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PHYSICOCHEMICAL CHARACTERIZATION AND FATTY ACID PROFILE OF PATIN (*Pangasius micronema*) FISH OIL AND HARUAN (*Channa striata*) FISH OIL CULTIVATED IN SAMARINDA

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ABSTRACT

Fish oil is one of the nutrients of choice to overcome the prevalence of stunting. Made from Pangasius micronemes, Patin and Haruan fish oil functions as a source of omega-3 or omega-6 fatty acids, in overcoming stunting. Although commercial fish oil production is on the rise, not all products meet International Fish Oil Standards. The study of the chemical properties and fatty acid profile of catfish and Haruan fish oil used experimental methods involving the extraction of fish flesh based on the dry pressing method to produce crude oil and pure oil. Analysis parameters include chemical properties (free fatty acids, peroxide, iodine, and saponification), and linear fatty acid profile. The fish oil RSA activity test was carried out with DPPH. Catfish oil yield 7.80%±0.15%, free fatty acids 1.86±0.02%, peroxide 6.66±0.26meq/kg sample, iodine 82.97±1.85g/100 g sample, and saponification 199.81±3.86 mg KOH/g. Analysis of the total fatty acid concentration of catfish oil, including saturated fatty acids (14.65%), unsaturated fatty acids (85.31%), omega-3 (3.66%), omega-6 (16.18%), and omega-9 (5.82%) and haruan fish oil with a yield of 1.82±0.13%, complete free fatty acids 1.89±0.04%, peroxide 7.13±0.09 meq/kg sample, iodine 111.57±9.4 g/100 g sample, and saponification value 193.28±5.24 mg KOH/g. The results showed that the total fatty acid concentration of haruan fish oil showed saturated fatty acids (23.65%), unsaturated fatty acids (21.31%), and omega-3 (8.3%). The RSA DPPH results were 20.94 ± 1.52% for catfish oil and 23.08% ± 0.50% for haruan fish oil. Based on the results of research, haruan and catfish oil have the potential active ingredients in emulsion preparation to overcome stunting.

Keywords: Physicochemical, Characterization, Fatty Acids, Fils Oil, *Pangasius microneme*, *Channa striata*

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INTRODUCTION

Malnutrition is a condition of imbalances in nutrient intakes such as calories, protein, vitamins, minerals, and other nutrients that are responsible for adequate growth, development, and a strong immune system. Regarded to World Health Organization child growth standards, malnutrition in children encompasses several clinical forms, including stunting (low height-for-age <-2 Standard Deviation (SD), wasting (low weight-for-age <-2 SD), and underweight (low weight-for-height <-2 SD). Based on basic causes of stunting, stunting affected an estimated 22.2% or 150.8 million children under 5 years old; and wasting was estimated to affect about 7.5% or 50.5 million children under 5 years old. Progressivity Indonesia's prevalence of stunting currently attracting world attention. Long-term lack of nutritional intake combined with particular cases of disease which are found in the community frequently causes progressivity of stunting prevalence rate.[1,2] It is supported by related research that the enormous economic progress and development witnessed in Indonesia in the last few decades, still more than 30% of Indonesian children under the age of five suffer from stunting, or low height for age.[2] Previous studies have demonstrated that malnutrition increases the risk of metabolic change.[3] Severe acute malnutrition in childhood: Hormonal and metabolic status at presentation, response to treatment and predictors of mortality and chronic. Stunting is characterized by chronic inflammation in Zimbabwean infants that can increase the risk of infection

