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Contributed Paper

Fatty Acid Composition, Physical Properties, Acute Oral Toxicity and Antioxidant Activity of Crude Lipids from Adipose Tissue of Some Commercialized Freshwater Catfish

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Abstract

Crude lipids extracted from the adipose tissues of some commercialized freshwater catfish, including the Mekong giant catfish (*Pangasianodon gigas*), striped catfish (*Pangasianodon hypophthalmus*) and hybrid catfish (*Pangasius larnaudii* x *Pangasianodon hypophthalmus*) were carried out. Their saturated fatty acid and unsaturated fatty acid contents were not much different, but their polyunsaturated fatty acid contents were obviously different. Their n-3 fatty acid contents were 15.58, 0.83 and 4.36 g per 100 g, respectively, and their eicosapentaenoic acid (EPA):docosahexaenoic acid (DHA) contents were 3.23:4.24, 0.07:0.13 and 0.65:2.72 g per 100 g, respectively. Their n-6 fatty acid contents were 6.36, 9.19 and 8.77 g per 100 g, respectively. They were in solid and semi-solid form at room temperature with certain different physical properties. No sign of toxicity was observed for 14 days in female Wistar albino rats after receiving a dose of crude lipids at 5000 mg/kg. Their antioxidant activities by ABTS assay were 3.21, 4.53 and 6.00 mM Trolox per 1 g, respectively.

Keywords: fish lipid, fatty acid, physical property, acute oral toxicity, antioxidant activity, *Pangasianodon gigas*, *Pangasianodon hypophthalmus*, hybrid catfish

1. Introduction

Nowadays, the entire freshwater catfish family (Pangasiidae), including the Mekong giant catfish (*Pangasianodon gigas*), striped catfish (*Pangasianodon hypophthalmus*) and hybrid catfish (*Pangasius larnaudii* x *Pangasianodon hypophthalmus*), is commercially produced throughout the Mekong River region and at inland freshwater aquaculture farms [1-3]. It has become one of the most popular freshwater fish species in European and US markets because its flesh is white and of mild flavor, it is virtually boneless and its price is extremely competitive [2]. In the preparation of fish for today's consumer market, up to 50% of the whole fish is commonly discarded as waste, but this waste could be better utilized in several ways, such as by methods of recovery of the flesh, in the extraction of oils, enzymes, vitamins, or flavour

materials, and through the production of gelatin or the intermediate moisture animal feed products [4]. Adipose tissue is a kind of fish waste that has not been properly or fully utilized. This tissue makes up 5-10 % of the total body weight of the fish, as was explained by Thai Panga Farm.

The crude fat content of the head and flaccid body fat of some freshwater catfish in the Mekong River region were reported at 44.62-67.44% [5]. However, the fatty acid composition of the fish lipids is affected by various factors, such as species, wild or cultivated types, age, diet, region, environment, season and by body portion [6-7]. For example, lipids in the muscle of the cultivated Mekong giant catfish contained 45.2-45.3% saturated fatty acids, 28.3-37.0% monounsaturated fatty acids and 17.7-26.5% polyunsaturated fatty acids [6]. Lipids extracted from the head and flaccid body fat of the striped catfish consisted of 34.8% saturated fatty acids, 40.9% monounsaturated fatty acids and 19.4% polyunsaturated fatty acids [5]. Lipids of the striped catfish cultivated in floating cages comprised 48.8% saturated fatty acids, 40.1% monounsaturated fatty acids and 10.1% polyunsaturated fatty acids, while those of the striped catfish that were cultivated in ponds were composed of 44.1% saturated fatty acids, 40.4% monounsaturated fatty acids and 13.5% polyunsaturated fatty acids [8]. The most predominant saturated fatty acid found in those lipids of the Mekong giant catfish and the striped catfish was palmitic acid (C16:0) followed by stearic acid (C18:0) and the most predominant unsaturated fatty acid was oleic acid (C18:1n-9) followed by linoleic acid (C18:2n-6). The contents of n-3 fatty acids : n-6 fatty acids of the Mekong giant catfish and striped catfish were 3.49-6.85: 13.34-18.68 and 0.40-1.96 : 9.7-16.62, respectively, while those of eicosapentaenoic acid (C20:5n-3, EPA): docosahexaenoic acid (C22:6n-3, DHA) were 1.49-3.46 : 0.00 and 0.00-0.20 : 0.00-0.43, respectively [5-6, 8].

