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Effects of wet rendering extraction on the fatty acid and physicochemical profiles of catfish (*Pangasius micronema* Blkr.), milkfish (*Chanos chanos* Forsskal.) and snakehead fish (*Chana striata* Bloch)

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Abstract

The catfish (*Pangasius micronema* Blkr.), milkfish (*Chanos chanos* Forsskal.) and snakehead (*Chana striata* Bloch.) are the most important fishing commodities in Indonesia. Its fatty acid content makes it a possible source of fish oil for nutritional supplement use. The extraction method influenced the quality of the fish oil produced. Wet rendering, also known as wet extraction, is the most popular extraction method for separating fish oil. The purpose of this research is to investigate how wet rendering extraction affects the fatty acid content and physicochemical profiles of catfish, milkfish and snakehead fish. The fatty acid profile was determined with a Gas Chromatography-Flame Ionization Detector (GC-FID). The quality of fish oil was determined according to the standard method of the Association of Official Analytical Collaboration (AOAC) International protocol. The results of this study indicated that the wet rendering extraction method produces various fatty acid content and physicochemical characterizations of catfish, milkfish, and snakehead fish oils. The acid value of catfish oil fulfilled the fish oil standards. In terms of peroxide value, milkfish and snakehead fish oil satisfied the criteria. In the iodine value parameter, snakehead fish oil matched the standard, while catfish oil and milkfish oil met the saponification value parameter. The highest content of omega-3 fatty acids was found in snakehead fish oil, while the highest levels of omega-6 and omega-9 were reported in milkfish oil and catfish oil, respectively.

1. Introduction

Indonesia is a maritime country that has many species of fish such as catfish, milkfish, and snakehead fish. Catfish is included in the top 5 Indonesian freshwater fishery products based on data from the Ministry of Fisheries and Marine Affairs, Republic of Indonesia (Sasongko *et al*., 2022). Catfish oil is potential as a source of omega-3 unsaturated fatty acids which can be seen from the analysis of the fat content of catfish, which is 31% (Hashim *et al*., 2015). The milkfish is one of the fish species that are distributed in tropical and subtropical waters of the Indo-Pacific. This fish can grow and live in various environmental conditions (Ali, 2017). Milkfish had high nutritional content, including 20.37% protein, 3.84% fat, and 4.02% ash (Arisky *et al*., 2021). Milkfish is considered a "fatty" fish because, in terms of body weight, this fish is considered to contain a lot of fat, so it includes fish that have the potential to produce fish oil well (Hidayah *et al*., 2022). Snakehead fish are a species of freshwater fish that is commonly utilized for medicinal purposes. The extract of snakehead fish fillet has been shown to have hepatoprotective properties in a rat model of oxidative stress (Suhartono *et al*., 2013), wound healing activity (Daisa *et al*., 2017), and by boosting the number of fibroblast cells (Atmajaya *et al*., 2019). Fish oil has been extensively researched in the production of dietary supplements and medicines. Many studies have discussed the benefits of fish oil, including for heart health, inflammation, cancer, diabetes, and other metabolic diseases (Keapai *et al*.,

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2016; Monk *et al*., 2021; Guo *et al*., 2022). Fish oil is a rich source of polyunsaturated fatty acids (PUFA) or double-bonded unsaturated fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Głowacz-Różyńska *et al*., 2016).

