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The Quality of Purified Eel Fish ((*Monopterus Albus Zuieuw*) Oil and Mackerel Tuna Fish (*Euthynnus Affinis*) Oil

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ABSTRACT

Fish oil nutrition has excellent potential in addressing stunting and other malnutrition. This study aimed to purify Eel fish oil (EFO) and Mackerel tuna fish oil (MTFO) extract using activated bentonite and measure the quality of the fish oil extract based on the international fish oil standard (IFOS). An experimental study with a randomized block was designed. The eel fish and Mackerel tuna were extracted with ethanol and n-hexane and then processed by degumming, neutralization, and bleaching with activated bentonite by concentration variations (2%, 4% and 6%). The quality of fish oil extract was characterized (peroxide number, anisidine number, free fatty acid, TOTOX number, vitamin D, fatty acid composition, and oil clarity). Data was analyzed using One-way ANOVA. The oil quality parameters from EFO and MTFO (peroxide value, free fatty acids, anisidine value, and TOTOX value) meet IFOS standards. EFO transmissions were the clearest (83.176 – 99.311). EFO's SFA was the highest (81.73%). The highest MUFA was in MTFO (33.6%). The highest PUFA was in EFO (30.5%). The highest vitamin D was in MTFO (5,7140 ppm). One-way ANOVA tested on all variables (P>1) showed that BP in fish oil did not significantly affect its quality. It is necessary to improvise purifying techniques to extract vitamin D and fish oil output.

Keywords: bentonite; eel fish; fish oil; mackerel tuna fish; purification

INTRODUCTION

Fish is an abundant Indonesian marine product. The Ministry of Maritime Affairs and Fisheries [(1)] recorded that national fish production was worth 23.16 million tons in 2020. Fish animal protein reached 20%. Meat and fish oil contain essential unsaturated fatty acids in the form of omega-3 (EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid)), omega-6 (linoleic and arachidonic acids (ARA)). Fish also contains vitamins (A, D, E) and minerals.[(2,3)]

Considering its nutritional content, fish can be used to overcome the problem of stunting and other malnutrition in Indonesia. For this reason, the Indonesian people must intensively consume fish. Based on a national socio-economic survey, the average national fish consumption in 2019 reached 53.79 kg/capita/year, lower than other countries in the Asia Pacific, such as Malaysia (70 kg/capita/year) and Japan (100 kg/capita/year). in 2014.[(4,5)] The low level of fish consumption per capita in Indonesia, especially for toddlers, is due to the public's opinion that eating fish causes worms, allergies, body odour, fishiness and breast milk to become less tasty.[(6)] Making fish oil capsules is a processing alternative expected to increase fish consumption.

Research results show that Omega-3 and Omega-6 in fish oil are needed for development (brain, fetus, nerves), cardiovascular function, anti-inflammatory, and immunomodulatory,[(7-9)] EPA and DHA play a role in healing keloid symptoms.[(10)] In fish oil, vitamins A, D, and E are antioxidants in treating rheumatoid arthritis, autoimmune thyroid disease, psoriasis, and maintaining vision.[(11-13)]

The Food and Agriculture Organization (FAO) states that world fish oil and meal production will reach 22 million tons annually in 2020.[(14)] Fish oil in Indonesia is generally imported. The value of fish oil imports 2017

reached 10,056,744 kg.[15] This value shows the high demand in the domestic market, which has not been able to be met by industry in Indonesia. It will be an opportunity to develop fish oil to compete in the international market.

