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Malaysia Snakehead Channa Striatus and Micropeltes: Physico-chemical Properties of Fillet Fish Oil and Water-soluble Extract

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Channa species is a local fish species in Malaysia. Channa striatus (Haruan) has been traditionally used for wound healing and reducing pain. This study was carried out to determine the availability of essential fatty acid (arachidonic acid) and other physico-chemical properties in the aqueous extract which has been proven to induce wound healing mechanism. Two Channa species were used in this study, which were Channa striatus (Haruan) and Channa micropeltes (Toman). Each sample was trimmed well to obtain the maximum fish fillet and extracted by using two different extraction methods. They were: 1) aqueous extraction and 2) Soxhlet extraction to procure the fish oil. The aqueous extract was characterised for its physico-chemical properties such as pH, moisture content, rheological properties (n = 3), amino acid composition and fatty acid determination (arachidonic acid) (n = 2) whilst fish oil was examined in terms of its colour, density, specific gravity, acid value (AV), free fatty acid (FFA) value, peroxide value (PV) (n = 3) and fatty acid composition (n = 2). Based on this study, Toman essence was found to be more neutral and had higher moisture content than Haruan essence ($p < 0.05$). They were considered having the same viscosity ($p > 0.05$). Both water extract of C. striatus and C. Both Haruan and Toman fish oils showed a relatively similar fatty acid profile and the arachidonic acid in a very low concentration. The physico-chemical properties of two Malaysia Channa species extracts revealed its potent ingredients (especially arachidonic acid) to induced wound healing process despite of its low concentration in the extracts. micropeltes were found to contain the essential amino acid, glycine and fatty acid (arachidonic acid) which aid in the process of wound healing despite their low concentration ($p > 0.05$). On the other hand, the highest yield of extracted fish oil (%, w/w) was obtained from C. micropeltes species, 9.51 ± 1.20 % ($p < 0.05$). The physico-chemical results of the fish oil from C. striatus and C. micropeltes, were as follows: colour – dark reddish with yellow of all species ($p < 0.05$), density - 0.99 ± 0.08 g/mL, 0.99 ± 0.00 g/mL ($p > 0.05$), specific gravity - 0.93 ± 0.10, 0.91 ± 0.03 ($p > 0.05$), AV - 21.78 ± 2.30 mg KOH/g, 5.55 ± 1.64 mg KOH/g ($p < 0.05$), FFA - 10.95 ± 1.16 %, 2.79 ± 0.83 % ($p < 0.05$) and PV - 18.00 ± 6.56 mEq/kg, 14.67 ± 3.79 mEq/kg ($p > 0.05$).

1. Introduction

Channa, or is more commonly known as snakeheads, has a conspicuous head similar to that of a snake, making this species very different from others (Mohd Husin, 2007). Channa is a group of freshwater and carnivorous fish that belongs to the family of Channidae. There is a total of 30 Channa spp. identified worldwide but only 7 species are known to be from Malaysia and it has been used for the treatment of different illnesses ever since by the traditional healers (Lee and Ng, 1994). The fish extract that is mostly used for wound healing application is secured from C. striatus or Haruan. The Haruan extract has the potential in tissue synthesis, wound healing and preventing the production of free radicals in regenerative medicines as well as an anti-aging agent, apart from the reported functions as human serum albumin substitute and pharmaceutical ingredients (Mudjiharto, 2007). Fish oil, which is the lipid fraction extracted from fish and its by-products are rich with essential fatty acids, especially polyunsaturated fatty acids (PUFA), decosahexaenoic acid (DHA)

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and eicosapentaenoic acid (EPA) (Abdulkadir et al., 2010). The presence of these certain PUFAs in fishes can regulate prostaglandin synthesis and therefore, induce wound healing mechanism (Bowman and Rand, 1980). This research described the study of different extracts from both Channa spp., which are C. striatus (Haruan) and C. micropeltes (Toman) and to determine the availability of essential fatty acid (arachidonic acid) and other physico-chemical properties which has been proven to induce wound healing mechanism. In wound healing process, both amino and fatty acids are important compounds where any paucity in these essential components will impede the recovery process (Mat Jais et al., 1994). Water based crude extracts and fish oil obtained were analysed for their physico-chemical properties as well as fatty acid and amino acid composition.