Fish oil is a component of fat in fish body tissues and is obtained by extraction (Fitriani *et al*., 2021). The quality of pure fish oil produced is influenced by the extraction method (Arisky *et al*., 2021). Measurement of physicochemical profiles (acid value, peroxide value, iodine value, and saponification value) is one way to assess the quality of fish oil (Sasongko *et al*., 2022). Nowadays, green extraction processes are widely used because they can reduce energy consumption, allow the use of substitute solvents and natural ingredients in a sustainable manner, and produce extracts or products of high quality while minimizing their impact on the environment (Runge *et al*., 2017). Several methods have been applied for the extraction of oils namely hydrolysis, rendering, ultrasound, microwave, and extraction with the help of pressure (Yusoh *et al*., 2022). Arisky *et al*. (2021) reported that the rendering method is the most used technique to get fish oil. The principle of the wet rendering method is boiling and pressing using water, while the dry rendering process does not require water to release the oil, but instead removes water from the material so that it is hoped that more oil will be obtained. The wet rendering method is commonly used because it is affordable, convenient to use, and can increase the extraction yield (Taati *et al*., 2018). Using literature review, the study on the comparison of fatty acid profile and quality of fish oil using the wet rendering method of several types of fish is limited. The novelty of this study is presenting the fatty acid properties of fish oil from catfish, milkfish, and snakehead fish. Therefore, this study aimed to find out how wet rendering extraction affects the fatty acid and physicochemical profiles of catfish, milkfish, and snakehead fish.

2. Materials and methods

2.1 Sample preparation

Catfish, milkfish, and snakehead fish weighing 400–800 g/head are purchased in the local fish market in Juwana Pati, Central Java, Indonesia. All fish are cleaned, sorted into flesh and intestines, and then cut into little pieces. All fish bodies were extracted separately for 60 mins at boiling temperature using aquadest solvent 1:3 and the wet rendering method. Using a separatory funnel, the extracted liquid is split into the oil and water phases. The solids that remain after filtering are pressed and the liquid that comes out is mixed with the liquid that needs to be separated. Before being refined, the crude fish oil is stored in a dark bottle at 2-8°C (Hastarini *et al*., 2012).

2.2 Fish oil refining

The crude fish oil thus produced is subsequently refined to produce pure fish oil. This purification method comprises the heating procedure, the addition of adsorbents, and vacuum filtration. When the temperature reaches 55-60°C, the oil is bleached using 1% bentonite by weight. Then, for 30 mins, the substance is heated until it reaches 80°C. The oil is filtered using a vacuum filter and the produced oil is weighed to determine the yield of pure fish oil (Hastarini *et al*., 2012).

2.3 Determination of fatty acid profile by Gas Chromatography-Flame Ionization Detector

Fatty acid methyl ester (FAME) was made from catfish, milkfish, and snakehead fish oils to find out what kinds of fatty acids they contained. A 1.5 mL of methanolic-sodium was mixed with 0.5 mL of oil sample. The solution was homogenized, heated for 5-10 mins at 60°C, and then cooled. After adding 2 mL of boron trifluoride (BF3), the mixture was heated for 5-10 mins at 60°C before being cooled. A volume of 1.0 mL heptane and 1.0 mL saturated NaCl were used to extract the sample. The top surface is carefully collected, and 1 L sample solution is fed into the Gas Chromatography-Flame Ionization Detector (GC-FID) (Rohman and Riyanto, 2020).

2.4 Measurement of physicochemical fish oil

The quality of fish oil was determined according to the standard method of the Association of Official Analytical Collaboration (AOAC) International protocol. The parameters measured are acid value, peroxide value, iodine value, and saponification value (AOAC, 2000).

2.5 Data analysis

The values are shown as the mean ± standard deviation (SD) of three separate measurements. Physicochemical values were analyzed statistically with an analysis of variance (ANOVA) followed by a post hoc LSD test, and a significance level of $p<0.05$ was established. Normality and homogeneity tests were carried out before the ANOVA test.

3. Results and discussion

In this research, the wet rendering method was used to process the fish oils from catfish, milkfish, and snakehead fish (Hastarini *et al*., 2012). Organoleptically, the fish oil produced looks yellow-brown, and the catfish oil looks clearer after the addition of 1% bentonite (Figure 1). The oils produced were then subjected to physicochemical characterization. Figure 2 reveals the