Fish oil can be used as a food product if it has a free fatty acid value ≤ 1.50%, peroxide value ≤ 3.75 mEq/kg, anisidine value ≤ 15 mEq/kg, total oxidation ≤ 20 mEq/kg.[16] Improving the quality of fish oil can be done mechanically, chemically, or by combining both methods. Mechanical methods are carried out through filtration, settling and centrifugation. Meanwhile, chemical methods involve neutralization, degumming (removing gum), bleaching (whitening color) and deodorizing (releasing aroma).[17]

According to research by Ayu et al.[18], a 2% concentration of bentonite used to purify catfish oil reduced 0.06 ± 0.01% free fatty acid content and peroxide value to 3.86 ± 0.06 meq/g. Andhiarto & Wijaya[19] used a concentration of 3% bentonite in purifying kite fish oil supplements to meet the International Fish Oil Standard (IFOS) requirements. Suseno et al.[20] purified sardine fish oil by combining attapulgite and bentonite, reducing the peroxide value to 2,250 meq/kg and anisidine to 0.348 meq/kg. To the best of our knowledge, currently there are no studies on extracting, purifying and measuring the quality of eel fish and mackerel tuna fish; this study aims to purify the extracts of EFO and MTFO by using activated bentonite and measure the quality of the fish oil extract based on IFOS.

METHODS

This study was experimental, with a randomized block design (RBD) with three replications (Table 1). The research samples were two types, namely eel fish and tuna fish. Eel fish was extracted with ethanol solvent, while tuna fish was extracted with n-hexane solvent. The fish oil extract obtained was then degumming (H_2O and NaCl), neutralization (NaOH) and bleaching with bentonite adsorbent activated by concentration variations (2, 4, 6%). The extract was characterized to determine the quality of fish oil extract (peroxide number, anisidine number, free fatty acid, vitamin D, fatty acid composition, and oil clarity).

The research was conducted at the Medan Health Polytechnic Integrated Laboratory and the Indonesian Oil Palm Research Institute (IOPRI) for two months. Analysis of the peroxide number,[21] free fatty acid content[22] using the titration method, analysis of the anisidine number[23] using a spectrophotometer, analysis of the total oxidation (TOTOX) number using the formula (total oxidation number is equivalent to twice the peroxide number plus the anisidine number). Oil clarity test[24] using a spectrophotometer (wavelength 450, 550, 620, 665, 700 nm). Analysis of vitamin D content using HPLC[25] fatty acid composition using GC-MS.[26] The resulting data was analyzed statistically with the one way- Anova test at a significance level of 5%.

Table 1. Randomized block design

Group	Treatment		
	I	II	III
Eel fish	EBI	EBII	EBIII
Mackerel tuna fish	MTBI	MTBII	MTBII

Note: E = Eel fish; M = Mackerel tuna fish; B = Bentonite; I = 2%; II = 4%; III = 6%

RESULTS

Dead fish samples are weighed wet, then washed, weeded, filleted, deboned, skin removed and considered again. Waste from weeding is in the form of heads, tail bones, belly flap meat, and stomach contents. Then, the fish fillets dried at a temperature of 60 - 70ºC for 48 hours. From the wet weight of Eel fish, 8.0715 Kg to 2.1714 Kg. At the same time, the damp weight of Mackerel Tuna was from 8.005 Kg to 1.8914 Kg.

Table 2. Wet weight and dry weight of fish samples

Type of fish	Wet weight (kg)	Dry weight (kg)
Eel fish	8.0715	2.1714
Mackerel tuna fish	8.0517	1.8914

The dried fish was extracted using the soxhletation method. Eel fish extraction was carried out using ethanol solvent, while tuna used n-hexane solvent. The tuna fish oil produced is dark brown, while the eel oil produced is brownish yellow. Both fish oils have a fishy aroma typical of fish oil.

Fish oil purification is carried out to improve the quality of fish oil so that it is suitable for consumption. The degumming stage is carried out to separate phospholipid impurities in the colloid phase, which consists of hydrated and non-hydrated phosphatides [3]. In this research, the degumming process was done in two stages. The first stage was by adding 5% v/v H_2O to the fish oil extract while stirring with a magnetic stirrer at 50 ºC for 20 minutes, centrifuged at 10,000 rpm for 10 minutes. The second stage was the addition of 5% w/v NaCl salt while stirring with a magnetic stirrer at a temperature of 50 ºC for 20 minutes. It was centrifuged at 10,000 rpm for 10

minutes. A salt solution can absorb fine particles suspended in the water, settle under the water, and attract the mucus formed in the fish oil to obtain mucus-free fish oil.(4)

The neutralization stage was carried out by adding NaOH adjusted to the amount of free fatty acids, stirring with a magnetic stirrer at a temperature of 50°C for 10 minutes, followed by centrifugation at 10,000 rpm for 10 minutes.