2. Materials and Methods

2.1 Raw material

Two Channa spp. were used as raw materials. This included Haruan and Toman. All samples were purchased from the local markets in Bandar Baru Bangi and a wet market in Kajang, Selangor, Malaysia. The fish species were then verified by the State Fisheries Department, Ministry of Agriculture, Malaysia.

2.2 Extraction process: aqueous extract

A pressure cooker set at 100 °C for 2 h (Mat Jais et al., 1994) was used for the extraction process. Boneless fish fillet which was cleaned with distilled water was weighed and placed into the pressure cooker. Then, distilled water with the ratio (fish: water volume) of 1 : 4 was added. When the fish fillet was cooked for 30 min, the distilled water was added to its initial volume before continuing the cooking process. This procedure was repeated every 30 min up to total 2 h cooking period. The fish fillet was removed whereas the liquid extract was collected, filtered with a filter paper and stored at 4 °C prior to further analysis.

2.3 Extraction process: fish oil

The boneless fish fillet obtained was weighed before it was cut into small portions and placed in a sterile plastic bag. The bag was clinched and moved into the freezer at -80 °C for 24 h. As soon as the fish fillet was removed, it was then shifted swiftly into the freeze-dryer for freeze-drying purposes. This step was carried out for 48 h in order to eliminate the entire water from the fillet. The dried fillet was weighed before the deformation process by a mechanical blender and stored at -20 °C (Lee et al., 2012). To acquire fish oil, Soxhlet extractor and n-hexane as solvent were used at 100 °C for 24 h. The collected fish oil was stored at -20 °C prior to physico-chemical analysis.

2.4 Aqueous extract pH, moisture content measurement & rheological attributes

(1) The pH meter; PHM210 Standard pH meter, MeterLab®, Radiometer Analytical S.A., France was used to determine the aqueous extract acidity/alkalinity (Febriyenti et al., 2008). (2) An oven drying method was employed to analyse the moisture content (AOAC, 2005). A total of 2 g sample was weighed on the aluminium plate and put into the Protech Force Air Convection oven at the temperature of 105 ºC for 16 h. The sample was then shifted to the desiccator for cooling purpose. (3) By employing rheometer (Anton Paar, Physica MCR 301) equipped with concentric cylinder CC27 measuring system, the rheological properties of each aqueous extract (20 mL) was analysed at room temperature of 25 ± 1 °C (Febriyenti et al., 2008). The viscosity of the respective aqueous extract was determined based on the shear stress versus shear rate profiles.

2.5 Aqueous extract amino acid composition determination

The amino acid composition of the aqueous extract was determined by high performance liquid chromatography (HPLC) AccQ. The hydrochloric acid and performic acid hydrolysis analysis on the aqueous extract were prepared according to the standard Sigma-Aldrich^{TM} instructions. All separations were carried out with an AccQ-Tag amino acid analysis column (3.9 mm x 150 mm). The column temperature was set at 36 ºC for acid hydrolysates, whereas for performic acid oxidised hydrolysates, the temperature of 31 ºC was used. With a flow rate set at 1 mL/min, 10 µL of the standard or hydrolysed sample was injected. The fluorescence detector was operated with 250 nm excitation and 395 nm emission wavelengths.

2.6 Aqueous extract fatty acid (arachidonic acid) determination

Approximately 2 g of water extract sample was weighed in a 20 mL test tube with screw cap. The sample then was dissolved in 2 mL hexane and followed by 4 mL of 2M methanolic KOH. Test tube was capped and vortexed for 2 min at room temperature. After centrifugation at 4,000 rpm for 10 min, the clear supernatant (hexane layer) was transferred into a 2 mL auto sampler vial and taken for GC analysis. Standard of FAMEs was utilised to determine the presence and quantity of a particular fatty acid, arachidonic acid.

2.7 Fish oil density determination

The bulk density of the fish oil was determined by using 25 mL measuring cylinder at 20 ± 1 ºC. The fish oil was poured into the 25 mL measuring cylinder and then the weight to volume ratio of the sample was recorded. The bulk density value was reported as g/mL (Huang and Sathivel, 2008).

