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The Effect of Adsorbents on the Quality of Refined Eel Fish (*Anguilla marmorata* [Q.] Gaimard) Oil as a Raw Material for Pharmaceutical Preparations

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Abstract: Eel fish (*Anguilla marmorata* (Q.) Gaimard) oil is a valuable source of health-promoting unsaturated fatty acids. Adsorbent selection during the bleaching stage of fish oil purification can significantly impact the final product quality. This study aimed to evaluate the impact of different adsorbents on the quality of refined eel fish (*Anguilla marmorata* (Q.) Gaimard) oil. Eel fish oil was extracted using Soxhlet extraction with diethyl ether and purified through degumming, neutralization, and bleaching stages. Various adsorbents (bentonite, magnesol XL, and zeolite) were employed at different concentrations (1% bentonite, 3% magnesol XL, and 5% & 10% zeolite activated by acid and base) to remove impurities. The refined oils were analyzed for physical, chemical, and microbiological properties. Results showed that all adsorbents effectively improved the quality of eel fish oil, meeting most *International Fish Oil Standard* (IFOS) and *Indonesian National Standard* (SNI - 8467:2018) criteria. However, density and iodine values were below the specified limits. Microbiological analysis confirmed the absence of contamination, but lead levels exceeded the permissible standard.

Keywords: Anguilla marmorata; Bentonite; Eel Fish oil; Magnesol XL; Refined; Zeolite

Introduction

Eel (*Anguilla marmorata* Q. Gaimard) holds significant economic value and is a prominent export within the fishery sector (Sudaryono et al., 2014). Consumed primarily during the silver phase, eels boast a unique nutritional profile heavily influenced by age and size. Notably, eels in the silver phase exhibit a higher content of fatty acids compared to their elver counterparts. Eel flesh surpasses even salmon and mackerel in its *docosahexaenoic acid* (DHA) content (1,337 mg/100 g vs. 820 mg/100 g and 748 mg/100 g, respectively). Similarly, eel *eicosapentaenoic acid* (EPA) content (742 mg/100 g) outshines both salmon (492 mg/100 g) and mackerel (409 mg/100 g). Interestingly, a study on eels from Palu River and Poso Lake revealed a disparity in *monounsaturated fatty acid* (MUFA) and *polyunsaturated fatty acid* (PUFA) content between the yellow and silver phases (Jamaluddin et al., 2018). Beyond direct consumption, eels offer immense potential for the production of fish oil rich in omega-3 fatty acids, particularly DHA and EPA. These essential fatty acids play a crucial role in promoting infant development, cognitive function, and heart health. They have also been linked to the prevention and management of various conditions, including cancer,

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Alzheimer's disease, and other cardiovascular ailments (Nitbani, 2018).

Extracting and purifying fish oil from eels is a crucial step in its preparation. Diethyl ether, employed in the Soxhlet extraction process, demonstrates a higher yield compared to n-hexane (Jamal et al., 2021b). However, the resulting crude fish oil contains contaminants that negatively impact its quality, leading to an unpleasant rancid odor and taste, reduced stability, and a shorter shelf life. Refining serves the critical purpose of eliminating these undesirable components, ultimately enhancing the oil's suitability for human consumption and further processing. The purification process typically involves several stages, including degumming, neutralization, and bleaching. Degumming removes impurities like mucus, while neutralization reduces free fatty acids (FFAs) and eliminates unwanted materials. Bleaching, a key stage, utilizes adsorbents to lighten the oil's color and absorb unwanted components such as phosphatides and gums (Bonilla-Méndez & Hoyos-Concha, 2018; Ketaren, 2012; Sari et al., 2015).

The selection of adsorbents in the bleaching process significantly impacts the quality of the final fish oil product. This study explores the potential of novel adsorbents for superior eel fish oil purification. Previous research has highlighted the efficacy of magnesol XL in lowering FFA content in cooking oil compared to conventional options like activated carbon and bleaching earth (Suseno et al., 2014; Yates et al., 1997). Notably, both magnesol XL and bentonite possess vast surface areas (700 m2/g and 305,957 m2/g, respectively), enabling them to effectively capture impurities within the oil (Nadhiro et al., 2018; Bardant et al., 2021). Zeolites, another class of adsorbents, are particularly interesting due to their activation with either bases (NaOH) or acids (HCl), potentially leading to enhanced adsorption capacity (Rosly et al., 2021).