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Protein deficiency resulting from low protein intake can disrupt diverse metabolic processes, change physiological responses, and induce cellular disturbances, especially in tissues with a high rate of protein turnover such as the hematopoietic system.[4] East Kalimantan's prevalence stunting rate in 2019 was 28.09 percent and positively back to 22.8 percent in 2021. On another hand, there are four regencies and cities which have the lowest stunting rates on the provincial average, namely, Kutai Kartanegara, Balikpapan City, Mahakam Ulu, and Samarinda East Kalimantan. Meanwhile, for six other districts/cities, East Kutai, PPU, Kukar, Bontang, Berau, and Paser, still out of stunting prevalence percentage of stunting rate is above the provincial average.[5] The related significant long-term impacts are focused on in adulthood, in the shape of reduced cognitive and physical development and a higher risk of metabolic disease.[6] Based on National health survey data released by the Indonesian Ministry of Health survey data indicated a stunting prevalence rate of 37% of Indonesia's population, higher than Malaysia's 20.7% stunting rate.[7] It requires an alternative solution if the stunting prevalence rate does not progress significantly. Several updated ideas for nutrition programs to overcome stunting rates based on the problem of malnutrition, such as using the implementation of biofortification, prebiotic foods, and remedial formulas such as ready-to-use therapeutic foods which require WHO standards are particular alternative solutions to the stunting prevalence rate.[8] The related finding indicates that The highest food groups consumed by toddlers are grains at 740.3 kcal/cap/day, and the lowest oily seeds fruit food group at 7.8 kcal/cap/day.[9] Fish is an animal food containing good quality protein because of its complete content of essential amino acids.[10] Patin fish (*Pangasianodon hypophthalmus*) fillet has an average protein content of about 17.79±0.20%, therefore it has the potential to be processed into fish protein concentrate (FPC) to produce a higher percentage of protein content.[11] Patin fish (*Pangasius micronema*) is well-known to have a high level of protein concentration and has a positive effect on malnutrition. Snakehead fish or haruan were categorized as Channa species under the family of Channidae. This species is a group of freshwater and carnivorous fishes and was very different from others due to its unique snake-head similarity shape. In fact, they have been widely found with a variety of species and consumed as food in many tropical countries such as Malaysia and other Southeast Asian countries.[12] High protein in this fish makes it potential to prevent stunting.[13] The high contents of albumin and omega-3 can accelerate the healing progress of a scratch wound as it is useful for forming new tissue during the growth period.[14] Gabus fish enhances the synthesis of different glycosaminoglycans in healing wounds and increases the rate of wound contraction leading to a quicker healing process. Omega-3 polyunsaturated fatty acid contained in Gabus fish oil (GFO) can regulate prostaglandin synthesis and also influence the immune system.[15] Protein content from Patin Fish protein concentrate was 81.06±0.55% was higher when compared to the average protein content of FPC from other fish species was 57-79%. Protein is a major component in body tissues. It is an essential nutrient in supporting optimal growth, development, weight management, and health.[16] Moreover, Fish oil is an essential fatty acid source that has antioxidant activity, therefore, fish oil could be considered a functional oil added in all types of food and pharmaceutical products. Fish oil has a significant impact on improving human health due presence of several bioactive compounds in fish tissue extracted in fish oil.[17] Moreover, the added value of fish oil supported Indonesia's policy through the stunting prevalence rate. Albumin and PUFA in haruan fish support the human body and significantly cure diseases caused by reducing blood protein found in Southern Kalimantan and Eastern Kalimantan varieties of snakehead fish are one of the groups of swampy waters in Southern and Eastern Kalimantan which are familiar completed by community as a complete snack of ketupat kandangan and nasi kuning.[18] The oxidation could also reduce the nutritional values and quality of food and pharmaceutical products, therefore stable antioxidants are needed. Antioxidants could be defined as any substances or materials capable of preventing oxidation of molecules.[19] Antioxidants in fish oil are influenced by extraction methods used to extract fish oils. Hydraulic pressing, Soxhlet extraction based on heat extraction, solvent extraction using non-polar solvents, and the latest extraction methods such as ultrasound-assisted extraction, supercritical fluid linearly several green extraction based on enzymatic methods.[20] Several extraction conditions also affected the extraction efficiency and it is necessary to select the appropriate extraction method to achieve high antioxidant activities in fish oil.[21] Several methods have been implemented for evaluating the antioxidant activities of fish oils in vitro including radical scavenging methods using 2,2'diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS), lipid peroxidation inhibition[22]

and metal-chelating efficacy.[23] Therefore, this study was aimed at exploring the physicochemical characterization and fatty acid profile of Patin (*Pangasius micronema*) fish oil and haruan (*Channa striata*) fish oil cultivated in Samarinda.

EXPERIMENTAL

Materials

Desa Yupa and other reagents at Kutai Kartanegara, East Kalimantan Patin easily found haruan fishes which contain chemicals of 2,2'-diphenyl-1picrylhydrazyl (DPPH) was purchased from Sigma (Aldrich, USA) solvents used for analysis were of analytical grade and collected from E. Merck (Darmstadt, Germany).