2.8 Peroxide value (PV) assessment

The peroxide value of the fish oil was examined as stated in American Oil Chemist's Society (AOCS, 1998). The sample, which weighed 5 g was placed in a 200 mL conical flask with 225 mL glacial acetic acid and 75 mL chloroform added. By whirling, the mixture mixed thoroughly. Prior to the addition of 30 mL distilled water, 0.5 mL saturated potassium iodide (KI) was added. Next, the mixture was titrated with 0.1 N sodium thiosulphate solution ($Na_2S_20_3$) with 1 mL of 1 % w/v soluble starch as an indicator until the blue colour then disappeared. Blank titration was performed without the presence of the sample.

2.9 Free fatty acid (FFA) value evaluation (colorimetric)

The free fatty acid (FFA) value of the sample was identified according to AOCS (AOCS, 1998). The fish oil sample which weighed 5 g was mixed and swirled with 75 mL of 95 vol% neutral ethyl alcohol. After adding 1 % w/v phenolphthalein as an indicator, the mixture was titrated with 0.1 N sodium hydroxide (NaOH). Pinkish colour was observed and stopped at the end point of titration process.

2.10 Acid value (AV) determination

Acid value is the number of milligrams of potassium hydroxide (KOH) needed to neutralise the free fatty acid present in 1 g of fat (Abdulkadir et al., 2010). An amount of 1 g fat sample was dissolved in carbon tetrachloride (CCl_4). Then, the solution was titrated with 0.05 M alkaline, using phenolphthalein as an indicator. A constant shaking was conducted until dark colour was observed.

2.11 Fatty acid composition of fish oil

The fatty acid composition of the extracted fish oil was determined by the preparation of FAME and this was followed by an analysis with gas chromatography mass spectrometry (GC-MS). For the preparation of FAME from transmethylation using 2 M potassium hydroxide (KOH) in methanol and n-hexane, the method described by Anderson (2000) with a slight modification was followed. Two drops of the fish oil were dissolved in 2 mL hexane before 4 mL 2 M methanolic potassium hydroxide (KOH) solution. Then, the tube was vortexed for 2 min at room temperature. After centrifugation (4,000 rpm for 10 min), the upper layer of n-hexane containing FAME was pipetted out from the solution, placed in 2 mL glass vial and stored at the temperature below -4 °C until GC-MS analysis was performed.

2.12 Statistical analysis

The data of the study was analysed by using SPSS software version 20.0. One way analysis of variance (ANOVA) was employed for the determination of the significant differences between the samples and the significant probability level was set at 95 % ($p < 0.05$).

3. Results and discussion

3.1 Fish oil Extract

Dry sample is required for the procurement of fish oil as lipid cannot be extracted effectively from moist food with solvent. Freeze-drying method which was carried out to removing water containing in a sample as it did not cause the sample to lose its biological activities (Zakaria et al., 2004). Around 39.40 g (20.87 %) of snakehead freeze-dried filler (FDF) was obtained from 188.8 g wet weight of the snakehead fillet after drying process, whereas for Toman fish, it was 43.58 g (24.31 %) FDF from 179.29 g wet weight of the Toman fillet. The lipids from C. striatus and C. micropeltes were extracted by solvent extraction process using n-hexane as an extracting solvent. It was found that C. striatus and C. micropeltes contained lipid of 5.50 ± 0.10 % and 9.51 ± 1.20 %. The previous report showed that C. striatus was approximately 5 % by weight, while C. micropeltes was about 9 - 11 % based on the total fat content (Zuraini et al., 2006), which had the similarities with the findings. The observed differences in the maximum oil yield might have been due to several factors. The fat content is basically influenced by species, geographical regions, age and diet (Pigott and Tucker, 1990). The snakehead fish used in this study were wild, young and were from a different geographical location compared to the Toman fish. The Toman fishes were old and reared in a pond. Toman fish are believed to obtain an adequate and consistent supply of food than wild snakeheads. In terms of diet, the more a fish eats, the greater the oil and other chemical compositions will be produced (Abdulkadir et al., 2010). Haruan and

Toman fish oils extracted in this study were not refined, bleached and deodorised (RBD). They were in the form of crude fish oils.

