



## Physico-Chemical Characteristics and Quality Evaluation of Malaysia *Haruan* (*Channa striatus*) and *Toman* (*Channa micropeltes*) Fish Oil: Preliminary Quality Analysis Prior to Therapeutic Consumption

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### ABSTRACT

The local *Channa* species used in this research were *Channa striatus* (*haruan*) and *Channa micropeltes* (*toman*). Both samples were extracted using soxhlet extraction to procure the fish oil and then examined on its physico-chemical properties. The *C. micropeltes* species shows the highest yield of extracted fish oil (% w/w) with dry weight basis ( $9.51 \pm 1.20\%$ ). The physico-chemical results of the fish oil from *haruan* and *toman* were as follows respectively; colour: yellowish dark red for both species, specific gravity:  $0.93 \pm 0.10$ ,  $0.91 \pm 0.03$ ; AV:  $21.78 \pm 2.30$  mg KOH/g,  $5.55 \pm 1.64$  mg KOH/g; FFA:  $10.95 \pm 1.16\%$ ,  $2.79 \pm 0.83\%$  and PV:  $18.00 \pm 6.56$  meq./kg,  $14.67 \pm 3.79$  meq./kg. Both fish oils showed a relatively comparable fatty acid profile. The availability of seven major enriched fatty acid constituents in both fish oils namely dodecanoic acid, 9-hexadecenoic acid, hexadecanoic acid, linoleic acid, oleic acid, octadecanoic acid, docosahexaenoic acid and an infinitesimal amount of arachidonic acid (the main precursor for wound healing process) may perhaps be requisite for therapeutic consumption.

**Keywords:** *Channa* species, Fish oil, Physicochemical analysis, Soxhlet extract, Wound healing.

### INTRODUCTION

Snakehead fish or *haruan* were categorized in *Channa* species under family of *Channidae*. This species are a group of freshwater and carnivorous fishes, and was very different from others due to its unique snake-head similarity shape (Mohd Husin, 2007). In fact, they had been widely found with variety of species and consumed as food in many tropical countries such as Malaysia and other Southeast Asian countries (Mat Jais, 2007; Mohd

Hasan, 2005). There is a total of 30 *Channa* spp. found worldwide but only 7 species are known from Malaysia. They are *Channa bankanensis*, *Channa gachua*, *Channa lucius*, *Channa maruloides*, *Channa melasoma*, *Channa micropeltes* and *Channa striatus* (Courtenay & Williams, 2004) or better known as *haruan* hutan, *haruan* todak, ikan bujuk, toman bunga, *haruan* palas, toman batik and *haruan* locally. The *haruan* extract from *C. striatus* species are mostly used today due to its potential in tissue synthesis and help in wound healing



(Mudjiharto, 2007) which are potentially due to its essence concoction broth or from its fish oil. The fish oil, which is the lipid fraction extracted from fish and fish by-products are rich with important fatty acids, especially polyunsaturated fatty acids (PUFA), decosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Faizal *et al.*, 2017; Kim and Mendis, 2006). The presence of certain PUFA in fish oil can be the regulators that affect the synthesis and activity of cytokines for promoting wound healing (Huang *et al.*, 2018). For that reason, the fish oil obtained through soxhlet method from both well-known *Channa* sp. which are *C. striatus* (*haruan*) and *C. micropeltes* (*toman*) was analyzed for their physico-chemical properties, fatty acid and its compositional profiles which could be the essential compound for therapeutic consumption mainly on wound healing.

## MATERIALS AND METHODS

### Raw materials

The species used in this study were *Channa striatus* (*haruan*) and *Channa micropeltes* (*toman*) that purchased from local markets in Bandar Baru Bangi and wet market Kajang, Selangor, Malaysia (Fig. 1). The fish species were then visually inspected by Malaysian Fishery Department (MFD), cross-referenced and vouched (JPM: 2002/627) for validation under the local fresh water fish species checklist.

