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Fatty Acid Profile and Antioxidant Capacity of Muscle and by Product Oil from Selected Fresh Water Fish

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Abstract Profile fatty acid compositions were determined for the 3 species of fresh water fish from Kedah, Malaysia. The fat content composition of patin (Pangasius pangasius), tilapia (Oreochromis niloticus), and keli (Clarias batrachus) was 11.03%, 4.47 %, 9.28% in muscle and 15.33%, 8.91%, 17,16% of their by-products, respectively, whereas the fatty acid compositions consisted of 60.12% to 97.14% saturated fatty acids (SFA), 0.90% to 19.28% monounsaturated fatty acid (MUFA) and 1.17% to 18.35% polyunsaturated fatty acids (PUFAs). The by-product consisted of 60.47% to 69.63% SFA, 13.53 to 30.69% MUFA, and 5.67% to 21.51% PUFA. The major component of PUFA (C18:3 n3) were fish by-products (1.20%-8.16%) and in patin muscle (1.82%). Then for MUFA and PUFA of by-product were higher than muscle. Then, tilapia by product was highest PUFA (omega-3) content of all fish examined. Antioxidant capacities of fish examined muscles for keli, tilapia and patin were 79.38%, 29.89%, and 27.83% respectively. Then, the by-products were 58.76% of patin, 5.17% of tilapia and 27.83% of keli.

Keywords Fresh Water Fish, Muscle, By-Product, Fat, Fatty Acid, Antioxidant Capacity

1. Introduction

There are 614 species of freshwater fish in Malaysia (Eli and Lei, 2008). Among them, patin (*Pangasius pangasius*), tilapia (*Oreochromis niloticus*), and keli (*Clarias batrachus*) are commercially cultivated by local people there.

These species are popular consumed by Malaysian people. We know, there is a growing world market demand for high-quality fish oils, and commercial fish oil production can be quite profitable if suitable raw materials are available. One alternative for fish oil production is to use material from freshwater fish and by products. By using these materials, the season factor as a constraint in the supply of raw material from sea water fish in the fish oil production can be solved.

Portion of fish meat called as muscle is a bit relatively. Fish fillet production leaves by-products those are head, viscera (heart, lever, and guts), skin, bones, and fins (Laila et al., 2013). The by-products are usually thrown away or processed as animal feed. However, by-products of freshwater fish are potential as fish oil source. It has been reported that fish oil can be produced from catfish viscera (Sathivel *et al*., 2003; Latip *et al,* 2013).

Then, there is a potential of producing fish oil from by product (Aidos et al., 2003). Major by-product of Salmon and Pollack industries in the USA were 90,000 and 700,000 metric ton in 2000 (Crapo and Bechthel, 2003). Many species of marine fish have been studied for fish oil production, but little attention has been paid to the oil production potentially of muscle and by product of fresh water.

Variation of lipid composition among fish species is mainly affected by fish diet. Higher content of *n*3 fatty acids is found in herbivorous freshwater fish species that use phytoplankton for nutrition (Vujković *et al*., 1999). The fish have a potential antioxidant, according Bragadóttir (2001) the antioxidative agents found in fish. The potentially of natural antioxidant from fish, It is important to produce a natural fish oil.

Numerous system has been reported maintain pro and antioxidative balancing in fresh fish. One of the systems is inhibition by antioxidant compounds, which naturally occur in fish (Bragadóttir, 2001). The ability of an antioxidative compound prevent oxidative reaction is determined as antioxidant capacity.

The aim of the current study was to determine the fatty acid composition of the lipids of three freshwater species in their muscles and by-products and to determine antioxidant capacity of them.

2. Materials and Methods

2.1. Materials

Fish materials used in this work consisted of 3 (three) species of fresh water fish were patin (*Pangasius pangasius*), tilapia (*Oreochromis niloticus*), and keli (*Clarias batrachus*) in Kedah, Malaysia waters. The fish samples were kept frozen at -20 ^{0}C until analyzed.