It is becoming apparent that regular consumption of long chain polyunsaturated n-3 fatty acids lowers the overall incidence of, as well as the rate of death from, cardiovascular heart disease. The biochemical basis for cardioprotective effects of n-3 fatty acids are probably multifactorial and may collectively result in increased heart rate variability (anti-arrhythmic), reduced atheroma development (anti-atherogenic), and decreased platelet reactivity/aggregation (anti-thrombotic), which may be mediated by the substrate competition between n-3 fatty acids and arachidonic acid (C20:4n-6) for cyclooxygenase (COX) enzymes that produce prostaglandins and thromboxanes [9]. In addition, n-3 fatty acids, namely EPA and DHA, still have other health benefits related to the immune system and renal disorders, as well as inflammation, allergies and cancer. DHA also plays an important role in brain development and retinal function of the fetus and in infants [10]. The official medical recommendation that people should eat fish twice a week in order to lower their chance of experiencing cardiovascular heart disease, was first announced by the United Kingdom Department of Health in 1994 [11], followed by the American Heart Association in 2002 [12]. Currently, it is recommended that the general public consume two fatty fish meals per week (0.3-0.5 g per day of EPA and DHA). Patients with coronary heart disease should have 1 g per day of EPA and DHA, whereas patients with hypertriglyceridemia should take 3 to 5 g per day of EPA and DHA under a physician's supervision [13].

Actually, n-3 fatty acids found in marine animals originate from their food sources. There are thousands of species of phytoplankton and algae, some producing no long chain n-3 fatty acids, and others with small or large proportions of EPA and DHA [14], and they are eventually transferred through the food web and incorporated into the lipids of marine

animals [15]. Freshwater fish could be seen as a good source of n-3 fatty acids, as well [16], however, freshwater fish oils contain n-6 fatty acids with totals roughly equal to the n-3 fatty acids, whilst marine fish oils have lower contents of n-6 fatty acids [17]. The contents of n-3 fatty acids of some freshwater fish in the USA and Thailand are 0.3–6.2 and 0.02–0.46 g per 100 g serving, respectively [18-19].

This work was aimed at the study of fatty acid composition, some physical properties, acute oral toxicity and the antioxidant activity of the crude lipids extracted from the adipose tissues, a kind of waste, of 3 commercialized freshwater catfish, including the Mekong giant catfish, striped catfish and hybrid catfish, to find out their potential to be used as raw materials for the manufacturing of nutraceutical products containing long-chain polyunsaturated n-3 fatty acids.

2. MATERIALS AND METHODS

2.1 Raw Materials

- Frozen adipose tissues of the Mekong giant catfish (*Pangasianodon gigas*) were acquired from Charun Farm, Chiang Rai, Thailand.

- Frozen adipose tissues of the striped catfish (*Pangasianodon hypophthalmus*) were acquired from Charoen Pokphand Food Farm, Vietnam.

- Frozen adipose tissues of hybrid catfish (*Pangasius lamaudii* x *Pangasianodon hypophthalmus*) were acquired from Thai Panga Farm, Kalasin, Thailand.

2.2 Lipid Extraction

Frozen adipose tissues were steamed at 80-100°C for 20 min. Hot adipose tissues were put in a filter sack and squeezed by screw compressor. Squeezed lipids were centrifuged at 4,000 rpm for 10 min to separate the solid particles. % Yield of crude lipids was calculated. Crude lipid samples were stored in a freezer until they were used for all analysis.

$$\% \text{ Yield} = 100x(W_{CL}/W_{FA})$$

Where: W_{CL} was weight of crude lipid
W_{FA} was weight of frozen adipose tissue

2.3 Fatty Acid Analysis

Crude lipid samples were sent to the Central Laboratory (Thailand) Co Ltd., Chiang Mai branch, for fatty acid composition analysis (both free fatty acid content and fatty acids released on triglyceride hydrolysis). An in-house method based on AOAC (2005) 996.06 (Hydrolytic extraction gas chromatographic method) [20] was used (Injection port: 250°C, FID Detector: 250°C, Oven Temperature profile: Initial temp. 140°C; hold 5 min., Increase temp. 3°C/min. to 250°C; hold 17 min., Run time: 55 min., Capillary column: Supelco SP-2560, GCFID: Agilent Model 6890N).