Extraction of Catfish and Haruan Fish Oil

The research approach involves multiple phases, starting with the preparation of wet catfish and haruan fish samples by cutting the flesh fish into pieces. Next, the samples are cleaned while being run underwater. Weigh the fish samples that were gathered in the interim, and use an oven set to 40–55°C for 24 hours to dry the wet fish meat. Weigh each dried fish piece that has been wrapped in a dried catfish sample using a filter cloth. Then, place it in the pressing machine's column and press it for two minutes at a pressure of 100 kN. Gathering oil into a 15 mL flacon is the final step. But to extract catfish oil, the collected filthy oil is weighed, and a cabinet dryer and pressing machine provide linear assistance in the dry rendering process.[24]

Purification of Patin and Haruan Fish Oil

When floating particles in fish oil are collected, separation is carried out using a centrifuge completed force of 5000 G for 30 minutes followed by sseparated the upper phase of shaping clear oil into other containers, and finally weight of pure oil collected was calculated.[25] The dirty catfish and haruan fish oil that has been collected is purified to separate impurities of pure oil. The purification method is carried out by flowing water through fish oil into anhydrous sodium sulfate and bentonite to separate water content and other impurity components.

Fatty Acid Profile Analysis Using Gas Chromatography

Various methods for analyzing fatty acid profiles have been developed and use gas chromatography. Chromatography is a separation method based on differences in the interaction of analytes completed by the stationary phase and the mobile phase. Gas chromatography has been widely used to analyze various components of a mixture simultaneously. In gas chromatography, the mobile phase used is an inert gas and the analyses used must be volatile. In the case of oil samples, fatty acids are found in free shape or bound to shape triglycerides. Meanwhile, in the gas chromatography system, triglycerides would be broken down into fatty acids which are then derivatized, finally fatty acids could easily evaporate. The final process concluded that one of the derivatization methods used is transesterification which would convert fatty acids into fatty acid methyl ester or FAME. Each analysis would evaporate and be carried away completed by carrier gas differently depending on the boiling point of fatty acid. Followed by analyses that interact and are completed by stationary phase and mobile phase in the shape of inert gas through column. Each analysis would elute and reach the detector at different times depending on interactions found along the column. The last phase of the process is a column of analyses that would reach the detector and then the detector sensor would analyze the type of analyses. Gas chromatography could be equipped with various types of detectors, including Mass Spectroscopy of MS, UV-Vis, and Flame Ionization Detector (FID). FID or flame ionization detector is an appropriate detector used in the analysis of organic compounds. The FID detector is capable of measuring carbon atoms from the results of ionization or decomposition of organic compounds. The carbon fragments would progressive conductivity of the FID sensor which is then recorded. Those parts of the process resulted in two practical benefits for the detector described as high sensitivity and not requiring a large number of samples. The chromatogram results collected were then compared and completed by literature to determine the fatty acid profile at each chromatogram peak.

Determination of the Free Fatty Acid Value

The Free Fatty Acid Value (FAV) is a measurement based on the AOAC basic technique. It is the number of milligrams of KOH needed to neutralize the free fatty acids in one gram of oil or fat. An indicator of 1

mL phenolphthalein was combined with 10 mL of ethanol and the oil samples in an Erlenmeyer flask. The mixture was titrated with 0.1 N KOH in ethanol and shaken continuously until the liquid's color changed from white to pink, which is known as the titration endpoint.[26]

Saponification Value Determination

The number of milligrams of KOH needed to neutralize the fatty acids produced by the full hydrolysis of one gram of sample is known as an oil's saponification value or SV. An Erlenmeyer flask weighed one gram of materials, to which 30 milliliters of KOH in ethanol were added. For the purpose of completing saponification, the Erlenmeyer was attached to an air condenser and boiled for 30 minutes. The warm solution was titrated with 0.5 N HCl after a few drops of phenolphthalein indicator were added, and the color changed from pink to colorless.[26]

Determining the Peroxide Value

The milliequivalents (meq) of radicals per kilogram of fat oil is the unit of measurement for the peroxide value, or PV. The approved AOAC technique was used to calculate PV (Ikhsan et al., 2021). In 3 milliliters of chloroform: acetic acid (2: 3) solution, one gram of oil sample was dissolved. After adding 0.5 mL of saturated KI solution, the mixture was placed in a dark area for one minute. Indicator starch (1.5%) was added, and the solution was titrated using 0.01 N sodium thiosulphate.