2.2. Lipid Extraction

Amount of 100 ml of chloroform and 200 ml of methanol were added to 100 g of samples and homogenized for 2 min. Another 100 ml of chloroform were added again and followed by blending for 30 s. Then it was filtered and the residue re-extracted with 100 ml of chloroform for 30 s and filtered. The filtrates were combined, and 100 ml of distilled water were added and mixed well. The mixture was allowed in cold room for separation and the chloroform layer-containing lipid was collected. The solvent was evaporated using a rotary evaporator at 40°C (Bligh and Dyer, 1959). Total lipid content was determined gravimetrically.

2.3. Fatty Acid Analysis

Fatty acids methyl ester was prepared using the method of Mondello *et al.* (2006). Crude lipid extract (20 µl) from samples were added by 200 µL of borontrifluoride-methanol (20% BF_3) reagent and heated at 100°C for 30 min. After cooling, 200 µl of n-hexane and 800 µl of distilled water were added to the mixture. Then it was agitated manually for 1 min and centrifuged for 2 min. Approximately 100 µL of the upper n-hexane layer was transferred to a 150 µl glass insert for 2 ml vials after diluting the extracted hexane to obtain a suitable chromatographic response. Fatty acids were identified by comparing the retention times of fatty acid methyl ester (FAME) mixture with the standard of fatty acids. Two replicate GC analyses were performed and the results were expressed in GC area % as mean values. The fatty acid composition of freshwater fishes lipid triacyglyserol was directly analyzed using Gas Chromatography (GC) after methyl-esterification. One µL of each FAME sample was injected (split ratio 15:1) into a GC 17 A-SHIMADZU Gas Chromatography (Shimadzu Scientific Inc., USA) with flame ionization detector (GC-FID). The column used, A BPX 70 (SGE, Australia), was consisted of a 30 m x 0.32 mm fused silica capillary coated with 70% cyanopropyl polysilphenylene-siloxane of 0.25 µm film thickness with Hydrogen as the carrier gas at constant linear velocity (28 cm/s). The injector temperature was 250°C and the detector temperature was 280°C. The oven was programmed as follows: 80°C for 2 min, 5°C/min to 200°C for 10 min and 10°C/min to 230°C for a further 10 min. Chromatographic peaks were identified by comparing retention times with the PUFA standard.

2.4. Analysis of Antioxidant Capacity

Antioxidant capacity was determined by free radical DPPH (1,1-diphenyl-2-picryl-hydrazil radical-scavenging). Amount of 2 ml of acetic buffer (pH 5.5) and 3.75 ml of methanol were added to 200 µl of DPPH 3mM in methanol. The tube was vortexed then added with 50 µl of sample. Then it was put in water bath at 37°C for 30 min. Sample solution absorbance was measured using spectrophotometer (λ = 517 nm). Control-negative was also required which was made by following above step and replacing sample with methanol. Sample antioxidant capacity was expressed as percentage relative to control-negative absorbance. Percentage of sample antioxidant capacity was gotten by using formula: difference between sample and control-negative absorbance divided by control-negative absorbance, then multiplied by 100% (Zzaman *et al.,* 2013; Kubo *et al.*, 2002; Molyneaux, 2004).

3. Result and Discussion

3.1. Fat Content and Fatty Acid Profile

Fat content and fatty acid profiles of three freshwater fish are presented in Table 1. Total fat content of patin, tilapia, keli respectively were 11.03%, 4.47 %, 9.28% in muscle and 15.33%, 8.91%, 17,16% of their by-products, respectively. Fish can be grouped into four categories according to their fat contents: lean fish (<2 %), low fat (2-4 %), medium fat (4-8 %) and high fat (>8 %) (Ackman, 1989). The fresh water fish examined were medium and high fat. Higher fat content of by-products on viscera and skin. Endinkeau and Kiew (1993) have reported that Malaysian *Oreochromis spp.* and *Clarius spp.* had 11.01 and 12.96g/100g fillet of lipid contents, respectively. Fat content percentage of Basa catfish was reported 24.4% in muscle and 20.6% in viscera (Mai, 1998) then, channel catfish species had 33.6% fat in viscera (Sathivel *et al*., 2002). Furthermore, Tra catfish (*Pangasius hypophthalmus*) had 2.55% fat in muscle (Ho and Paul, 2009).

