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Chemical and Fatty Acid Characterization of the Penja Fish (*Sicyopterus parvei*) Oil in Budong-Budong River Estuary, West Sulawesi, Indonesia

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ABSTRACT

The Penja, *Sicyopterus parvei*, is an endemic fish species in the Budong-Budong River, Central Mamuju, West Java, Indonesia. This species has a high nutritional content for human health. However, there is no research regarding the characterization of penja fish. This study aimed to determine the chemical quality and characteristics of the penja fish oil (*Sicyopterus parvei*) from the Budong-Budong River Estuary. Penja fish samples were extracted with 96% ethanol, purified and bleached with 3% bentonite. The yield and chemical characteristics of penja fish oil, including acid number, free fatty acid number, anisidine and peroxide value were determined. The characteristics of penja fish fatty acid were assessed using GCMS (Gas Chromatography-Mass Spectrometry). All chemical characteristics of penja fish crude oil were higher than penja fish pure oil, including oil yield (8.55% vs 2.83%), acid number (50.75% vs 19.40%), free fatty acids (19.35% vs 9.56%), peroxide value (8.51 vs 2.58 meq/kg), anisidin value (4.14 vs 2.38 meq/kg) and total oxidation (20.95 vs 7.38 meq/kg). The fatty acid of penja fish recorded 10.62% PUFA, 19.03% MUFA and 35.3% SFA. Besides, penja fish contain omega 3, viz., DHA and EPA. The quality of the penja fish oil after purification was better than that before purification. Free fatty acids did not meet either the Indonesian National Standard (SNI) nor the International Fish Oil Standards (IFOS), while the peroxide value, anisidin number and total oxidation met both standards.

INTRODUCTION

Penja (*Sicyopterus parvei*) is an endemic aquatic organism in the Budong-Budong River, Central Mamuju, West Java, Indonesia. *Sicyopterus parvei* is a typical small pelagic fish species usually caught by fishermen in West Sulawesi, especially, Mamuju **(Muthiadin *et al*., 2020)**, Polewali Mandar **(Rahman *et al*., 2020)** and Central Mamuju **(Astuti *et al*., 2022)**. Penja fish use plankton and other microalgae as a growth-promoting diet. They are commonly found in river estuaries and along the coast. Many rivers that flow into the sea transport abundant nutrients for aquatic organisms such as zooplankton and fish. Fish resources offer enormous potential to improve the nation's consumption of animal protein. The high level of fish fat adds value to the fish oil. Fish oil derived from fisheries' products contains long-chain unsaturated fatty acids, with 14-24 carbon atoms and I-6 double bonds, including EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) **(Permana & Citroreksoko, 2003)**.

Fish are the best food source of fatty acids (EPA and DHA), especially sardines, mackerel, anchovies and various salmon species. These fish have an n-3 to n-6 fatty acid ratio close to 7. Fish synthesize fatty acids by consuming algae and plankton. The availability of natural food is the most important factor in the growth and development of river fish. Natural foods haven't been entirely substituted until now. Although there are considerable interspecific and intraspecific variations in the fatty acid composition, fish represent a significant omega-3 (PUFA) source in the human diet. Several factors have been shown to influence the amount of PUFA **(Falk-Petersen *et al.*, 1998)**.

Penja fish are a food ingredient with a high nutritional value. The fat content of Penja fish ranges from 8 to 10% **(Jayadi & Rahman, 2018; Fajriana & Ma'rifatullah, 2019)**. Fish have different fatty acid profiles based on their species, age, diet, sex, reproductive cycle and environmental parameters, such as habitat, salinity, temperature, season and climate **(Všetičková *et al.*, 2020)**. Fish oil is a fish product essential for human nutrition. Fish oil is easily obtained naturally or by taking fish oil supplements from sea fish. To the best of our knowledge, there is no research regarding the characterization of penja fish oil, especially in the Budong-Budong River, West Sulawesi, Indonesia. Therefore, this study aimed to determine the chemical quality and fatty acid characteristics of penja fish (*Syciopterus parvei)* in the Budong-Budong River Estuary.