Iodine Value Determination

The total number of double bonds in fats and oils is measured by the iodine value, or IV. An Erlenmeyer flask with a cork was used to weigh a 0.3 g sample. The samples were dissolved in 25.0 mL of Wijs reagent (1% iodine chloride in glacial acetic acid) and 10 mL of chloroform. After adding 10 mL of KI 15% solution and 15 mL of water, the mixture was left in the dark for 30 minutes. Afterward, sodium thiosulphate 0.1 N was added and titrated until the yellow tint nearly vanished. After adding a few drops of 0.5% starch indicator, the titration was carried out until the blue tint vanished. The blank test was conducted using the same methodology.[27]

DPPH Free Radical Scavenging Assay

The DPPH radical scavenging assay was finished with a few adjustments.[28] For every 50 µL of fish oil sample, 1 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) 0.4 mM was added, and 5.0 mL of finished ethanol was diluted. For half an hour, the solution mixture was left to stand at room temperature in the absence of light. A spectrophotometer (Hitachi, U-2900, Japan) was used to measure the absorbance of the evaluated solutions at 515 nm.[29,30,31] The absorbance was then adjusted using blank solutions that contained solvent and the examined samples. To determine the DPPH radical scavenging activity, the absorbance of control solutions containing DPPH solution was also tested. The result was:

$$\%\text{RSA} = \frac{(\text{Abs control} - \text{Abs sample})}{(\text{Abs control})} x100\%$$

RESULTS AND DISCUSSION

Table-1: Yield and Characterization of Patin Fish Oil

Sample	Yield (%)	Free Fatty Acid Value(%)	Peroxide Value (meq/kg sample)	Iodine Value (g/100 g sample)	Saponification Value (mg KOH/g)
1	7.91	1.88	6.76	82.86	202.87
2	7.63	1.84	6.86	84.87	201.08
3	7.86	1.85	6.37	81.18	195.48
Mean ±SD	7.80 ± 0.15	1.86 ± 0.02	6.66 ± 0.26	82.97 ± 1.85	199.81 ± 3.86

With free fatty acids at 1.86 ± 0.02%, peroxide at 6.66 ± 0.26meq/kg sample, iodine at 82.97 ± 1.85g/100 g sample, and saponification at 199.81 ± 3.86 mg KOH/g, the yield of patin oil is 7.80% ± 0.15%. Chemometrics and FTIR spectroscopy together may be a trustworthy method for measuring and differentiating PFO and PFO tainted with PO.[22]

A sample of haruan fish oil yielded 1.82 ± 0.13%, 1.89 ± 0.04% completed free fatty acids, 7.13 ± 0.09 meq/kg sample, 111.57 ± 9.4 g/100 g sample of iodine, and 193.28 ± 5.24 mg KOH/g of saponification.

A study of haruan fish oil yielded results of 1.82% (± 0.13%), 1.86% (± 0.02) for completed free fatty acids, 7.13 (± 0.09) for peroxide, 111.57 (± 9.4) for iodine, and 193.28 (± 5.24) for saponification.

Table-2: Yield and Characterization of Haruan Fish Oil

Sample	Yield (%)	Free Fatty Acid Value (%)	Peroxide Value (meq/kg sample)	Iodine Value (g/100 g sample)	Saponification Value (mg KOH/g)
1	1.92	1.92	7.19	113.91	189.67
2	1.67	1.85	7.03	119.57	199.29
3	1.88	1.89	7.18	101.22	190.88
Mean ± SD	1.82 ± 0.13	1.89 ± 0.04	7.13 ± 0.09	111.57 ± 9.4	193.28 ± 5.24

When compared to haruan, the soxhlet extraction yield of toman yielded more oil ($p<0.05$). The two fish oils had similar specific gravity values ($p>0.05$) and were colored dark red and yellow ($p<0.05$). Given that it exhibits lower levels of peroxide value (PV), free fatty acid (FFA) value, and acid value (AV), all of which indicate a good and healthy nutritional content obtained, toman fish oil was thought to be a higher quality by-product than haruan fish oil. The fatty acid profiles of the toman and haruan fish oils were comparatively comparable. Even though arachidonic acid is the primary precursor for wound healing, the presence of seven major fatty acid constituents—dodecanoic acid, 9-hexadecenoic acid, hexadecanoic acid, linoleic acid, oleic acid, octadecanoic acid, and docosahexaenoic acid—in both fish oils may make them essential for therapeutic application (such as wound healing).

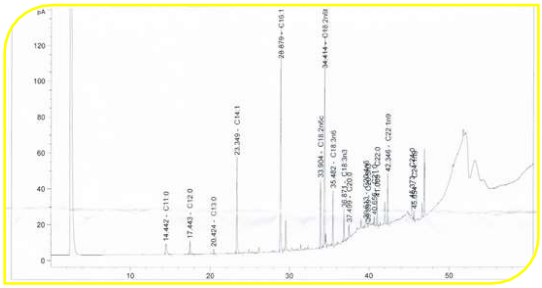

Fig.-1: Identification of Fatty Acid Methyl Ester (FAME) From Patin Fish Oil

One of the derivatization methods used is transesterification which would convert fatty acids into Fatty Acid Methyl Ester (FAME). Identification of the FAME collected was carried out by comparing the peak retention time of each FAME of the Patin fish oil sample completed by the standards used. Various fatty acids that were detected were then calculated for their relative concentrations in Fig.-1. Each fatty acid could be grouped based on its type. The dominant fatty acids found were palmitoleic acid, linoladiic acid, and myristoleic acid completed relative concentration percentages of 25.91%, 24.68%, and 11.14 respectively, Total Fatty Acid concentration is shown in Table-3.