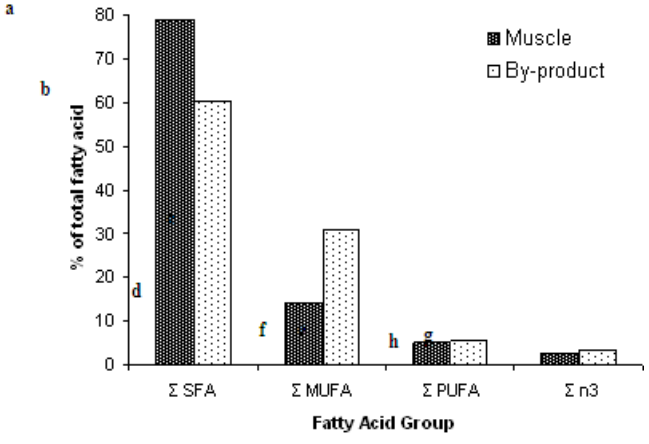

Figure 1. Fatty acid profile of patin (Pangasius pangasius)

Fatty acid profile of patin (*Pangasius pangasius*) is shown in Figure 1. The fatty acid compositions in muscle of three

freshwater fish species ranged from 60.12% to 97.14% saturated (SFA), 0.90% to 19.28% monounsaturated (MUFA), and 1.17% to 18.35% polyunsaturated (PUFA). In addition, their by-product consisted of 60.47% to 69.63% SFA, 13.53 to 30.69% MUFA, and 5.67% to 21.51% PUFA.

Fatty acids with high proportion in muscle and by-products were myristic acid (C14:0; 1.86%-4.82% and 2.06%-2.78%), palmitic acid (C16:0; 23.55%-46.80% and 21.98%-29.76%), palmitoleic acid (C16:1; 5.56%-12.05% and 4.01%-8.61%), stearic acid (C18:0; 23.98%-41.22% and 24.14%-42.63%), oleic acid (C18:1 *n*9; 5.62%-7.56% and 3.32%-26.28%), and linolenic acid (C18:3 *n*3; 1.82%-2.13% and 1.2%-8.16%). Fatty acid profile of tilapia (*Oreochromis niloticus*) and keli (*Clarias batrachus*) are shown in Figure 2 and 3.

Stearic acid (C18:0) was the primary SFA in muscle of tilapia (23.98%) and patin (41.22, then also similar to by-product of tilapia (26.96%) and keli (42.63%). In exception, patin by-product had palmitic acid (29.76%) as the highest SFA, also keli muscle (46.80%). The major components of MUFA were C16:1 and C18:1 *n*9, which values of varied among three fish species both in muscle and by-product. The highest C18:1 *n*9 were on by-product of patin and keli. The content of PUFAs was very low on three freshwater. The major component of PUFA (C18:3 *n*3) were on three fish by-products (1.20%-8.16%) and in patin muscle (1.82%). Fat content and fatty acid profiles of three freshwater fish are presented in Table 1

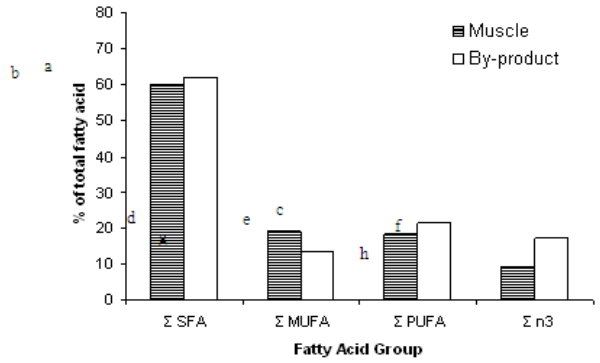

Figure 2. Fatty acid profile of tilapia (Oreochromis niloticus)