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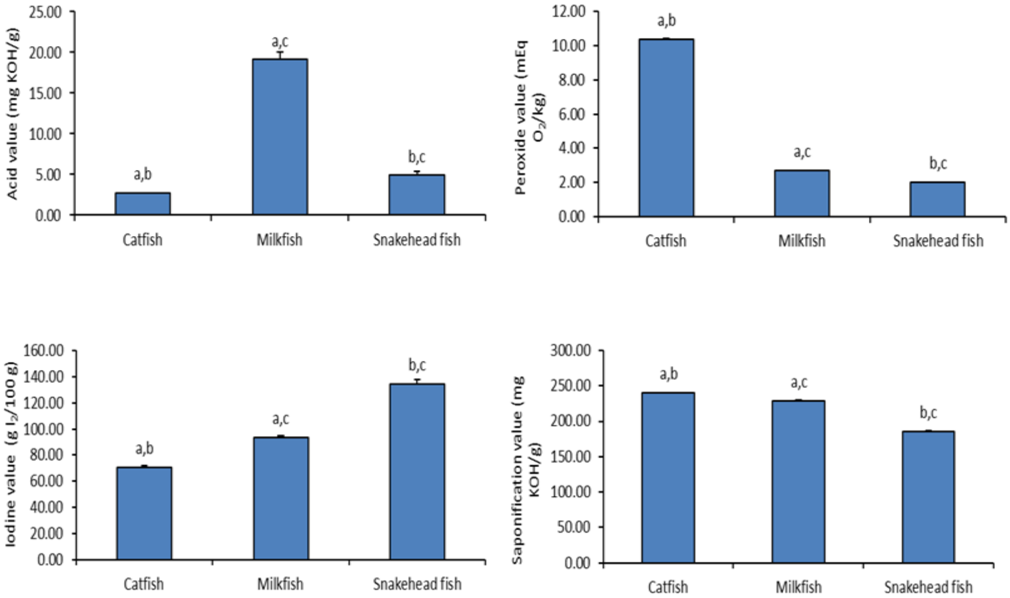

Figure 1. Physicochemical properties of catfish, milkfish, and snakehead fish. The mean values with the same letters showed no significant difference ($p \geq 0.05$).

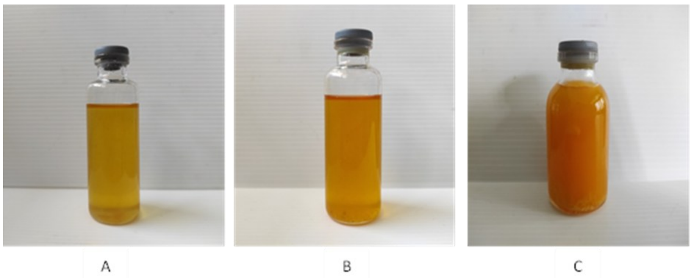

Figure 2. The fish oil extraction results after the refining process. (A) catfish, (B) milkfish and (C) snakehead fish.

characteristics of the physicochemical properties of fish oils. Acid value, saponification value, iodine value, peroxide value, and fatty acid are parameters of oil quality (Syifa *et al*., 2022). There are significant differences in each physicochemical parameter in catfish, milkfish, and snakehead fish oils ($p<0.05$). The acid value is a measure of free fatty acid (FFA) content in the oil or fat. FFA are produced by the triacylglycerol hydrolysis process (Hidayah *et al*., 2022). A low acid number indicates an acceptable quality of fish oil (Syifa *et al*., 2022). The acid number is a value that expresses the number of mg of KOH required in 1 g of oil or fat (Putri *et al*., 2020). The results of the acid number in catfish oil, namely 2.72±0.01 mg KOH/g; milkfish oil is 19.11±0.96 mg KOH/g; and snakehead fish oil, which is 4.89±0.47 mg KOH/g. According to FAO (2017) concerning fish oil standards, the value of a good acid number is 3 mg KOH/g (FAO, 2017). Based on the results, catfish oil had the best acid number.