Refined fish oil undergoes rigorous testing to determine its physical, chemical, and microbiological properties. Physical characteristics evaluated include organoleptic properties (taste and odor), clarity, solubility, density, and viscosity. Chemical quality assessment involves parameters like p-anisidine value, peroxide value, total oxidation value, acid number, heavy metal content, and FFA profile. The *International Fish Oil Standard* (IFOS) establishes crucial benchmarks for refined fish oil quality, including peroxide value (<3.75 meq/kg), FFA content (<1.5%), p-anisidine value (<15.0 meq/kg), and total oxidation value (<20.0 meq/kg). These standards safeguard against fat oxidation, a process that can lead to rancidity, taste alterations, quality degradation, and potential health risks associated with compromised nutritional value. Microbiological evaluation involves analyzing mold, yeast, and total plate counts to assess potential contamination (Bako et al., 2017); (Bako et al., 2017; Shahidi, 1994).

Fish oil that meets these stringent quality standards finds diverse applications within the pharmaceutical industry. Examples include gelatin capsules, concentrated EPA and DHA formulations, and kid-friendly emulsions. This study aims to address the challenges associated with eel fish oil quality through a novel purification approach employing various adsorbents. The analysis of the physicochemical and microbiological properties will be conducted in accordance with the established IFOS protocols and Indonesian National Standard (SNI) 8467:2018.

Method

Materials

Type of research

This research was a laboratory-based experiment where pure eel oil was characterized in terms of physical, chemical, and microbiological properties. Organoleptic, clarity, viscosity, density, and solubility were among the physical characteristics examined. Free fatty acids, heavy metal content, fatty acid profile, total oxidation value, peroxide value, p-anisidine value, iodine value, and acid value were all included in the chemical parameters. Total plate count (TPC) as well as *total yeast and mold count* (TYMC) were determined for microbiological testing.

Equipment used

The equipment used in this study were stem mixer, tube rack, tube reaction (Iwaki Pyrex®), measuring flask (Iwaki Pyrex®), measuring glass (Iwaki Pyrex®), jar, dropper, cutting board, chemical glass (Iwaki Pyrex®), round bottom flask, desiccator (Duran®), spoon horn, ruler scale, measuring glass (Labnet®), ruler, scissors, knife, blender, thermometer, analytical balance (AE Adam Nimbus®), rotary evaporator (Eleya®), centrifuge tube, centrifuge (C2 Series®), stand and clamp, cutter, pH meter, magnetic heater stirrer (Suntex®), porcelain cup, tweezers, mortar and pestle, soxhlet (Thermo Scientific®), oven (Shel Lab®), Brookfield viscometer, pycnometer, vortex mixer (Labnet®), UV-Vis spectrophotometer (CECIL CE7410 7000 Series®), spectrophotometer atomic absorption (SSA) instrument, autoclave (Hiarayama HVE-50®), Petri dish, syringe, bunsen burner, incubator (Shel Lab®), spoon horn, vial, Erlenmeyer flasks, colony counter (Suntex®) and laminar air flow (Streamline®).

Research materials

The chemicals used for testing included diethyl ether (Merck®), n-hexane (Merck®), 1% bentonite,

magnesol XL, zeolite, PP indicator, red methyl indicator, potassium hydroxide (KOH) (Merck®), ethanol (C_2H_5OH) 95%, acetic acid (CH_3COOH) (Merck®), chloroform ($CHCl_3$) (Merck®), potassium iodide (KI) (Merck®), distilled water (H_2O), sodium thiosulfate ($Na_2S_2O_3$) (Merck®), 1% starch (Merck®), hydrochloric acid (HCl) (JT Beaker®), isooctane (Merck®), p-anisidine (Merck®), methanol, BF_3, NaOH (Merck®), citric acid (Merck®), acetone, Wijs solution, nutrient agar (GranuCult™), potato dextrose agar (GranuCult™), NaCl infusion (Widatra®).