2.4 Physical Property Analysis

The following physical properties of the crude lipid samples were measured or analyzed with 3 replications.

- Colour (L*, a*, b*) at room temperature (30 ± 5°C) and 40°C was measured by Tri-Stimulus Colorimeter (JUKI: model JC801)

- Viscosity at 40°C was measured by Viscotester (ROIN: model VT-04) and a measuring time indicated when 50 ml oil sample flowed through a 0.2 mm orifice at 30 cm height

- Turbidity at 40°C was measured by Turbidimeter (HACH: model 2100 N)

- Specific gravity at 40°C was measured by 100 ml Specific-gravity bottle

- Melting point was analyzed by modified AOSC method Cc 3-2521]] or softening (open capillary tube melting) point method

- Smoke point was analyzed by modified AOSC method Cc 9a-48 [21] or Cleveland open cup method

2.5 Acute Oral Toxicity Study

The acute oral toxicity study was conducted

using the limit dose test of the up and down procedure (UDP) according to OECD Test Guideline 425 [22]. Female Wistar albino rats were used. The rats were fasted overnight prior to being dosed. The crude lipid sample at a dose of 5000 mg/kg was administered using the gastric feeding tube to five rats. Distilled water was feed for control group. Each rat was observed for signs of toxicity every 15 min in the first 4 h, then monitoring continued for 14 days after dosing with normal feeding. Signs of toxicity include behaviour pattern, changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems.

2.6 Antioxidant Activity Study

The scavenging activity of ABTS (2,2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic) radicals was measured according to the method described by Re *et al.* [23] with some modifications. The ABTS reagent was prepared by mixing 5 ml of 7 mM ABTS with 88 µl of 140 mM $K_2S_2O_8$. After the mixture was kept in the dark at room temperature for 16 h to allow the completion of radical generation, it was diluted with 95% ethanol. To determine the scavenging activity, 1 ml ABTS reagent was mixed with 10 µl of crude lipid sample or negative control and the absorbance was measured at 734 nm 6 min after the initial mixing, using ethanol as a blank. The inhibition percentage of the sample was calculated by the following equation:

%ABTS radical scavenging activity =
(A734 blank-A734 sample)x100/A734 blank

Where: A734 was the absorbance at 734 nm

Trolox, a derivative of vitamin E, was used as a standard. All determinations were carried out in triplicate. The EC50 was calculated from their dose-response curves and the antioxidant activity of each sample was expressed as Trolox equivalent antioxidant capacity (TEAC), which represented the concentration (µM) of Trolox as having the same activity as 1 g of sample.

2.7 Statistical Analysis

Data of physical properties, acute toxicity and EC50 (antioxidant activity) were analyzed by analysis of variance (Completely Randomized Design, CRD) and their means were compared by Tukey (a)'s w test. All statistical analysis was done by SPSS 16.0 Family.

3. Results and Discussion

3.1 Yield of Crude Lipids

Yields of crude lipids extracted from the adipose tissues of the Mekong giant catfish, striped catfish and hybrid catfish were 39.4, 48.0 and 47.6% (w/w), respectively. Frozen crude lipid samples were thawed in a water bath at 40°C because crude lipid samples would become solid or semi-solid form at room temperature (30±5°C), almost all analysis was done at 40°C.

3.2 Fatty Acid Composition

Solid or semi-solid form of the lipid samples could be explained by almost equal amounts of saturated and unsaturated fatty acids in Table 1. Their saturated fatty acid and unsaturated fatty acid contents were not much different, but their polyunsaturated fatty acid contents were obviously different.