3.2 Aqueous extract pH, moisture content and rheological attributes

The water based crude extracts of Channa fish species are serve to treat wounds, so their pH value should be as neutral as possible as to minimise pain and irritation of patients. The pH of water extract Haruan and Toman were close to the neutral pH ($p > 0.05$). The pH value of aqueous extract Haruan was significantly different from that of Toman (6.34 ± 0.01) than Haruan essence (6.59 ± 0.01) ($p < 0.05$). Meanwhile, the aqueous extract of Toman (97.3 ± 0.01 wt%) exhibited higher moisture content than Haruan (98.9 ± 0.01 wt%) ($p < 0.05$) which was comparable with the previous study of 91.1 % to 97.5 wt% (Wu and Shiau, 2002). Two water based crude extracts of the Haruan and Toman essence behaved like a Newtonian fluid with the viscosity of 1.50 ± 0.31 mPa.s and 1.10 ± 0.13 mPa.s ($p > 0.05$).

3.3 Aqueous extract amino acid composition and fatty acid (arachidonic acid)

Both essences were found to contain at least eighteen amino acids even with a low concentration and had insignificant differences ($p > 0.05$) among the samples except for the amino acid histidine, lysine and hydroxyproline. The main essential amino acids of snakehead essence were glutamic acid (0.18 ± 0.05 mg/g), glycine (0.15 ± 0.01 mg/g) and lysine (0.12 ± 0.01 mg/g), while the Toman essence was composed of glutamic acid (0.13 ± 0.02 mg/g), alanine (0.11 ± 0.01 mg/g) and glycine (0.10 ± 0.01 mg/g) predominantly. The same amount (mg) of arachidonic acid (fatty acid) were determined in Haruan (3.72×10^{-4} ± 0.23 µg) and Toman (7.20×10^{-4} ± 0.55 µg) essence ($p > 0.05$). The quantity of arachidonic acid was minuscule as it was highly diluted during sample preparation. The results of this study showed that C. striatus and C. micropeltes water based crude extracts contained the amino acid required in wound healing, which are glycine and glutamic acid. Glycine plays an important role with arachidonic acid from the fatty acids group in the recovery process. It is one of the main components of human collagen which works together with other amino acids (such as proline, alanine, arginine, isoleucine, phenylalanine and serine) in the effort of forming polypeptide that promotes tissues repair and healing process (Witte et al., 2002).

3.4 Fish oil density

The C.striatus and C.micropeltes fish oil density were 0.99 ± 0.08 g/mL and 0.99 ± 0.00 g/mL at 20 ± 1 ºC ($p > 0.05$). Both extracts were quite similar to that of the reported salmon fish oil (Sathivel, 2005).

3.5 Peroxide value (PV)

The PV of an oil or fat is an indication of rancidity, which occurs by autoxidation (volatile secondary oxidation by-products). The higher the PV, the higher the chances of triggering autoxidation (Lee et al., 2012). The same PV of Haruan (18.00 ± 6.56 mEq/kg) and Toman fish oil (14.67 ± 3.79 mEq/kg) was evaluated ($p > 0.05$) and within the PV of any crude fish oil of 3 and 20 mEq/kg (Young et al., 1993). Due to its higher amount of PV, both species have higher chances of triggering autoxidation.

3.6 Free fatty acid (FFA) value

The FFA of C. striatus and C. micropeltes lipids was found to be in the ranges of 10.95 ± 1.16 % and 2.79 ± 0.83 % ($p < 0.05$). The percentages of FFA which were above 1.5 - 3 % signified the unsuitability of the lipid for edible intention (Molla et al., 2007). Both Haruan and Toman fish oils were unsuitable to be consumed because of the presence of natural lipase and bacteria in fat. The fish oils deteriorated very quickly as lipase and bacteria hydrolysed fat to FFA and sulphur (Abdulkadir et al., 2010). These lipids might not be suitable for edible purposes.

3.7 Acid value (AV)

The lipid of C. striatus (21.78 ± 2.30 mg KOH/g) contained 5-fold higher than that of C. micropeltes (5.55 ± 1.64 mg KOH/g) ($p < 0.05$). Both acid values of the fish oils exceeded the standard quantity, which is 5 - 8 mg KOH/g (Adeniyi and Bawa, 2006). AV signifies the acidity of oil where a low AV demonstrates high oxidative stability (Essien et al., 2012). Both the fish oils procured in this study showed low oxidative stability. A high AV value is usually correlated with the lipase activity arisen from microorganisms or biological tissues (Boran et al., 2006). Since the all samples were not sterilised nor studied under aseptic circumstances, there were possibilities that some enzymes or microorganism contaminations might have occurred during the removal of the samples for laboratory works (Norziah et al., 2009).