MATERIALS AND METHODS

1. Time and location of research

This research was conducted in June-August 2022. Penja fish samples were collected from the Budong-Budong River Estuary, Babana village, Budong-Budong sub-district, Central Mamuju, West Sulawesi, Indonesia (Fig. 1).

2. Penja fish oil extraction

Fish oil was extracted using maceration or immersion method with 96% ethanol **(Handayani & Nurcahyanti, 2015)**. The fish were weighed as much as 250g and then dried in the oven at 50ºC for 5h. The dried samples were then weighed and blended to obtain the yield of fish meal. Furthermore, the fish meal was soaked in 96% ethanol for 24h. The solvent was separated using a separatory funnel coated with filter paper no. 41. The filtrate was then evaporated using a rotary evaporator until obtaining a crude extract. Purification of fish oil using the method of **Suseno *et al*. (2014)**, which was modified.

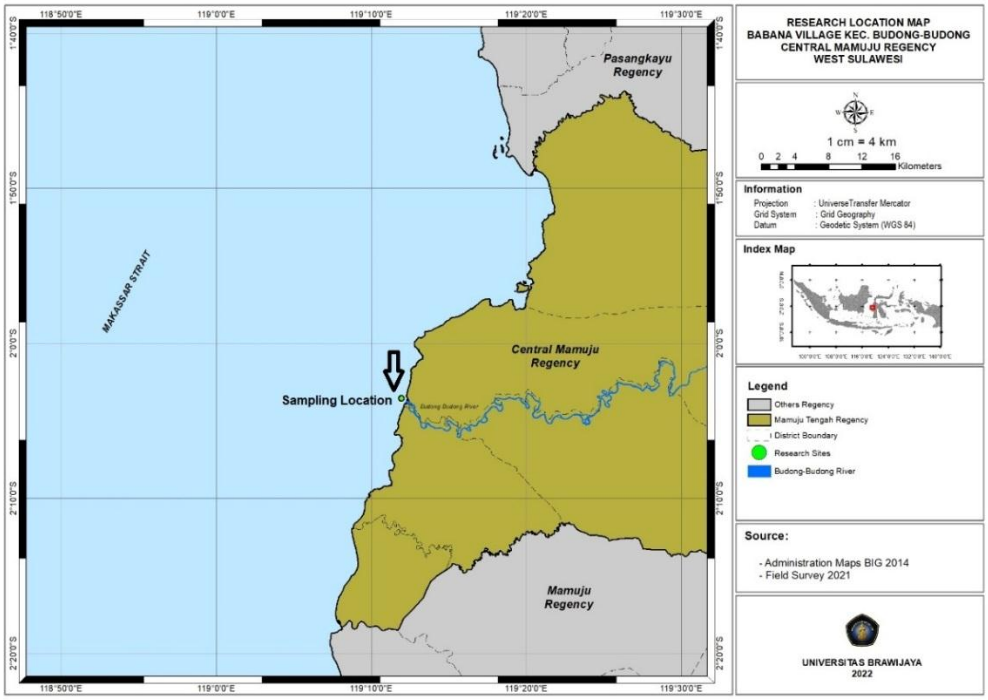

Fig. 1. Sampling location (arrow) of penja fish in Budong-Budong River Estuary, Central Mamuju, West Sulawesi, Indonesia

3. Chemical characteristics

The yield and chemical characteristics of penja fish oil were carried out at the Ujung Pandang State Polytechnic and Brawijaya University, Malang. The chemical characteristics of penja fish oil, including acid number, free fatty acid number, anisidine and peroxide value were determined.