The main saturated and unsaturated fatty acids found in this study were similar to those of previous reports [5-6, 8]. They were palmitic acid followed by stearic acid, and oleic acid followed by linoleic acid, respectively, except for the crude lipids of the Mekong giant catfish in this present study, of which 7.84% a-linolenic acid (C18:3n-3) was found to be more than 5.33% cis-9,12-linoleic acid (C18:2n-6) (Table 1). Compared to the fatty acid composition of the giant catfish muscle in the previous work [6],

Table 1. Fatty acid composition of crude lipids of 3 commercialized freshwater catfish.

Fatty acid composition (g per 100 g, %)	Mekong giant catfish	Striped catfish	Hybrid catfish
Capric acid (C10:0)	-	0.01	-
Undecanoic acid (C11:0)	0.01	-	-
Lauric acid (C12:0)	0.32	0.44	0.31
Tridecanoic acid (C13:0)	0.13	-	0.01
Myristic acid (C14:0)	5.41	3.86	3.60
Pentadecanoic acid (C15:0)	1.13	0.13	0.26
Palmitic acid (C16:0)	27.86	30.52	28.21
Heptadecanoic acid (C17:0)	1.28	0.14	0.34
Stearic acid (C18:0)	8.19	9.30	10.44
Arachidonic acid (C20:0)	0.50	0.20	0.21
Heneicosanoic acid (C21:0)	0.14	0.04	0.06
Behenic acid (C22:0)	0.48	0.09	0.13
Tricosanoic acid (C23:0)	1.81	0.26	0.34
Lignoceric acid (C24:0)	0.17	0.07	0.09
Saturated fatty acid	**47.43**	**45.06**	**44.00**
Myristoleic acid (C14:1)	0.05	0.02	0.03
Palmitoleic acid (C16:1n7)	4.43	0.90	1.77
cis-10-Heptadecenoic acid (C17:1n10)	0.04	-	-
trans-9-Elaidic acid (C18:1n9t)	0.39	0.06	0.08
cis-9-Oleic acid (C18:1n9c)	16.07	37.28	34.47
cis-11-Eicosenoic acid (C20:1n11)	3.34	1.15	1.04
Erucic acid (C22:1n9)	0.72	0.05	0.10
Nervonic acid (C24:1n9)	0.11	0.05	0.11
Monounsaturated fatty acid	**25.15**	**39.51**	**37.60**
trans-Linolelaidic acid (C18:2n6t)	0.05	-	-
cis-9,12-Linoleic acid (C18:2n6)	5.33	8.37	8.37
g-Linolenic acid (C18:3n6)	0.37	0.26	0.10
a-Linolenic acid (C18:3n3)	7.84	0.59	0.93
cis-11,14-Eicosadienoic acid (C20:2)	0.60	0.41	0.36
cis-8,11,14-Eicosatrienoic acid (C20:3n6)	0.51	0.52	0.26
cis-11,14,17-Eicosatrienoic acid (C20:3n3)	0.27	0.04	0.06
Arachidonic acid (C20:4n6)	0.10	0.04	0.04
cis-13,16-Docosadienoic acid (C22:2)	-	0.08	-
cis-5,8,11,14,17-Ecosapentaenoic acid (C20:5n3)	3.23	0.07	0.65
4,7,10,13,16,19-Docosahexaenoic acid (C22:6n3)	4.24	0.13	2.72
n-3 fatty acid	**15.58**	**0.83**	**4.36**
n-6 fatty acid	**6.36**	**9.19**	**8.77**
Polyunsaturated fatty acid	**22.54**	**10.51**	**13.49**
Unsaturated fatty acid	**47.69**	**50.02**	**51.09**
Trans fatty acids* and others**	4.88	4.92	4.91

* Trans fatty acids could occur during hydrolytic extraction.

** The amount was less than the limit of detection (LOD) at 0.01 g per 100 g.

the differences of the saturated fatty acid, mono and polyunsaturated fatty acids and the EPA contents of the crude lipids of the Mekong giant catfish were not obvious, but the amounts of the n-3 fatty acids (15.58% compared to 3.49-6.85%) and DHA (4.24% compared to 0%) in this present study were higher, while the amounts of n-6 fatty acids (6.36% compared to 13.34-18.68%) were lower.