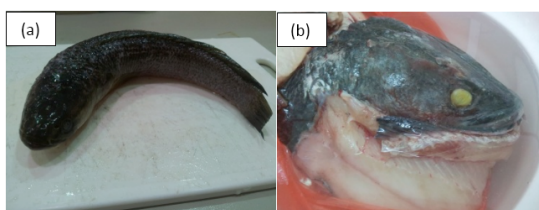


Fig. 1. Pictorial of (a) *Channa striatus* (*haruan*) and (b) *Channa micropeltes* (*toman*) confirmed and vouched by the Malaysian Fishery Department (MFD)

### Pre-treatment of raw material

After collecting the fish samples, they were cleansed by discarding their bones, liver, stomach and viscera manually (Fig. 2). Only edible muscles with skin were needed prior to physico-chemical characteristic evaluation. In preparation prior to aforementioned analysis, frozen samples (frozen at  $-10^{\circ}\text{C}$ ) were freeze-dried (48 h), thawed, rinsed and cleansed with distilled water twice and air-dried at  $25^{\circ}\text{C}$  prior to grinding and fish oil extraction process.



Fig. 2. Cleaned boneless fillet of (a) *Channa striatus* (*haruan*) and (b) *Channa micropeltes* (*toman*) prior to soxhlet extraction process

### Chemicals

N-hexane (System<sup>®</sup>, ChemAR<sup>®</sup>) was used as solvent to extract oil from all fish samples. Other analytical chemicals employed include hexane (LiChrosolv<sup>®</sup>), methanol (J.T.Baker), potassium hydroxide (R&M Chemicals), glacial acetic acid (System<sup>®</sup>), chloroform (System<sup>®</sup>), potassium iodide (QR $\text{\u00c}$ C<sup>TM</sup>), sodium thiosulphate (Merck KGaA), 1% (w/v) soluble starch (Acros Organics), 95% (v/v) ethyl alcohol neutral (HmbG<sup>®</sup> Chemicals), 1% (w/v) phenolphthalein (HmbG<sup>®</sup> Chemicals), sodium hydroxide (R&M Chemicals) and carbon tetrachloride (QR $\text{\u00c}$ C<sup>TM</sup>). All reagents used for the extraction and chemical analyses were of analytical grade, unless specified otherwise.

### Fish oil soxhlet extraction process

The frozen boneless fish fillet was freeze-dried for 48 h beforehand and deformed by a mill grinder (Panasonic MK-5078, Japan) for 5 minutes. The soxhlet extractor and n-hexane as solvent were used to acquire fish oil for 2 h of extraction process (solvent-to-solid ratio of 1:10 ml/g) ( $n = 3$ ). The fish oil was collected eventually and stored at  $-20^{\circ}\text{C}$  prior to physico-chemical analysis.

### Physical properties of extracted fish oil

#### Colour analysis

The colour of extracted fish oil was measured by using Lovibond<sup>®</sup> Tintometer Colourimeter Model F (Minolta, Japan), where  $L^*$  value indicated degree of lightness to darkness,  $a^*$  value depicted degree of redness to greenness and  $b^*$  value epitomized degree of yellowness to blueness. This analysis was conducted in triplicates to obtain the average of colour profiles value ( $n = 3$ ).

#### Fish oil specific gravity

The specific gravity of the unrefined fish oils was determined using a 25 mL glass-measuring cylinder ( $n = 3$ ) (Bako *et al.*, 2017). The weight-to-volume ratio of oil (g/mL) was divided by the weight-

to-volume ratio of water (g/mL) at  $20 \pm 1^\circ\text{C}$  to obtain the specific gravity. Finally, the specific gravity of fish oil was determined and calculated as density of fish oil per density of water.

### Chemical properties of extracted fish oil

#### Peroxide value (PV) analysis

The peroxide value of fish oil was examined based on the prior work of AOCS protocols (AOCS, 1998). The mixture was titrated with 0.1 N sodium thiosulphate solution ( $\text{Na}_2\text{S}_2\text{O}_3$ ) with 1 mL of 1% (w/v) soluble starch as indicator until blue colour disappeared in reference to blank ( $n = 3$ ).