3.1. Fish oil yield

Yield is the percentage of oil produced from the sample processing process. Oil yield can be calculated using the following formula:

$$Oil\ yield(\%) = \frac{Produced\ oil}{Total\ sample} \times 100\%$$

3.2. Acid number

The acid number is the amount of free fatty acids calculated by the size of the fatty acid molecule or a mixture of fatty acids. The acid number was expressed as 0.1 N KOH mg used to neutralize free fatty acids in 1g of oil. The acid value was calculated using the following formula:

$$Acid\ number\ (^{meq}/_{kg}) = \frac{A \times N \times 56.1}{Amount\ pf\ sample\ (g)}$$

Where,

A : Number of Mol KOH for titration;

N : Normality of KOH solution, and

56.1 : Molecular weight of KOH

3.3. Free fatty acids analysis

Free fatty acids are fatty acids that exist as unbound free acids as triglycerides. Free fatty acids produced by hydrolysis and oxidation processes usually combine with neutral fats.

$$\%\ of\ free\ fatty\ acid = \frac{25.6 \times V \times N}{W} \times 100\%$$

Where,

25.6 : Mr of oil, and

W : Sample weight

3.4. Peroxide value

Peroxide value (PV) is the most important value to determine the degree of damage to the oil or grease. Saturated fatty acids can bind oxygen to their double bonds to form peroxides. Peroxide can be determined by the iodometry method with the following formula:

$$Peroxide\ Value\ ({}^{meq}/_{kg}) = \frac{A \times N \times 1000}{Number\ of\ samples\ (g)}$$

Note:

A = $Na_2S_2O_3$ volume (ml);

N = $Na_2S_2O_3$ solution normality

3.5. Anisidine value

The p-anisidine value is one of the parameters for determining the number of aldehydes in enal and dienal forms in oil, providing information on the number of secondary oxidation products. The aldehyde compound is formed from the oxidation process in oil.

$$Anisidine\ value\ ({}^{meq}/_{kg}) = \frac{25\ mL \times (1.2 \times A2 - A1)}{m}$$

Where,

A1 = absorbance of the test solution 1;

A2 = absorbance of the test solution 2, and

m = fish oil sample weight (g) = 2 g

3.6. Total oxidation

The total oxidation value (TOTOX) determines all oil oxidation parameters. TOTOX is determined from the sum of 2 times the primary and secondary oxidation in the oil, as follows:

$$Total\ oxidation\ value\ ({}^{meq}/_{kg}) = (2PV + AnV)$$

Where,

PV : Peroxide value (meq/kg), and

AnV : Anisidine value (meq/kg)

4. Characterization of fatty acids of penja fish oil

The characteristics of penja fish fatty acid were determined using the GCMS (Gas Chromatography Mass Spectrometry). Shimadzu Gas Chromatography (GC-2010) used had a flame ionization detector (FID) and a Thermo Scientific column (TR-FAME) with a length of 30m, a diameter of 0.25mm, and a film thickness of 0.25µm. The column temperature started at 120°C for 7 min, and then increased to 250°C with a temperature rise of 10°C/ min and maintained for 20min at a constant temperature of 250°C. The detector temperature is 270°C. The injection temperature was heated to 260°C, equipped with a 1/10 split injector, and helium carrier gas pressure set at 75 kPa. 1µL sample was added to the injection site **(Pontoh, 2019)**.

RESULTS AND DISCUSSION

1. Yield and chemical properties of penja fish oil (*Sicyopterus parvei*)

The fish oil extraction process could separate oil or fat from fish meat with high-fat content. The results of the extraction of penja fish oil can be seen in Fig. (2). The color and odor of fish oil before purification were yellowish brown and had a very pungent odor influenced by the penja fish species. While after purification, the color became darker, influenced by the purifying agent. Natural materials (3% bentonite) were used during purification. After purification, the odor of fish oil was no longer pungent **(Sabar *et al*., 2015)**. The coloring matter in fish oil waste consisted of two groups: natural coloring matter and coloring matter from the degradation of natural coloring matter. Natural coloring matter is naturally present in materials containing oil and is extracted together with oil during extraction **(Ketaren, 1986)**. Although good quality oil is golden yellow, the characteristic quality of fish oil after purification is better than before purification.