In the case of the striped catfish, the saturated fatty acid, polyunsaturated fatty acid, n-3 fatty acid, n-6 fatty acid, EPA and DHA contents were different from those of the head and flaccid body fat that were reported in the previous study [5]. The results in this study showed the higher content of saturated fatty acids (45.06% compared to 34.8%) and the lower content of polyunsaturated fatty acids (10.51% compared to 19.4%), n-3 fatty acids (0.83% compared to 1.8%), n-6 fatty acids (9.19% compared to 16.62%), EPA (0.07% compared to 0.20%) and DHA (0.13% compared to 0.43%). But all of these totals did not differ much from those of the cultivated striped catfish that were reported in another previous study [8].

For the hybrid catfish (*Pangasius lamaudii* x *Pangasianodon hypophthalmus*) of black ear catfish and striped catfish, higher contents of n-3 fatty acids (4.36% compared to 0.40-1.96%), EPA (0.65% compared to 0.00-0.20%) and DHA (2.72% compared to 0.00-0.43%) were found compared to the striped catfish [5, 8].

Emphasis on the contents of n-3 fatty acids, especially EPA and DHA, crude lipids of the Mekong giant catfish could be considered as the best, followed by those of hybrid catfish and striped catfish, respectively, because of the species, feedings and conditions of cultivation. However, contents of n-3 fatty acids, especially EPA and DHA, of the crude lipids reported in this present study (0.83-15.58 g per 100 g) were still lower than those in the fish oils from other popular fish species (19.7- 36.65 g per 100 g), such as menhaden, herring, cod, capelin, sardines, skipjack tuna, butterfish, yellowtail flounder, winter flounder, haddock, halibut, mackerel, and salmon [9].

All the above results showed the possibility of increasing the contents of n-3 fatty acids, especially EPA and DHA in the lipids of commercialized freshwater catfish by the appropriate farming methods, such as through feeding and crossbreeding. Changes in the composition of animal lipids could be manipulated through feeding [24]. Therefore, n-3 fatty acids contents of commercialized freshwater catfish may be improved by farming with supplemented feeds, which contain n-3 fatty acids, especially EPA and DHA, such as some species of *Chlorella*, *Gonyaulox*, *Phaeodactylum* or *Spirulina* [25-26]. In addition, the supplementation of *Spirulina* in the feed of the Mekong giant catfish resulted in a higher weight gain, red blood cell count and the number of mature brood stock, which resulted in a significant benefit to reproduction [27]. In the case of crossbreeding, this present study showed different profiles of fatty acid compositions between hybrid catfish and its breeder, as mentioned above.

In addition, the methods applied for separating the unwanted fatty acids, such as chromatography, fractional or molecular distillation, enzymatic splitting, low-temperature crystallization, the solubility differences of fatty acid salts, supercritical fluid extraction, and urea complexation [10, 25] could be applied to use them as raw materials for the manufacturing of nutraceutical products containing long-chain polyunsaturated n-3 fatty acids.

3.3 Physical Properties

Physical characteristics of lipids were dependent on their composition [24]. Because of the different composition, almost all of their physical properties were significantly different ($p<0.05$) except for a* of liquid form and melting point (Table 2).

For example, crude lipids in the solid form of the Mekong giant catfish were found to be a more intense yellow, as shown in Figure 1, and were found to have a higher b* value, as shown in Table 2. Crude lipids at 40°C of striped catfish were found to be rather viscous compared to those of hybrid catfish and the Mekong giant catfish, respectively. And the smoke point of the crude lipids of striped catfish was lower than those of hybrid catfish and the Mekong giant catfish, respectively (Table 2).

In case of their melting points, it was found to be at about 30°C. The main reason for these high melting points could be described by the fact that their compositions contained 27.86–30.52% palmitic acid, 8.19–10.44% stearic acid and 3.60–5.41% myristic acid (Table 1), and for which the melting points of palmitic acid, stearic acid and myristic acid are 62.9, 69.9 and 54.4°C, respectively [28].