#### Free fatty acid (FFA) value analysis

The free fatty acid (FFA) value of sample was identified according to the AOCS protocols (AOCS, 1998). Approximately of 1% (w/v) phenolphthalein was added into the mixture and titrated with 0.1 N sodium hydroxide (NaOH) until pinkish color was observed ( $n = 3$ ).

#### Acid value (AV) determination (mg KOH/ g)

The acid value was determined based on the prior work by Abdulkadir *et al.*, (2010). Approximately of 1 g fat sample was dissolved in carbon tetrachloride ( $\text{CCl}_4$ ) and titrated with 0.05 M alkaline, using phenolphthalein as indicator until dark colour was observed ( $n = 3$ ).

#### Fatty acid compositional analysis

The fatty acid methyl esters (FAME) composition of the extracted fish oil was determined by using GC-MS (Shimadzu QP2010 quadrupole) equipped with carbowax capillary column (30 cm x 0.25 mm internal diameter; 0.25 in film thickness) and flame-ionization detector with 2.0  $\mu\text{L}$  of both oils was injected into the column. Fatty acid (%) were calculated from the proportion of each fatty acid within the sample; these percentages were used to create a fatty acid profile for each oil used ( $n = 3$ ).

#### Statistical analysis

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

## RESULTS AND DISCUSSION

### Fish oil extract

Pre freeze-drying method was able to minimize sample final moisture without losing its biological activities, thus maintain high product

quality (Mohammad *et al.*, 2012). Around 39.40 g (20.87%) of *haruan* freeze-dried filler (FDF) was obtained from 188.8 g wet weight of snakehead fillet after drying process, whereas for toman fish, it was 43.58 g (24.31%) FDF from 179.29 g wet weight of toman fillet. *Haruan* dan *toman* fish oils extracted in this study were in the form of crude fish oils. As shown in Fig. 3, it was found that *haruan* and *toman* contained lipid of  $5.50 \pm 0.10\%$  and  $9.51 \pm 1.20\%$  respectively, which is slightly higher than the prior work (Zuraini *et al.*, 2005). The observed differences in maximum oil yield may have been due to different species, geographical regions, age and diet.

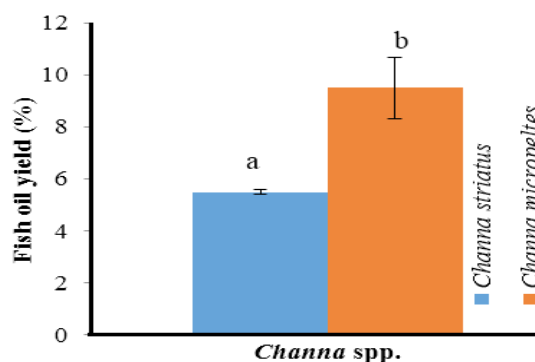


Fig. 3. Fish oil yield (% dry basis) of both *Channa* spp. extracted using soxhlet method. Results are the mean value of three replicates  $\pm$  S.D. ( $n = 3$ ). a-b Mean with different superscript letter are significantly different ( $p < 0.05$ )

### Physical properties of extracted fish oil

Generally, it was observed that the oils extracted from two of the *Channa* fish species (Table 1) were tolerable to the standard values (specific gravity and peroxide value), nevertheless some were above the normal ranges (free fatty acid and acid value) (Abdulkadir and Abubakar, 2010). It was actually due to the fact that most of the standard values from the prior work results were reliant on geographical locations, seasons and intention to which the oil was produced for (Ahmed *et al.*, 2017).