Table-3: Total Fatty Acid Concentration of Patin Fish Oil

Description	Relatif Consentration (%)
The total concentration of saturated fatty acid	14.65
Total concentration of unsaturated fatty acids	85.31
Total concentration of omega-3 fatty acid	3.66
Total concentration of omega-6 fatty acid	16.18
Total concentration of omega-9 fatty acid	5.82

The analysis reveals the total fatty acid concentration in patin oil, including saturated fatty acids (14.65%), unsaturated fatty acids (85.31%), omega-3 (3.66%), omega-6 (16.18%), and omega-9 (5.82%).

The various fatty acids that were detected were then calculated for their relative concentrations shown in Fig.-2. Each fatty acid could be grouped based on its type. The dominant fatty acids found were Hexadecanoid acid, and Palmitic acid completed relative concentration percentages of 21.24%, and 10.16% respectively.

The analysis of total fatty acid concentration in haruan fish oil reveals saturated fatty acids (23.65%), unsaturated fatty acids (21.31%), and omega-3 (8.3%). Using a Shimadzu QP2010 quadrupole equipped

with a carbowax capillary column (30 cm x 0.25 mm internal diameter; 0.25 in film thickness) and a flame-ionization detector, the fatty acid methyl esters composition of the extracted fish oil was ascertained.

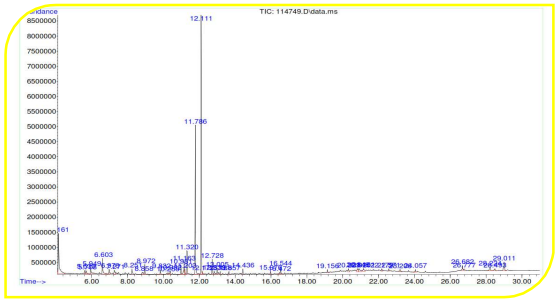

Fig.-2: Identification of Fatty Acid Composition of Haruan Fish Oil

Table-4: Total Fatty Acid Concentration of Haruan Fish Oil

Description	Relatif Consentration (%)
The total concentration of saturated fatty acid	23.65
Total concentration of unsaturated fatty acids	21.31
Total concentration of omega-3 fatty acid	8.3

Two milliliters of each oil were injected into the column. The percentage of each fatty acid in the sample was used to compute the fatty acid (%), which was then utilized to produce a fatty acid profile for each oil used (n = 3).

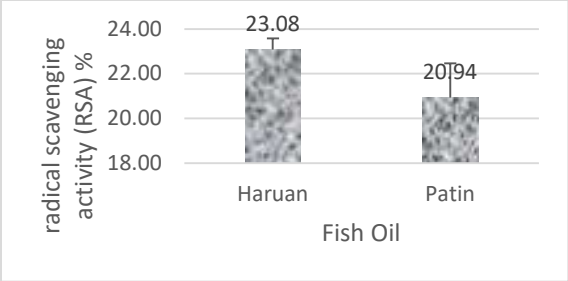

Fig.-3: Radical Scavenging Activity (RSA) Test Completed DPPH in the Fish Oil

Figure-3 shows that RSA DPPH results are 20.94±1.52% for patin oil and 23.08 ± 0.50% for haruan fish oil.

CONCLUSION

The dry rendering pressing method is used to extract oil from fish meat, producing both crude and refined oil. The fish oils are both yellow. When compared to haruan fish oil, patin fish oil is thought to be of higher quality because it exhibits a lower free fatty acid number (FFA) and peroxide value (PV), both of which signify that a good and healthy nutritional content is reached. Hexadecanoid acid and palmitic acid are the predominant fatty acid profiles in Haruan oil, whereas palmitoleic acid, linoleic acid, and myristoleic acid predominate in Patin oil. Catfish oil has an RSA of 20.94 ± 1.52% and haruan fish oil of 23.08 ± 0.50%. These results suggest that fish oil may be used as a nutritional supplement in cases of malnutrition in infants.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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