Bentonite is an aluminosilicate mineral used as a catalyst, buffer, bleach and adsorbent. In the bleaching process, several adsorbents were added, which can absorb dyes and impurities in the oil.(6) Bentonite activation was done to increase the adsorption surface area, thus optimising adsorbents.(7)

The yield is the percentage of fish oil obtained from the initial fish oil after going through the refining process. After purification, it goes through the degumming, neutralization and bleaching stages. The oil yield produced by eel fish oil is 11.71%, while that of tuna fish oil is 12.12%.

Table 3. Fish oil levels produced during the extraction process

Fish oil	Sample weight	Oil yield	% oil
Eel fish	10.0017	1.171	11.71
Mackerel tuna fish	10.002	1.212	12.12

this difference in yield occurs due to differences in the oil content of each fish and differences in extraction solvents; eel fish is extracted using ethanol, while mackerel tuna fish is extracted using n-hexane.(9) The percentage of oil produced by Mackerel Tuna is higher than Eel fish.

Tabel 4. Fish oil characteristics

No	Parameters	EFO			MTFO		
		BI	BII	BIII	BI	BII	BIII
1	Peroxide number (meq/Kg)	2.2	2.09	2.31	2.2	2.09	2.42
2	Free fatty acid (%)	1	0.96	1	1.05	1.09	1
3	Vitamin D (ppm)	0.851	0.8719	0.6413	5.714	4.8521	4.017
4	Anisidine number (meq/Kg)	1.46	1.48	1.45	1.4	138	1.41
5	TOTOX number	5.86	5.66	6.07	5.8	5.56	6.25
	%T at λ 450 nm	95.499	97.051	95.94	23.604	21.928	30.408
	%T at λ 550 nm	97.498	97.498	96.605	65.013	62.23	68.707
6	%T at λ 620 nm	97.498	83.176	96.827	81.846	80.353	83.368
	%T at λ 665 nm	97.051	98.85	98.174	88.92	88.308	89.949
	%T at λ 700 nm	98.175	99.311	97.723	91.833	90.782	92.469

The emergence of free fatty acids in oil is due to the hydrolysis of triglycerides; the content value is influenced by high temperatures, which accelerate the oxidation process during the refining process (14). The free fatty acid content obtained in eel fish oil during the purification process with bentonite (2, 4, 6%) were 0.96 - 1%, while the free fatty acids of MTFO were 1 - 1.09%. This free fatty acid value is still lower than that set by IFOS < 2.25%.

The anisidine compound is a secondary oxidation product (aldehyde, ketone, alcohol, acid and hydrocarbon compounds). This compound is the decomposition of hydroperoxide compounds. The anisidine number indicates the secondary oxidation value of fish oil. The anisidine number of EFO with purified bentonite concentration (2, 4, 6%) were 1.45 - 1.48 meq/Kg. Meanwhile, the anisidine value in MTFO were 1.38 - 1.41 meq/kg.

Total oxidation analysis (TOTOX) was carried out to determine the formation of primary and secondary oxidation products. This value is obtained by adding two times the peroxide value and one time the anisidine value. The TOTOX number in eels with purified bentonite concentrations (2, 4, 6%) is 5.66 - 6.07 meq/Kg. Meanwhile, the TOTOX number in purified bentonite tuna fish oil concentrations (2, 4, 6%) is 5.56 - 6.25 meq/Kg.

In our study, the clarity values of EFO and MTFO were measured using a spectrometer at various wavelengths (λ) (450, 550, 620, 665, 720 nm). Transmission percentage of EFO with bentonite purification concentrations (2, 4, 6%) at λ 450 nm (95.499 – 97.051), at λ 550 nm (96.605 – 97.498), at λ 620 nm (83.176 – 97.498), at λ 665 nm (97.051 – 98.85) and λ 720 nm (97.723 – 99.311). Transmission percentage of MTFO by bentonite purification concentrations (2, 4, 6%) at λ 450 nm (21.928 – 30.408), at λ 550 nm (62.23 – 68.707), at a wavelength of 620 nm (80.353 – 83.368), at λ 665 nm (88.308 – 89.949) and λ 720 nm (90.782– 92.469).