In addition, the results showed that penja fish crude oil yield was higher than pure oil (8.55% vs 2.83%) (Table 1). The yield is the percentage of the primary raw materials that can be used as the final product or the ratio of the final product to the primary raw materials. The higher the yield indicated, the more oil produced. The quality of the resulting oil extraction process is generally inversely proportional to the yield presentation **(Apituley *et al*., 2020)**. Fish oil was extracted with 96% ethanol because it has a wide solubility from non-polar compounds to polar compounds, and compounds in the oil are included in the compounds that dissolve in non-polar solvents **(Jayadi & Rahman, 2018; Luthfi & Jerry, 2021)**.

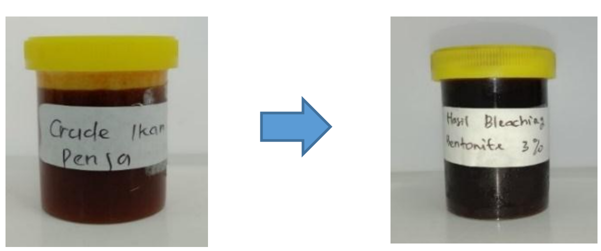

Fig. 2. Penja fish oil (*Sicyopterus parvei*)

Analysis of acid number, free fatty acid, peroxide value and anisidin value was carried out to determine the quality of fish oil. The crude penja fish oil exhibited a higher acid than pure oil (50.75% vs 19.40%) (Table 1). While, the crude penja fish oil also has a high free fatty acid (FFA) level, compared to pure fish oil (19.35% vs 9.56%). The peroxide value, anisidin number and total oxidation were also high in crude fish oil than in pure oil (Table 1). The quality of crude and pure penja fish oil did not meet fish oil quality standards due to high free fatty acid content. The high free fatty acid in crude fish oil was caused by foodstuffs' damage with high moisture content. When fish is handled improperly, microorganisms can grow quickly, simplifying the oxidation process. The activity of microorganisms on the food material enhanced oil's free fatty acid levels greatly. Microorganisms produce lipase enzymes as biocatalysts for oil hydrolysis reactions to produce glycerol and free fatty acids **(Rahman *et al*., 2020)**.

Table 1. Chemical properties of penja fish oil (*Sicyopterus parvei*)

Chemical Property	Penja fish oil	
	Crude	Pure
Oil yield (%)	8.55 ± 0.08	2.83 ± 0.29
AN (%)	50.75 ± 0.07	19.40 ± 0,48
FFA (%)	19.35 ± 0.27	9.56 ± 0.33
PV (meq/kg)	8.51 ± 0.29	2.58 ± 0.33
AV (meq/kg)	4.14 ± 0.11	2.38 ± 0.39
TOTOX (meq/kg)	20.95 ± 0.75	7.38 ± 0.50

Note: Data were expressed as mean ± standard deviation. OY: Oil yield, AN: Acid number, FFA: Free fatty acids, PV: Peroxide value, AV: Aniside value, TOTOX: Total oxidation.

The lipase enzyme can break down fat into FFA and glycerol. In the digestive system of fish, the activity of this lipase enzyme is strongly influenced by the protein content in fish food **(Ramlah *et al*., 2017)**. The acid number describes the amount of FFA content in the oil, which is formed due to the hydrolysis reaction of the oil triacylglycerol. The international fish oil standard for acid value in oil is ≤ 3 mg KOH/g **(WHO, 2017)**. A large acid number indicates the formation of a large FFA from oil hydrolysis. Acid number and peroxidase value are directly inversely correlated with the quality of fish oil. The higher the acid number and peroxidase value, the lower the quality of the fish oil **(Panagan *et al*., 2011)**.

The extraction method and extraction time had no significant effect on the acid number of fish oil (**(Panagan *et al*., 2011)**. However, oil damage can occur during the handling process and storage. Fat or oil damage causes a rancid odor and bad taste, reducing quality and nutritional value. Therefore, to avoid this damage, the oil must be purified. The process of purifying penja fish oil is carried out in 2 stages, including centrifugation and bleaching with absorbent (bentonite).