Table 1: Physico-chemical characteristics of both *haruan* and *toman* fish oils

Physico-chemical properties	Haruan	Toman
(a) Color Profiles:		
L*	22.65 $\pm$ 0.19a	26.34 $\pm$ 1.27b
a*	7.26 $\pm$ 0.15a	5.21 $\pm$ 0.49b
b*	3.38 $\pm$ 0.11a	5.91 $\pm$ 0.27b
(b) Specific gravity		
(c) Peroxide value (meq./kg)	18.00 $\pm$ 6.56a	14.67 $\pm$ 3.79a
(d) Free fatty acid value (%)	10.95 $\pm$ 1.16a	2.79 $\pm$ 0.83b
(e) Acid value (mg KOH/g)	21.78 $\pm$ 2.30a	5.55 $\pm$ 1.64b

Results are the mean values of triplicates  $\pm$  S.D ( $n = 3$ ). a-b Mean with different superscript letter are significantly different ( $p < 0.05$ ) in between species

### Colour profiles

The average values of  $L^*$ ,  $a^*$  and  $b^*$ , for both fish oil samples were shown in Table 1. Generally, they were in dark red with yellow colours. These colour parameters for *haruan* were significantly different ( $p < 0.05$ ) with those of *toman*. Lower  $L^*$  value was determined in *haruan* fish oil sample, stated the overall darker tone of *haruan* fish oil compared to that of *toman*. In spite of that, there was actually not much differences when observing the lightness of the samples. Prior works have been reported that high temperature could actually induce browning reactions that can caused a decrease in lightness value, which frequently perceived to be happen when thermal treatment executed (Leadley, 2009; Patras *et al.*, 2009). Meanwhile, the  $a^*$  (red-green colour) and  $b^*$  (blue-yellow colour) values also showed significant difference ( $p < 0.05$ ) between the samples. *Haruan* fish oil was more red in colour ( $a^*_{haruan} = 7.26 > a^*_{toman} = 5.21$ ) but less yellowish ( $b^*_{haruan} = 3.38 < b^*_{toman} = 5.91$ ) than *toman* fish oil. Brighter yellow colour of *haruan* fish oil was an indication of sample browning, confirming the tendency observed for lightness value (Ortiz *et al.*, 2013).

### Specific gravity

From Table 1, it was calculated that there was insignificant difference between the specific gravity of *haruan* fish oil and *toman* fish oil ( $p > 0.05$ ). The standard specific gravity of the fish oil was in between 0.907 to 0.915 (Adeniyi and Bawa, 2006). Specific gravity value of *haruan* fish oil ( $0.93 \pm 0.10$ ) was just short of the above range. Hence, the results was in range of the prior work as to indicate the efficiency of the soxhlet extraction process and its processing parameters used (Paul *et al.*, 2013).

### Chemical properties of extracted fish oil

#### Peroxide value (PV)

Peroxide value of an oil or fat is an indication of rancidity, which occurs by autoxidation (Lee *et al.*, 2012). Autoxidation is the most remarkable reason which causes fish oils to deteriorate in terms of quality as volatile secondary oxidation products were produced (Venkateshwarlu *et al.*, 2004). The unsaturated nature of lipids and strong pro-oxidative system in fish tissues could cause the lipids to oxidize during the process of processing and storing. Even very low peroxide value was detected at the early oxidation stage or during the induction period, undesirable flavors and odors emerged. Oxidation

of lipids not only produced rancid odors and flavors, but also can degrade the nutritional quality and safety of food through the formation of secondary products (Pak, 2005). However, there was an insignificant difference ( $p > 0.05$ ) of PV between *haruan* fish oil ( $18.00 \pm 6.56$  meq./kg) and *toman* fish oil ( $14.67 \pm 3.79$  meq./kg). PV values of all samples were less than 20 meq./kg, within the acceptable limit. This suggested that the extracted fish oils have low rate of lipid oxidation. Low PV value was also supported by the fact at which the oils were only left for few hours before analyses. The lesser the period for the oil to be expose to the atmosphere, the slower the rate of oil oxidation (Ab Kadir *et al.*, 2017; Adeniyi and Bawa, 2006). Moreover, the PV limit which posed by the regulatory agencies for quality and acceptability of oils for human consumption is 7-8 meq./kg (Boran *et al.*, 2006). Fish oils in this research might not suitable for consumption purposes as they were in crude oil form which did not underwent the process of refining, bleaching and deodorizing (RBD) (Jurid *et al.*, 2020).