The fatty acids measurement of fish oil was done by using chromatography-mass spectra (GC-MS). The mechanism of this examination involves separating several compounds based on differences in their respective solubility in the moving solvent and differences in the absorption of each mixture into the stationary phase. The initial stage is converting the oil's fatty acids into methyl esters.(27)

Table 5. EFO fatty acids profile

Fatty acids composition	Unit	Bentonite concentration		
		2%	4%	6%
Lauric acid (C12:0)	%	0.3	0.3	0.2
Myristic acid (C14:0)	%	1.5	1.3	1.1
Palmitic acid (C16:0)	%	58.5	55.1	48.9
Stearic acid (C18:0)	%	17.0	16.6	15.0
Arachidic acid (C20:0)	%	0.7	0.6	0.5
Behenic acid (C22:0)	%	3.7	6.9	6.1
Total	%	81.7	80.9	81.73
Palmitoleic acid C16:1)	%	5.0	4.7	3.3
Oleic acid (C18:1)	%	11.0	11.3	21.5
Eicosenoic acid (C20:1)	%	6.4	5.9	5.4
Total	%	22.4	21.9	30.2
Linoleic acid (C18:2)	%	5.3	9.3	21.5
Linolenic acid (C18:3) (Omega- 3)	%	0.6	0.6	9.0
Total	%	5.9	9.9	30.5

Eleven fatty acids were identified in EFO. These were divided into several types of fatty acids based on the double bonds of their carbon atoms. Six SFA with the highest percentage at BP 6% was 81.73%. Three MUFA with the highest percentage at BP 6% was 30.2%. Two PUFA with the highest percentage at 6% BP was 30.5%. In PUFA, omega-3 was found as linolenic fatty acid with the highest percentage in the purification of 4% bentonite with a value of 9.9%.

Table 6. MTFO fatty acids profile

Fatty Acids Composition	Unit	Bentonite concentration		
		2%	4%	6%
Lauric acid (C12:0)	%	0.3	0.3	0.3
Myristic acid (C14:0)	%	0.7	0.6	0.7
Palmitic acid (C16:0)	%	51.5	50.9	52.4
Stearic acid (C18:0)	%	5.9	5.7	5.7
Arachidic acid (C20:0)	%	0.4	0.4	0.4
Total	%	58.8	57.9	59.5
Oleic acid (C18:1)	%	32.4	33.6	32.0
Eicosenoic acid (C20:1)	%	0.1	0.1	0.1
Total	%	32.4	33.6	32.0
Linolenic acid (C18:3) (Omega- 3)	%	0.1	0.1	0.1
TOTAL	%	0.2	0.2	0.2

Eight fatty acids were identified in MTFO. Five SFA with the highest percentage in 6% bentonite purification is 59.5%. One MUFA with the highest percentage in 4% bentonite purification is 33.6%. Two PUFA with the same percentage in bentonite purification variations (2, 4, 6%), was 0.2%. In PUFA, omega-3 was found in the form of linolenic fatty acid.

According to the results of the High-Performance Liquid Chromatography (HPLC) instrument examination, it was found that the vitamin D content of eel fish oil, when purified using 4% bentonite, was 0.8719 ppm. Meanwhile, the highest vitamin D content in tuna fish oil was found when purified using a 2% bentonite concentration of 5.7140 ppm.

Tabel 7. Vitamin D content in eel fish oil and tuna fish oil

Parameter	EFO at BP			MTFO at BP		
	2%	4%	6%	2%	4%	6%
Vitamin D (ppm)	0.8510	0.8719	0.6413	5.7140	4.8521	4.0170

The measurements of peroxide value, free fatty acids, vitamin D, anisidine number, TOTOX number and transmittance of fish oil resulting from bentonite purification with varying concentrations of 2, 4, and 6% are critical to determining the quality and composition of the oil produced. The one-way ANOVA statistical test results on all measurement parameters showed p-value >1, meaning bentonite purification of fish oil did not significantly affect the quality of fish oil.