Pure fish oil has a low free fatty acid (9.56%), peroxide value (2.58 meq/kg) and anisidin number (2.38 meq/kg) (Table 1). Oil purifying can reduce the percentage of acid number and free fatty acid level but does not meet fish oil quality standards. Purifying fish oil by centrifugation and bleaching methods with 3% absorbent (bentonite) can reduce free fatty acid value by 9.56%. Previous research stated that purification by centrifugation and bleaching methods with bentonite could reduce FFA in sardine fish oil by 16% **(Suseno e*t al*., 2014)**.

Likewise, the peroxide value is a parameter to determine the quality of fish oil since peroxide value is the most important value in determining the degree of oil damage. Unsaturated fatty acids can bind oxygen to the double bond to form peroxides which cause rancidity. The smaller the peroxide, the better the quality of the oil. The extraction method affects the peroxide value **(Putri *et al.,* 2020)**. In addition, peroxidase value is formed due to heating which causes damage. High peroxides can accelerate the process of causing rancid odors and unwanted flavors from food ingredients. The international fish oil standard for peroxidase value is ≤ 5meq/ kg **(WHO, 2017)**. If the amount of peroxide in food exceeds 100meq/ kg, it will be toxic and cannot be consumed **(Rahman *et al*., 2020)**.

Apart from peroxidase value, fish oil's anisidin (AV) number and total oxidation after purifying were also decreased. The anisidine value determines the amount of secondary oxidation products. The anisidin value does not always correspond to the high peroxide value **(Guillén & Cabo, 2002)**. However, the high peroxide value can cause the anisidin number to increase if the process given to the fish oil allows for further degradation **(Pramestia *et al*., 2015)**.

The anisidin value of penja fish oil was very good (Table 1). The anisidine values of crude penja fish oil were 4.14meq/ kg and 2.38meq/ kg after purification. This is in accordance with the international fish oil standards (≤ 20meq/ kg) **(WHO, 2017)**. Centrifugation and bleaching purification methods with bentonite affect greatly the decrease in anisidin numbers. These results align with previous studies reporting that the oil purification using bentonite can reduce the anisidine number in catfish oil **(Nurbayasari *et al.,* 2017; Sembiring *et al*., 2018)**. Purification materials using clay minerals (bentonite) heated and dissolved in a certain amount and duration of time were able to maximize the adsorption power of impurities in accordance with the natural structure of bentonite **(Dari *et al*., 2017; Meirawaty *et al*., 2021)**.

The total oxidation value is a determinant of all oil oxidation parameters. Total oxidation of penja fish oil before and after purifying was 20.95 and 7.38meq/ kg, respectively. Purification by centrifugation and bleaching (bentonite) stages reduced the total oxidation of penja fish oil. **Feryana *et al.* (2014)** and **Sembiring *et al.* (2018)** stated that pure fish oil has lower total oxidation compared to crude oil. The total oxidation of penja fish oil is in accordance with the finding of **Bimbo (1998)**, which was 10- 60meq/ kg, and that of **WHO (2017)**, which was ≤ 26meq/ kg.

2. Fatty acid characterization of fish oil

Fish oil generally consists of various types of triacylglycerol in the form of a molecule composed of glycerol and fatty acids. The fatty acid chains in fish oil have more than eighteen carbon atoms and 5 or 6 double bonds. Penja fish individual has a high fatty acids' content, such as saturated fatty acids (SFA 38.00%), monounsaturated fatty acids (MUFA 18.82%) and polyunsaturated fatty acids (PUFA 11.92%) (Table 1). The high values of the saturated fatty acids in fish coincide with those recorded in the study of **Osman *et al*. (2001)** who elucidated that the most abundant saturated fatty acids were found in fish fat. The main fatty acids present in the fish body are palmitic acid and DHA **(Josephus *et al*., 2019)**. The variation in fish fatty acid profiles differed among species.