The measurement of peroxide number is used as an indicator of the levels of peroxides and hydroperoxides formed in the early stages of the fat oxidation reaction (Salih *et al*., 2021). The peroxide number is one of the indicator parameters of fish oil quality. The peroxide number is used to determine the level of degradation or rancidity of fish oil, where the higher the peroxide number, the lower the quality of the oil (Putri *et al*., 2020). If the peroxide number is higher, then the oil is not safe for consumption because it is easily degraded and becomes rancid. In this test, the results for the peroxide value of catfish oil were 10.36±0.09 mEq O_2/kg; milkfish oil was 2.68±0.04 mEq O_2/kg; and snakehead fish oil was 2.02±0.01 mEq O_2/kg. Based on the Food and Agriculture Organization (FAO), the standard fish oil peroxide value is < 5 mEq O_2/kg (FAO, 2017). The milkfish and snakehead fish oil samples in this study met the standards of the FAO. The iodine value is a measure of the amount of unsaturated fatty acids that indicate the degree of unsaturation in the oil (Salih *et al*., 2021). The higher the iodine value, the higher the degree of unsaturation and the better the quality of the oil (Indah *et al*., 2022). The iodine value obtained by catfish oil was 70.69±1.03, milkfish oil was 93.20±1.41, and snakehead fish oil was 134.74±3.26. Based on the International Fish Oil Standard (IFOS), the iodine value is said to be good, namely 95-118 g I_2/100 g oil (Indah *et al*., 2022). From the test results, fish oil that has a good iodine number is snakehead fish. The saponification value is a value that expresses the amount of potassium hydroxide (KOH) used to make 1 g of fat or oil. The saponification value measures the molecular weight of the fatty acids contained in the oil (Putri *et al*., 2020). Oils composed of short carbon chain fatty acids have relatively small molecular weights so that they have a large saponification number, and vice versa. The saponification values obtained for catfish oil were 240.17±0.75 mg KOH/g; milkfish oil, which is 228.94±1.49 mg KOH/g; and snakehead oil, which is 185.61±1.02 mg KOH/g. According to the Indonesian Food and Drug Authority, the standard for fish oil saponification is at least 195 mg KOH/g (Sasongko *et*

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al., 2022). Thus, the oils whose saponification number meets the standard are catfish oil and milkfish oil.

Table 1 shows the fatty acid composition of catfish, milkfish, and snakehead fish oils. Catfish oil contains fatty acids detected in large amounts such as linoleic acid (41.88%), c-oleic acid (38.08%), palmitic acid (22.88%), and stearic acid (8.08%). In milkfish oil, detected in large amounts are palmitic acid (27.59%), c-oleic acid (24.13%), and linoleic acid (11.63%). Meanwhile, in snakehead fish oil, eicosapentaenoic acid (35.92%), c-oleic acid (35.68%), heptadecanoic acid (14.89%), and palmitic acid (14.14%). The amounts of omega-3, omega -6, and omega-9 in catfish oil were 1.73%, 12.99%, and 38.12%. Milkfish were 3.98%, 13.89%, and 24.28%. Meanwhile, snakehead fish oil amounted to 8.72%, 9.90%, and 35.72%. The results showed that omega-3 fatty acids are often claimed to have the most pharmacological effects on snakehead fish oil. Omega-3 fatty acids are precursors to anti-inflammatory compounds that have beneficial effects on chronic inflammatory disorders such as diabetes, cancer, and ischemic heart disease (Saini and Keum, 2018; Shahidi and Ambigaipalan, 2018; Yang *et al*., 2019). The source of fish and the method of extraction greatly affect the fish oil produced (Sasongko *et al*., 2022). Another study showed the omega-3 content in catfish oil by maceration method (3.08%) (Putri *et al*., 2019), and dry rendering (0.79%) (Lestari *et al*., 2020). Omega-3 contents in snakehead fish oil using the maceration method (7.83%) (Putri *et al*., 2019), direct pressing method (4.52%)

Table 1. Wet rendering extraction method on fatty acid profile of catfish, milkfish, and snakehead fish oils.