DISCUSSION

Drying is a method of removing some of the water from a material by evaporating the water using heat energy from an oven. It can be seen in Table 2. the water content of sample significantly decreased about 73,09% in Eel fish and 76,51% in Mackerel Tuna Fish. Apart from reducing the water content, this drying is also a

preservation process because microorganisms and enzyme activities that can cause decay will stop. Thus, the dried material can have a long shelf life, making the soxhletation process easier.(1) Eels fish and Mackerel Tuna fish have different weights because the heating process until they reach a constant weight results in a loss of water content in the fish.

Besides water content, color changes are also part of the physical changes in oil during fish extraction. The color change in fish oil occurs because the natural dyes in the fish containing the oil are also extracted. Natural dyes usually found in fish include α and β carotene, xanthophyll, chlorophyll, and anthocyanin. This dye causes the oil to turn yellow, brownish-yellow, greenish, and reddish. Meanwhile, the color resulting from the degradation of natural dyes consists of several colors, namely dark, brown, and yellow. Apart from color, aroma must also be considered in the fish oil extraction process.(2)

The EFO produced after undergoing the degumming, neutralization, and bleaching process with varying bentonite concentrations (2%, 4%, 6%) is brownish-yellow in odour and more apparent than the crude oil extract after extraction. Meanwhile, MTFO has a brown obvious, but the concentration level is reduced compared to before. The fishy odour of EFO and MTFO decreased after the bleaching process with bentonite compared to fish oil before bleaching.

The color of fish oil extracted from this study differs from that produced by flying fish oil in Andhiarto and Wijaya's research.(19) Flying fish oil after refining has a clear yellow color, and the fishy odor is reduced compared to fish oil before refining. Differences in the physical appearance of flying fish oil after refining using bentonite at the bleaching stage at concentrations of 1%, 3%, and 5%.

The results of calculating the yield of fish oil in both eel and tuna show that a lot of fresh fish was needed to produce fish oil in this study. The results of this study are still lower when compared with research by Kamini et al.(28), who stated that the extraction of by-products from catfish processing has the characteristics of catfish oil resulting from dry rendering extraction at a temperature of 70-80ºC resulting in a yield of 72.50-76.48%. This difference in yield is likely due to differences in the oil content of the fish, extraction methods, and purification provided (29). The higher the fish fat content, the more fish oil produced will increase.

The ability of bentonite as a bleaching agent can be increased by activation in an acidic environment because it will modify the structure and increase the surface area of bentonite. According to the Regulation of the Head of the Food and Drug Supervisory Agency Number HK.00.06.52.0100 concerning the supervision of organic processed food, bentonite is a food additive and other material that is permitted to be used in the production of organic processed food (8). In this study, the adsorbent used was bentonite activated with 3N H_2SO_4 with varying concentrations (2, 4, 5%) with a temperature of 105 – 110ºC. This variation in bentonite concentration is a development of research conducted by Andiartho and Wijaya, which used bentonite variations of 1%, 3%, and 5%.(19)

The peroxide number is a value to determine the degree of oil damage. What can cause damage to oil is the oxidation process of oxygen atoms in the air against unsaturated fatty acids in oil during processing and storage.(11) Sari(12), in his research on the purification of catfish oil by-products of fish smoking, used 1% and 3% bentonite at 1.60 and 4.88 meq/Kg. Andhiarto & Wijaya(19) obtained the peroxide value in fish oil supplements purified using bentonite at concentrations (1%, 3%, 5%) which met the IFOS standard at a concentration of 3%, namely 3.9953 ± 0.0742 meq/kg.

