (Osman *et al*., 2001)
SFA	46.89	48.11	20.7	32.20	20.8
MUFA	38.13	37.75	43.34	25.21	19.43
PUFA	11.99	11.32	29.74	36.92	59.8

The total amount of n-3 fatty acids from each refining step was higher than the amount of n-6 fatty acids and it was in this order: crude > degummed > neutralized > bleached > deodorized. These results indicated that refined Patin oil is suitable to be used in various food industries and for human consumption. The n-3 and n-6 PUFAs are two families of essential fatty acids that cannot be synthesized by the human body and must be provided through foods (Kaur *et al*., 2012). Moreover, intake of a higher ratio of n-3 to n-6 fatty acids is more desirable for human health as it affects the gene expression (Simopoulos, 1996), prevention from many of the chronic diseases, cytokine production, and eicosanoid metabolism (Simopoulos, 2002).

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