Fatty acid profile	Fish oil (%)		
	Catfish	Milkfish	Snakehead fish
Linoleic Acid (C 18:2)	41.88±0.01	11.63±0.01	8.00±0.01
C-Linoleic Acid (C 18:2)	11.83±0.11	11.63±0.01	8.00±0.01
C-Oleic Acid (C 18:1)	38.08±0.08	24.13±0.10	35.68±0.02
Eicosapentaenoic Acid (EPA) (C 20:5)	0.33±0.01	1.34±0.01	0.32±0.02
Heptadecanoic Acid (C 17:1)	0.12±0.01	0.65±0.01	0.89±0.01
Palmitoleic Acid (C 16:1)	1.37±0.04	3.79±0.04	4.40±0.09
Arachidonic Acid (C 20:4)	0.43±0.01	0.96±0.01	1.26±0.01
Pentadecenoic Acid (C 15:1)	0.04±0.01	0.12±0.01	0.27±0.01
Eicosatrienoic Acid (C 20:3)	0.74±0.01	1.30±0.01	0.65±0.01
Omega-6 Fatty Acids	12.99±0.12	13.89±0.01	9.90±0.02
Myrisroleic Acid (C 14:1)	0.07±0.01	0.20±0.01	0.23±0.01
Eicosadienoic Acid (C 20:2)	0.75±0.01	1.54±0.01	0.51±0.01
Tridecanoic Acid (C 13:0)	0.02±0.01	0.05±0.01	0.04±0.01
Omega 3 Fatty Acids	1.73±0.01	3.98±0.01	8.72±0.02
Linolenic Acid (C 18:3)	0.86±0.01	0.87±0.01	6.13±0.01
Lignoceric Acid (C 24:0)	0.05±0.01	0.09±0.01	0.05±0.01
Polyunsaturated Fat	15.47±0.13	19.40±0.01	19.13±0.04
Docosahexanoic Acid (DHA) (C 22:6)	0.45±0.01	1.50±0.02	1.83±0.03
Stearic Acid (C 18:0)	8.08±0.02	6.17±0.03	3.86±0.01
Eicosatrienoic Acid (C 20:3)	0.08±0.01	0.27±0.01	0.44±0.01
Heptadecanoic Acid (C 17:0)	0.26±0.01	0.82±0.01	14.89±0.02
Erucic Acid (C 22:1)	0.04±0.01	0.15±0.01	0.04±0.01
Palmitic Acid (C 16:0)	28.88±0.02	27.59±0.01	14.14±0.01
Unsaturated Fatty Acid	56.31±0.01	50.71±0.04	61.55±0.03
Omega 9 Fatty Acids	38.12±0.08	24.28±0.10	35.72±0.02
Pentadecanoic Acid (C 15:0)	0.22±0.01	0.96±0.01	1.21±0.01
AA	0.43±0.01	0.96±0.01	1.26±0.01
Myristic Acid (C 14:0)	3.92±0.01	6.50±0.03	1.30±0.01
Lauric Acid (C 12:0)	0.24±0.01	4.24±0.04	0.11±0.01
Eicocenoic Acid (C 20:1)	1.13±0.01	2.27±0.02	0.90±0.01
Monounsaturated Fatty Acid	40.84±0.12	31.31±0.03	42.42±0.07
Capric Acid (C 10:0)	-	0.06±0.01	-
Arachitic Acid (C 20:0)	0.21±0.01	0.31±0.01	0.32±0.01
Saturated Fatty acids	41.88±0.01	46.79±0.05	35.92±0.02

(Syifa *et al*., 2022), and pressurized boiling (3.25%) (Pasaribu *et al*., 2020).

4. Conclusion

The results of this study show that the wet rendering method still makes fish oil that does not meet standards for physicochemical properties. The highest content of omega-3 fatty acids is found in snakehead fish oil, omega-6 in milkfish oil, and omega-9 in catfish oil. In future studies, it is necessary to optimize a wider range of fish sources in order to obtain more complete physicochemical properties and fatty acid compositions. In addition, the type and amount of adsorbent greatly affect the quality of the fish oil produced.

Conflict of interest

The authors declare no conflict of interest.

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