3.8 Fatty acid composition of fish oil

The GC chromatogram profile of both C. striatus and C. micropeltes fish oils had produced a total of 21 to 26 peaks with a difference in the amount (%) of fatty acids (Figure 1). Both the fish oils showed a relatively similar fatty acid profiles since they were originated from the same fish species, which is Channa species.
Fish oil is made up of a long chain of fatty acids with a minimum chain length from C12 to C24 (Muhamad and Mohamad, 2012). The results of this study were in line with the previous study where both fish oils produced fatty acids with a chain length ranging from C12 to C22 (Table 1). The fish oil yield (%) of some major fatty acid compounds of the Toman fish oil (C. micropeltes) was significantly higher than that of the Haruan ($p < 0.05$). Arachidonic acid which plays an important role in wound healing was undetected/depleted in both fish oils possibly due to the freeze-drying process which has induced the degradation of smaller molecules due to the flash dehydration via freeze burning mechanism (Mat Jais et al., 1998).

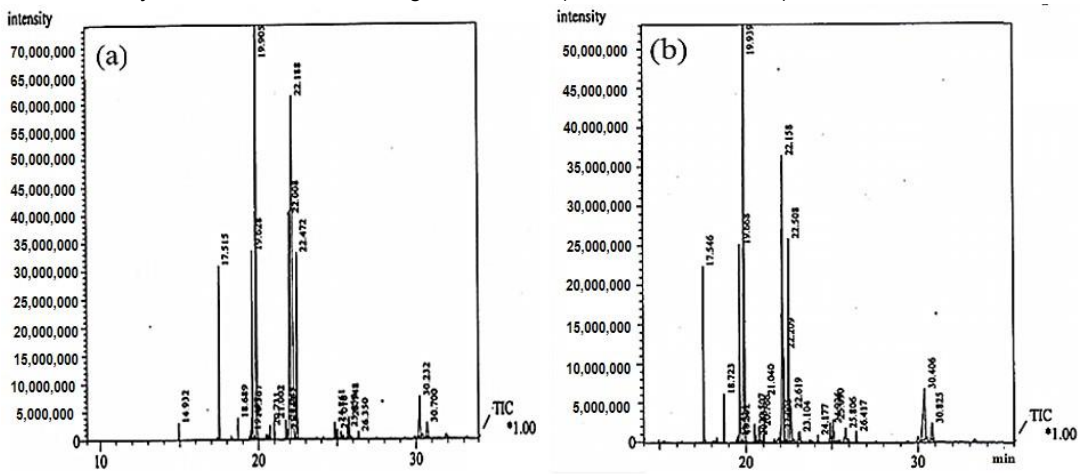

Figure 1: Fatty acids GC chromatogram profiles of: (a) Haruan and (b) Toman fish oil

Table 1: The content (%) of some major fatty acids of C. striatus and C. micropeltes fish oils. Results were the mean values of replication ± S.D (n = 2). Means within row were significantly different with the different superscript letter (p < 0.05)

Compound name	Symbol	Molecular weight	Molecular formula	C. striatus fish oil (wt%)	C. micropeltes fish oil (wt%)
Dodecanoic acid, methyl ester	12 : 0	214	$C_{13}H_{26}O_2$	3.62 ± 0.11^a	5.62 ± 0.08^b
Hexadecanoic acid, methyl ester	16 : 0	256	$C_{16}H_{32}O_2$	24.07 ± 0.47^a	29.11 ± 0.31^b
Octadecanoic acid, methyl ester	18 : 0	298	$C_{19}H_{38}O_2$	8.13 ± 0.31^a	11.77 ± 0.48^b

4. Conclusion

The water based crude extracts of Toman was more neutral and contained higher moisture content than that of Haruan ($p < 0.05$). As for viscosity, both the aqueous extracts had the same viscosity ($p > 0.05$) and shown Newtonian based fluid profiles. Toman produced higher fish oil yield as compared to C. striatus ($p < 0.05$). In fact, it has a better quality of fish oil due to its low peroxide value (PV), free fatty acid (FFA) value and acid value (AV) ($p < 0.05$). Both fish oils depicted a relatively similar fatty acid profiles and essential arachidonic acid was not discovered due to the flash dehydration during processing (freeze-drying). Both essences contained sufficient amount of amino acids. Traces of essential fatty acid (arachidonic acid) was merely available in the aqueous extract which are principally needed in wound healing process.

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