#### Free fatty acid (FFA) value

The AV between *toman* and *haruan* fish oil was noticeably incongruent of  $21.78 \pm 2.30$  and  $5.55 \pm 1.64$  mg KOH/g respectively (Table 1) ( $p < 0.05$ ). In fact, both fish oils surpassed the tolerable standard value of 5 mg KOH/g which demonstrated low oxidative stability (Essien *et al.*, 2012). The tolerable oil standard values are heavily dependent on geographical locations, seasons, processing method and purpose of which the oil is used for (Ab Kadir *et al.*, 2017; Adeniyi and Bawa, 2006). For that reason, the low AV was commonly correlated with the microorganisms or biological tissues lipase activity that affect the freshness and spoilage attributes (Park, 2005). As the prepared samples were not sterilized nor prepared under aseptic micro-environment, the chances of having potential enzymatic or microorganisms adulterations during the removal of internal organs and pre-processing of carcasses for the extraction and physico-chemical analysis works was undeniable attainable (Norziah *et al.*, 2009).

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#### Fatty acid composition of fish oil

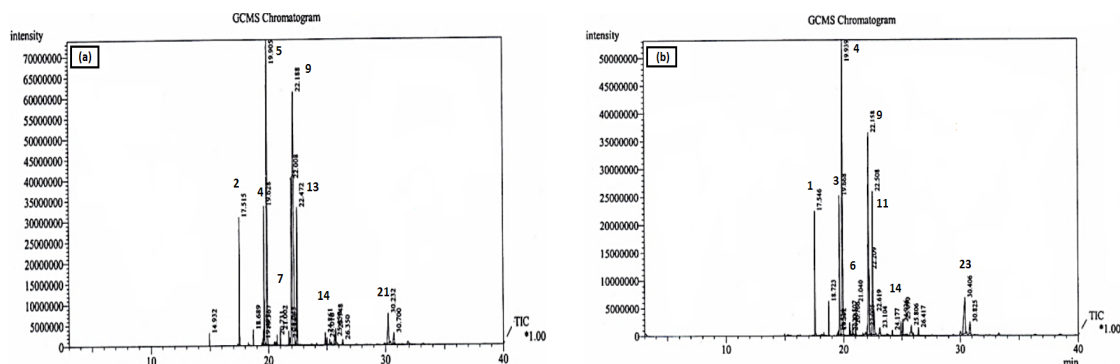
Figure 4a and Figure. 4b illustrated the fatty acid profiles for haruan and toman fish oils respectively. There were relatively similar fatty acid profiles for *haruan* and *toman* fish oils since they were originated from the same fish species, which is *Channa* species. Each with total peaks from 21-26 (*haruan*) and from 22-23 (*toman*)

respectively. Table 2 revealed that the content (%) of some seven major fatty acid compounds of toman fish oil was significantly higher than that of haruan fish oil ( $p < 0.05$ ) except for arachidonic acid as both extracts contained miniscule amount of it ( $p > 0.05$ ) (Peak No. 14). The fatty acid compounds obtained for both species in this research was also higher from the prior studies (Omar *et al.*, 2010; Muhamad and Mohamad, 2012). In fact, the fatty acid composition of the fishes were greatly influenced by food, habitat, climate and whether the fish is reared or not (Muhamad and Mohamad, 2012). In this investigation, the arachidonic acid availability in both extracts is essential as it plays an important role in wound healing a(to promote wound healing by initiating collagen synthesis and re-epithelialisation in damaged tissues) and tissue regenerative in therapeutic consumption (Haniffa *et al.* 2014). However, the present of arachidonic acid in both haruan and toman fish oils were infinitesimal this finding has also been reported on the previous studies due to its minute concentration and was below the GC-MS detection limit (Muhamad and Mohamad, 2012).