In addition to the determination of the peroxide number, another important parameter for assessing the quality of fish oil is the analysis of the free fatty acid content. Fatty acid levels are low in eel and tuna fish oil because the non-oil fractions in both fish oils are also saponified. Bija et al.(15) explained that the degumming and neutralization stages in fish oil facilitate the neutralization process, thereby facilitating the purification process and have an impact on reducing the value of free fatty acids in sardine fish oil, with the neutralization stage at a temperature of 50°C having the lowest percentage of free fatty acids, namely 0.24%.

The results of Anisidine analysis addition activated bentonite can be seen in Table 4. Anisidine value in both fish oils meets IFOS <15 meq/Kg. Andhiarto & Wijaya(19) in their study purified layfish (*Decapterus ruselli*) oil supplements with a bentonite concentration of 1, 3, 5%, obtaining anisidine value (9.9625 ± 2.7789 - 13.6624 ± .9574 meq/Kg) and still meets the value set by IFOS <15 meq/kg.

The advancement of the oil's degradation process can be measured using the total oxidation value, which can also reveal information about the formation of primary and secondary oxidation products. The TOTOX number in this study still meets IFOS <20 meq/kg. Bija et al.(17), in refining sardine fish oil with 5% magnesol (13.80 ± 2.56 – 54.63 ± 1.10) with a treatment temperature of 50 – 80ºC shows that not all refining stages have a total oxidation value according to IFOS <20 meq/kg.

Clarity is what attracts consumers to choose fish oil. The transmission value of EFO is higher at each wavelength measured when compared to the transmission value of MTFO, which tends to vary. It was because of the clarity in terms of visual appearance; EFO is much clearer than MTFO. The high transmission value is close to the highest light transmission percentage of 100%; this value is close to the light transmission of commercial oil, so it has good clarity.(16) The clearer the fish oil, the fewer impurities there are in the fish oil. Andhiarto & Wijaya(13) obtained the highest clarity of kite fish oil after purification at a bentonite concentration of 3% with a

transmission percentage at wavelengths (λ) 450, 550, 620, 665, and 700 nm respectively, namely 74.6996±1,9363; 94.4950±1.5724; 95.1060±1.667; 95.6906±1.4371, and 95.9356±1.3427 %T.

The fat found in different fish body sections has different fatty acid compositions. Differences in the fatty acid composition and levels of fish are influenced by various factors and are associated with multiple aspects such as species habitat, size, age.[30] The results of this study are not in line with those carried out by Suseno et al. [17]; when examining fatty acids in 8 imported commercial fish oils (soft gels) in Central Java, it was found that the SFA values ranged from 4.91 – 15 28%, MUFA 0.03 – 23.68% and PUFA 1.41 – 87.66%.

The SFA and MUFA values of MTFO extract are close to the SFA and MUFA values of red snapper fish oil extract, respectively 43.4% and 25.6%, but the PUFA value of MTFO is much lower compared to the PUFA and Omega-3 of red snapper oil. (33.3 and 26.8%).[18] The fatty acids in fish fat have been the subject of most research, particularly omega-3 PUFA. This is believed to have many health benefits, including infant development, cancer, cardiovascular disease, and, more recently, various mental illnesses, including depression, attention deficit disorder, hyperactivity, and dementia.[31,32] Fulfilling international fish oil quality standards is not a relatively good outcome because the large amount of fresh fish needed to produce fish oil created limitations in this research. More detailed considerations and calculations are required in developing mass fish oil production.

CONCLUSION

The Oil quality parameters of EFO and MTFO after refining process, such as peroxide value, free fatty acids, anisidine value, and TOTOX value, meet IFOS standards. EFO transmissions are much more precise than MTFO. An examination of fish oil fatty acids found that the SFA composition in EFO contained behenic fatty acids while MTFO did not. The percentage of EFO's SFA was higher than MTFO, MTFO's MUPA was higher than EFO's MUPA, while EFO's PUFA was higher than MTFO's PUFA. The highest vitamin D was in EFO. A one-way ANOVA test on all variables (P>1) showed that BP in fish oil did not significantly affect fish oil quality. Improvised purification methods must be carried out to obtain fish oil yield's and vitamin D.

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