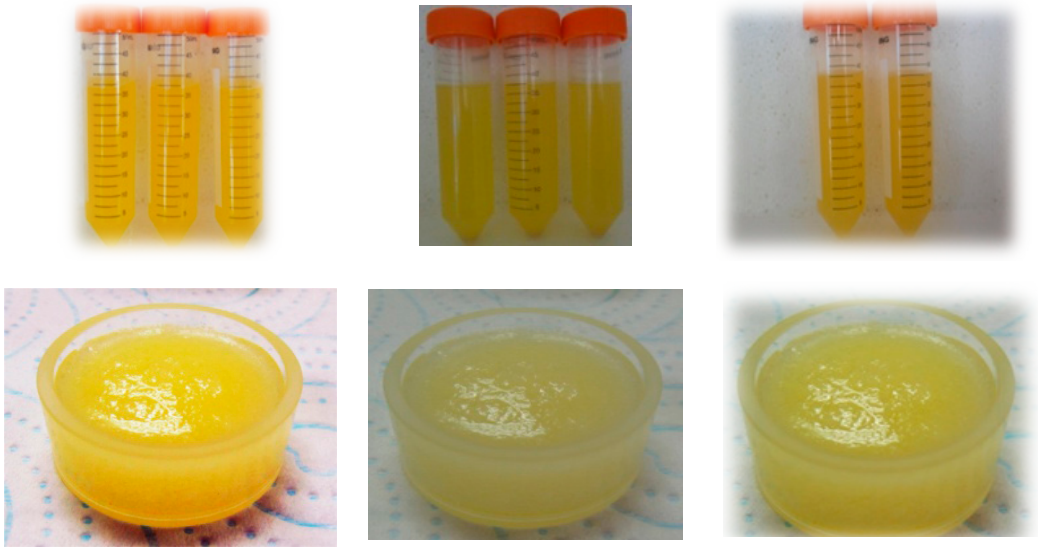
3.4 Acute Oral Toxicity

In terms of the acute oral toxicity, no sign of toxicity, both behavioral and physical manifestations, was found from all rats over 14 days after crude lipid dosing. The body weight of the rats fed with all 3 crude lipids was also not significantly different ($p>0.05$) when compared to those of the control group. The acute oral toxicity results showed that all 3 crude lipids were safe for consumption.

Table 2. Some physical properties of crude lipids of 3 commercialized freshwater catfish.

	Mekong giant catfish	Striped catfish	Hybrid catfish
Colour			
Solid form at room temperature (30 ± 5°C)			
L*	$84.30^{b} \pm 0.91$	$97.36^{a} \pm 1.00$	$82.66^{b} \pm 3.10$
a*	$38.16^{b} \pm 0.29$	$39.20^{a} \pm 0.54$	$34.55^{c} \pm 0.88$
b*	$83.18^{a} \pm 0.11$	$60.39^{c} \pm 0.63$	$68.33^{b} \pm 1.51$
Liquid form at 40°C			
L*	$58.12^{b} \pm 0.14$	$58.57^{a} \pm 0.20$	$58.29^{b} \pm 0.03$
a* ^{ns}	-13.76 ± 0.34	-13.74 ± 0.34	-14.10 ± 0.23
b*	$- 40.77^{a} \pm 0.05$	$- 40.69^{ab} \pm 0.17$	$- 40.47^{b} \pm 0.12$
Viscosity at 40°C			
Viscotester (Poise)	$0.30^{c} \pm 0.00$	$0.40^{a} \pm 0.00$	$0.35^{b} \pm 0.00$
Orifice (sec)	$24^{b} \pm 3$	$65^{a} \pm 5$	$57^{a} \pm 4$
Turbidity at 40°C (NTU)	$4.64^{a} \pm 0.27$	$3.27^{b} \pm 0.24$	$4.48^{a} \pm 0.26$
Specific gravity at 40°C (g per cm^2)	$0.9142^{a} \pm 0.0016$	$0.9078^{b} \pm 0.0017$	$0.9027^{c} \pm 0.0031$
Melting point (°C) ^{ns}	29.9 ± 2.0	32.1 ± 1.2	33.0 ± 1.5
Smoke point (°C)	$204.3^{a} \pm 5.5$	$168.0^{c} \pm 11.3$	$192.3^{b} \pm 8.5$

a, b, c Means with different superscript in the same row are significantly different ($p < 0.05$).
ns Means in that raw are not significantly different ($p > 0.05$).