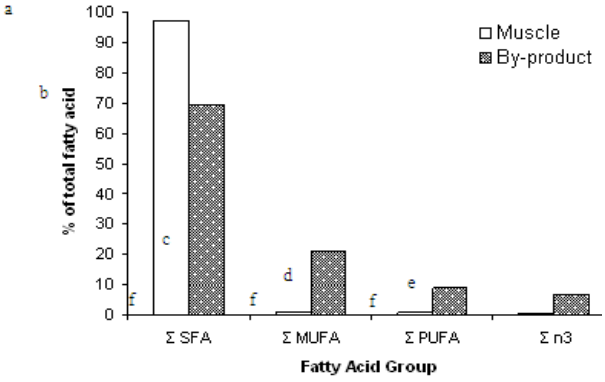

Figure 3. Fatty acid profile of keli (Clarias batrachus)

Table 1. Fat content and fatty acid profiles of three freshwater fish

%	Pangasius pangasius		Oreochromis niloticus		Clarias batrachus	
	Muscle	By-product	Muscle	By-product	Muscle	By-product
Fat Content	11.03±0.02	15.33±0.16	4.47±0.15	8.91±0.02	9.28±0.08	17.16±0.08
Fatty acid						
C 8:0	0.28±0.01	0.14±0.01	0.32±0.01	0.18±0.01	0.16±0.01	0.15±0.01
C 10:0	0.43±0.01	0.33±0.00	0.34±0.02	0.18±0.00	0.16±0.01	0.14±0.00
C 12:0	0.13±0.01	1.30±0.01	0.24±0.02	0.22±0.04	0.33±0.01	0.16±0.01
C 13:0	0.27±0.00	0.05±0.06	0.25±0.00	0.16±0.01	5.32±0.01	0.10±0.00
C 14:0	1.86±0.02	2.32±0.01	3.04±0.01	2.78±0.02	4.82±0.01	2.06±0.01
C 15:0	0.86±0.01	0.52±0.01	1.40±0.02	1.01±0.01	0.22±0.01	0.26±0.01
C 16:0	32.17±0.01	29.76±0.01	23.55±0.78	25.82±0.01	46.80±1.11	21.98±0.01
C 17:0	0.46±0.01	0.26±0.00	1.68±0.01	1.32±0.02	0.00±0.01	0.28±0.01
C 18:0	41.22±0.01	24.14±0.20	23.98±0.01	26.96±0.01	30.00±0.15	42.63±0.52
C 20:0	0.26±0.01	0.22±0.01	0.82±0.00	0.44±0.01	2.86±0.10	0.44±0.01
C 21:0	0.24±0.01	0.57±0.01	0.56±0.00	0.36±0.01	1.56±0.01	0.44±0.01
C 22:0	0.38±0.01	0.22±0.01	0.78±0.01	0.83±0.00	4.72±0.01	0.12±0.00
C 23:0	0.29±0.01	0.26±0.00	0.94±0.01	0.48±0.02	0.12±0.02	0.17±0.00
C 24:0	0.18±0.01	0.38±0.01	2.22±0.01	1.22±0.01	0.07±0.01	0.68±0.01
Σ SFA	79.03	60.47	60.12	61.98	97.14	69.63
C 14:1	0.46±0.01	0.26±0.01	0.52±0.02	0.96±0.01	0,02±0.01	0.06±0.01
C 15:1	0.25±0.00	0.12±0.01	0.43±0.01	0.32±0.01	0,20±0.03	0.04±0.01
C 16:1	5.56±0.01	4.01±0.00	12.05±0.07	8.61±0.13	0,01±0.00	5.38±0.01
C 17:1	0.13±0.01	0.02±0.01	0.66±0.01	0.32±0.01	0,67±0.03	0.18±0.01
C 18:1 n9	7.56±0.00	26.28±0.01	5.62±0.18	3.32±0.01	0,00±0.01	15.36±0.02
Σ MUFA	13.96	30.69	19.28	13.53	0.90	21.02
C 18:2 n6 cis	1.15±0.07	0.86±0.01	4.24±0.02	2.76±0.00	0	1.12±0.01
C 18:3 n3	1.82±0.01	1.20±0.01	2.13±0.03	8.16±0.01	0,22±0.01	2.52±0.01
C 20:2	0.68±0.01	0.56±0.00	2.64±0.01	0.74±0.01	0,14±0.01	0.54±0.01
C 20:3 n3	0.28±0.01	1.20±0.22	1.54±0.05	6.75±0.35	0,16±0.02	1.55±0.07
C 20:3 n6	0	0	0	0	0	0
C 20:4 n6	0.31±0.01	0.38±0.01	0.28±0.01	0.28±0.01	0	1.04±0.01
C 20:5 n3 (EPA)	0.56±0.01	0.90±0.00	5.40±0.01	2.13±0.00	0,15±0.01	2.38±0.01
C 22:2	0.32±0.02	0.52±0.01	2.07±0.01	0.65±0.00	0,34±0.05	0.12±0.01
C 22:6 n3 (DHA)	0	0.05±0.00	0.05±0.01	0.04±0.01	0,16±0.00	0.02±0.01
Σ PUFA	5.12	5.67	18.35	21.51	1,17	9.29
PUFA/SFA	0.06	0.09	0.30	0.35	0.01	0.13
Σ n6	1.46	1.24	4.52	3.04	0	2.16
Σ n3	2.66	3.35	9.12	17.08	0.69	6.47
n6/n3	0.55	0.37	0.50	0.18	0	0.33
DHA/EPA	0	0.06	0.01	0.02	1.07	0.01
unidentified	1.89	3.17	2.25	2.98	0.79	0.06