In addition, water environment with different ecological conditions is an important source of various nutritional components.

Table 1. Characterization of penja fish fatty acids (*Sicyopterus* spp.)

Fatty acid	Name of fatty acid	Maceration	
		Estuary %	**River %**
Saturated Fatty Acids (SFA)			
C:14-0	Myristic Acid	4.66	0.67
C:15-0	Pentadecanoic Acid	0.61	0.27
C:16-0	Palmitic Acid	23.78	10.66
C:17-0	Heptadecanoic Acid	1.00	0.00
C:18-0	Stearic Acid	7.95	4.34
Total		38	15.94
MUFA + PUFA			
C:16-1	Palmitoleic Acid	1.59	3.42
C:18-1	Oleic Acid W-9	16.33	14.00
C:20-3	Eicosatrienoic Acid W-3	0.21	0.65
C:20-4	Arachidonic Acid W-6	0.90	0.00
C:20-5	Eicosapentaenoic acid W-3 (EPA)	1.21	1.9
C:22-6	Decosahexaenoic Acid W-3 (DHA)	10.5	9.37
Total Monounsaturated Fatty Acids (MUFA)		18.82	17.42
Total Polyunsaturated Fatty Acids (PUFA)		11.92	11.92
Total saturated + unsaturated fatty acids (MUFA+PUFA)		30.74	29.34

Fishing season, size and reproductive status of individuals of the same species living in an area influence this variation. Moreover, the cultivation conditions and the feed used in fish farming cause variations in the fatty acid composition of fish **(Taşbozan & Gökçe, 2017)**. Phytoplankton is a primary source of nutrients in fish food due to its high number of phytonutrients and biologically active ingredients, including fatty acids, amino acids, sterols, organic minerals, enzymes, carotenoids, chlorophyll, trace elements and vitamins. Regarding valuable phytoplankton fatty acids, PUFA accounts for the largest proportion of fatty acids in freshwater and marine chlorophytes, freshwater and marine cryptophytes and marine diatoms (PUFA>SAFA>MUFA), while being the smallest in freshwater cyanobacteria (SAFA>MUFA> PUFAs). This shows how important phytoplankton's structure is in assessing its nutritional value **(Napiórkowska-Krzebietke, 2017)**.

Palmitic acid in penja fish was high, compared to DHA and EPA. This might be attributed to the consuming of phytoplankton and zooplankton by the penja fish, as indicated by plankton identification in the Budong-Budong River between the penja fishing season and before the fishing season. The identification results showed that phytoplankton abundance was higher than zooplankton, especially during the penja fishing season. Fish fed on phytoplankton experience many benefits, such as a significant increase in growth, feed consumption, health and survival. By increasing the nutritional value of all the components in the aquatic food web, phytoplankton is regarded as an important source of fats, especially omega-3 (n-3) and omega-6 (n-6) PUFAs, sterols in addition to essential amino acids.

GC-MS analysis showed that penja fish have omega-3 compounds, such as EPA and DHA (Table 1). The content of DHA in penja fish was higher than EPA. Penja fish consume zooplankton with high DHA content **(Nugraha & Hismayasari, 2011)**. Plankton availability in the Budong-Budong River and the penja fishing season was abundant. The availability of natural food in the river is a special attraction for fish. The abundance of plankton is relatively high during the penja fishing season. This might be due to the rainy season, which brings nutrients from the river flow and then supports the availability of plankton, which uses nutrients as the main factor for phytoplankton growth **(Rahman *et al*., 2020)**.

CONCLUSION

Penja fish oil has free fatty acids above the threshold of quality standards (SNI and IFOS). The peroxide value, anisidin number and total oxidation are in accordance with fish oil quality standards (SNI and IFOS). *Sicyopterus parvei* has high saturated fatty acids (SFA) and unsaturated fatty acids (MUFA and PUFA). Omega 3 fatty acids found in penja fish were docosahexaenoic acid (9.20%) and eicosapentaenoic acid (1.21%).

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