(a) (b) (c)

Figure 1. Crude lipids in liquid and solid forms of the Mekong giant catfish (a), striped catfish (b) and hybrid catfish (c).

3.5 Antioxidant Activity

The antioxidant activity of the fish oil has been reported in several previous works [29-31]. In this present study, the scavenging activity of the ABTS radicals of 3 crude lipids, compared to Trolox and their antioxidant activity expressed as the Trolox equivalent antioxidant capacity (TEAC) or EC50 are shown in Figure 2 and Table 3, respectively. Crude lipids of the hybrid catfish showed significantly higher antioxidant activity ($P<0.05$) than those of striped catfish and the Mekong giant catfish, respectively, and it was found that the antioxidant activity results showed the positive correlation ($r = 0.97$) with the unsaturated fatty acid contents of the 3 crude lipids, but in-depth study should be conducted.

4. Conclusions

Yields of crude lipids extracted from the adipose tissues of the Mekong giant catfish, striped catfish and hybrid catfish were 39.4, 48.0 and 47.6% (w/w), respectively. They were proved that they contained n-3 fatty acids (0.83-15.58 g per 100 g) and n-6 fatty acids (6.36-9.19 g per 100 g), but crude lipids of the Mekong giant catfish was the best source of n-3 fatty acids, followed by those of hybrid catfish and striped catfish, respectively. Their physical properties were quite different and all of them were safe for consumption. The antioxidant activity of crude lipids of hybrid catfish was better than those of striped catfish and the Mekong giant catfish, respectively, and the positive correlation between antioxidant activity and the unsaturated fatty acid was found.

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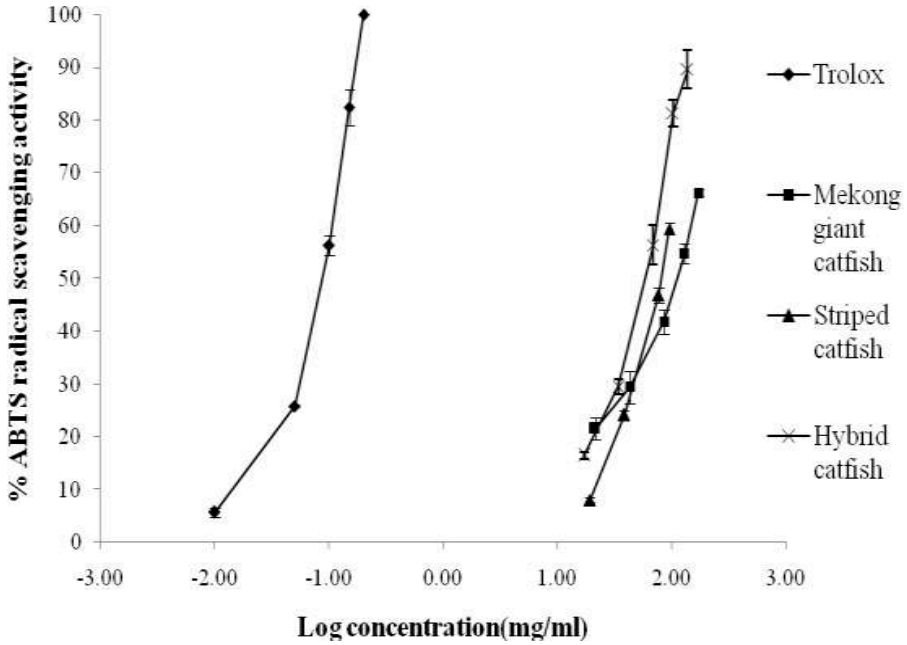

Figure 2. The scavenging activity of ABTS radicals of crude lipids of 3 commercialized freshwater catfish compared to Trolox**.**

Table 3. Trolox equivalent antioxidant capacity (TEAC) or EC50 of crude lipids of 3 commercialized freshwater catfish.

Crude lipid	TEAC or EC50 (mM Trolox per 1 g of sample)
Mekong giant catfish	$3.21^{c} \pm 0.08$
Striped catfish	$4.53^{b} \pm 0.19$
Hybrid catfish	$6.00^{a} \pm 0.36$

a, b, c Means with different superscript are significantly different ($p < 0.05$).

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