According to Table 1, patin muscle showed higher SFA value than by product. Then for MUFA and PUFA of by-product were higher than muscle. Then, tilapia by product was highest PUFA (omega-3) content of all fish examined. Accord According to Suloma et al. (2008), tilapia had 44.76% PUFA and 27.07% omega-3. At keli muscle was highest SFA content of all fish examined. Lipid levels and fatty acid composition vary with species, sex, and age, season of the year, food availability, salinity and water temperature (Stansby, 1981 and Monsen, 1985). The fatty acid composition of different fish from the same species can vary because of diet, location, gender and environmental conditions (Gruger, 1967).

PUFA/SFA ratio of patin, tilapia, keli by-products were 0.09, 0.35, and 0.13, respectively. The rasio of by product were higher than the muscles. At *Oreochromis sp*. and *Clarias sp*. has been reported by Endinkeau and Kiew (1993) that the ratio of PUFA/SFA was 0.6. For DHA content of fish examined is very lower than EPA. All ratio of DHA/EPA was less than one; except for of keli muscle was 1.07. The highest of EPA content values were obtained from tilapia muscle (5.4%) and keli by-product (2.38%).

Generally, the by-products had PUFAs *n*3 higher than in muscles. However, PUFAs *n*6 were distributed at muscles (1.46-4.52%), and then their by-products were 1.2 to 3.04% except for keli muscle which PUFA *n*6 was undetected. On the other hand, PUFAs *n*3 were higher in by-products (2.66%-9.12%) than muscles. Therefore, *n*6/*n*3 ratios of patin and tilapia muscles were 0.55 and 0.49. They are higher than their by-products.. Whereas, the ratio of keli muscle was undetected and its by-product was 0.33. Although a low *n*6/*n*3 ratio is desirable, FAO recommends the *n*6/*n*3 ratio should be 5:1 in total daily diet (Vujković *et al*., 1999).