**Table 2: Eight major fatty acids composition (%) including arachidonic acid (wound healing precursor) of *haruan* and *toman* fish oil discovered based on chromatogram Fig. 4**

Peak No. <i>Haruan</i>	Peak No. <i>Toman</i>	Compound name	Symbol	Molecular weight	Molecular formula	Haruanfish oil (%)	Tomanfish oil (%)
2	1	Dodecanoic acid, methyl ester	12:0	214	C <sub>13</sub> H <sub>26</sub> O <sub>2</sub>	3.62 ± 0.11 <sup>a</sup>	5.62 ± 0.08 <sup>b</sup>
4	3	9-hexadecenoic acid, methyl ester	14:0	242	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	7.88 ± 0.13 <sup>a</sup>	6.11 ± 0.07 <sup>b</sup>
5	4	Hexadecanoic acid, methyl ester	16:0	256	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	24.07 ± 0.47 <sup>a</sup>	29.11 ± 0.31 <sup>b</sup>
7	6	9,12- octadecatrienoic acid, methyl ester (linoleic acid)	18:2 (n-6)	268	C <sub>17</sub> H <sub>32</sub> O <sub>2</sub>	0.82 ± 0.01 <sup>a</sup>	0.18 ± 0.01 <sup>b</sup>
9	9	Cis-9-octadecenoic acid, methyl ester (oleic acid)	18:1 (n-6)	296	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	18.20 ± 0.12 <sup>a</sup>	15.45 ± 0.03 <sup>b</sup>
13	11	Octadecanoic acid, methyl ester	18:0	298	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	8.13 ± 0.31 <sup>a</sup>	11.77 ± 0.48 <sup>b</sup>
14	14	5,8,11,14-eicosatetraenoic acid, methyl ester (arachidonic acid)	20:4 (n-6)	318	C <sub>21</sub> H <sub>34</sub> O <sub>2</sub>	0.16 ± 0.01 <sup>b</sup>	0.18 ± 0.08 <sup>b</sup>
21	23	4,7,10,13,16,19 - Docosahexaenoic acid (DHA)	22:6 (n-3)	419	C <sub>23</sub> H <sub>36</sub> O <sub>2</sub>	1.52 ± 0.12 <sup>b</sup>	1.47 ± 0.40 <sup>b</sup>

Results are the mean values of duplicates ± S.D (n = 2). <sup>a-b</sup>Mean with different superscript letter are significantly different ( $p < 0.05$ ) in between species



**Fig. 4. GC separation of fatty acid methyl ester of (a) *haruan* fish oil and (b) *toman* fish oil with miniscule content of arachidonic acid (precursor for wound healing) and seven other major fatty acids constituents which are dodecanoic acid, 9-hexadecenoic acid, hexadecanoic acid, linoleic acid, oleic acid, octadecanoic acid and docosahexaenoic acid (numbered and circled in red)**

## CONCLUSION

The soxhlet extraction yield of toman procured a higher amount of oil as compared to *haruan* ( $p < 0.05$ ). Both fish oils were in the color of dark red and yellow ( $p < 0.05$ ) with equally the same specific gravity values ( $p > 0.05$ ). The *toman* fish oil was considered to be a better quality by-product than *haruan* fish oil as it shows lower peroxide value (PV), free fatty acid (FFA) value and acid value (AV) in which signify a good and healthy nutritional content attained. The *haruan* and *toman* fish oils depicted a relatively similar fatty acid profiles. The availability of seven major fatty acid constituents which are dodecanoic acid, 9-hexadecenoic acid, hexadecanoic acid, linoleic acid, oleic acid, octadecanoic acid and docosahexaenoic acid presented in both fish oils would possibly be an essential compound for therapeutic application (e.g. wound healing) even though the arachidonic acid (the main precursor for wound healing process) was presented with an

infinitesimal concentration. For that reason, it is strongly suggested that to utilize both of the aqueous and soxhlet extraction technique sequentially as to retain and optimize the availability of the arachidonic acid and other aforementioned fatty acids prior to any efficacy studies on wound healing for therapeutic consumption (e.g. 3-D biomimicry cell culture).

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## Conflicts of Interest

No data were used elsewhere to support this study and it was entirely a new set of data.

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