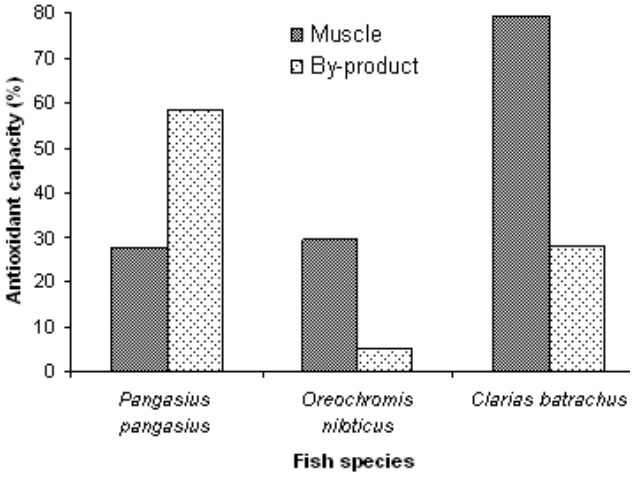

Figure 4. Antioxidan capacity of freshwater fish species in muscle and by-product

3.2. Antioxidant Capacity

Antioxidant capacities of muscles and by-products were also observed. Antioxidant capacities of fish examined muscles for keli, tilapia and patin were 79.38%, 29.89%, and 27.83% respectively. Antioxidant capacities of the by-products were 58.76% of patin, 5.17% of tilapia and 27.83% of keli (Figure 4).

By-products lipid of tilapia and keli had lower antioxidant capacity than muscle lipid. By product lipid was more susceptible to oxidation than muscle lipid, probably due to a slightly higher level of PUFA, than muscle. The result contrast with Zhong et al, 2007. The difference may be caused by the diet that fish receive, as culture fish might have their feed supplemented with vitamin E and others. It could be happened as high level of PUFA in by-products made antioxidant compounds worked to prevent PUFA oxidation *in vitro* during analysis. Besides, post mortem muscle tissue could reduce the ability of system to keep antioxidant in reduced state because reducing compounds was less (Bragadóttir, 2001). According to Zhong *et al (*2007), Muscle lipid of steelhead trout had higher concentration of α-tocopherols and total carotenoids than visceral lipid. However, visceral lipid was more stable than muscle. The carotenoids might exhibit prooxidant activity lipid (Zhong *et al*., 2007). Antioxidative agents found in fish are ascorbic acids, tocopherols, carotenoids, and ubiquinone (Bragadóttir, 2001).

Ascorbic acid generally is low in fish muscle and it may be considered as negligible (Nettleton and Exler, 1992). Tocopherols, known as Vitamin E, in fish tissue are related to its diet since fish are unable to synthesize the vitamin (Ackman and Cormier, 1967). Carotenoids in fish are found in the skin, flesh, eggs, gonads, milt, liver and eyes. Lutein is a dominant pigment in freshwater fish (Simpson, 1982). Carotenoids are less widely distributed in muscle than in integument (Haard, 1992). Ubiquinone or Coenzyme Q_{10} (CoQ_{10}) is a benzoquine with an isoprenoid side chain (Kagan *et al*., 1996). Almost every cell of a living organism contains CoQ (Lambelet *et al*., 1992). The richest sources of CoQ_{10} ranged from 4-27 mg/100 g in fish (Bragadóttir, 2001).

4. Conclusions

Freshwater fish (muscle and by product) were potential as source of PUFA *n*3 fatty. The PUFA content of by-products was higher than in muscle, mainly for tilapia (*Oreochromis niloticus*), it could be considered for recovering edible fish oil production. Antioxidant capacity values showed that fish by-product lipid content high PUFA. It was higher than muscle lipid, so need to be added antioxidants in the by-product oil, to keep the stability of oxidation.

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