Sampling technique

The purposive sampling approach was the sampling strategy employed. This method involves choosing samples from the population depending on the researcher's preferences, such as type, weight, size, and collection location (Solimun et al., 2018). The fish sample used was the adult *Anguilla marmorata* (*silver eel*) with a length ranging from 60 to 150 cm from the river of Central Sulawesi.

Extraction of eel fish oil

Fifty grams of eel fish samples were weighed and placed within the sleeve socket. Diethyl ether was added to a round-bottom flask in an amount of 250 mL. The extraction process lasted 5 hours at 60°C. In a round-bottom flask, the mixture of oil and solvent was evaporated until the solvent was completely evaporated. Oil in a round-bottom flask was incubated at 105°C for approximately one hour, followed by a 30-minute desiccator cooling period. The oil-filled round-bottom flask was then weighed until the weight remained constant. The procedure was carried out in triplicate (Jamal et al., 2021) .

Purification of the extracted crude eel oil

a) Purification using zeolite

The extracted crude fish oil was heated at 60°C for 1 minute, then a 3% citric acid solution was added and heated at 60°C for 20 minutes while stirring using a magnetic stirrer. The oil was then cooled to room temperature and then centrifuged at a speed of 2,600 rpm for 10 minutes.[16] After undergoing the degumming process, the *crude fish oil* was then neutralized. The oil was placed into a beaker, and 9.5% NaOH solution was added and heated at 60°C for 20 minutes while stirring with a *magnetic stirrer*. This was followed by cooling to room temperature and then centrifugation at 6,000 rpm for 10 minutes. In the *bleaching process*, the neutralized oil was added to 5 or 10% zeolite, activated by acid and base, respectively, while stirring with a magnetic stirrer for 30 minutes. The oil was cooled to room temperature and then centrifuged at 10,000 rpm for 30 minutes. Purified oil was the result of the filtrate that was removed from the impurities (Sembiring et al., 2018; Euglene et al, 2014).

b) Purification using 3% magnesol XL

The crude oil was heated at 70°C for 1 minute. An aliquot of 3 mL of 3% citric acid solution was then added while stirring and heated at 70°C for 1 minute. The oil was then cooled and centrifuged at 20°C for 10 minutes at a speed of 2,600 rpm. The oil that resulted from the degumming process was added to 9.5% of the NaOH solution and stirred at 65°C with a magnetic heater stirrer for 20 minutes. The oil was cooled to room temperature and then centrifuged at a speed of 5,000 rpm for 30 minutes. The resulting oil from the neutralization process was added to 3% magnesol XL adsorbent, stirred at 29°C for 20 minutes in *a* magnetic heater stirrer, then centrifuged at a speed of 10,000 rpm at a temperature of 10°C for 10 minutes (Suseno et al., 2016).

c) Purification using 1% bentonite

The crude oil was heated at 70°C for 1 minute. An aliquot of 3 mL citric acid solution (3%) was added, then heated at 70°C for 1 minute, while stirring. The oil was cooled to room temperature and then centrifuged at 20°C for 10 minutes at a speed of 2,600 rpm. Oil resulting from the *degumming* process was added to 9.5% of the NaOH solution and stirred at 65°C for 20 minutes. The oil was then cooled to room temperature and then centrifuged at a speed of 2,600 rpm at 20°C for 10 minutes. After that, the oil was rinsed three times with distilled water in a 1:2 oil to distilled water ratio to obtain a mixture between the oil and the soap. An aliquot of 1% bentonite was added to the oil and stirred at 70°C for 20 minutes. Then, the oil was separated by centrifugation at a speed of 10,000 rpm at 10°C for 10 minutes (Euglene et al, 2014).

Physical characterization of the purified eel oil

a) Organoleptic test

An organoleptic evaluation was conducted, taking into account the turbidity, color, and aroma [19]. The organoleptic test employed a scoring system with 30 non-standard panelists who satisfied the requirements. In this method, the quality of the oil was rated on a scale from 1 to 9, where 1 was the lowest quality level and 9 was the highest. Evaluation of the sample quality was documented on the evaluation sheet. The data obtained from the assessment sheet was tabulated, and the quality was assessed by determining the average result of each panelist with a 95% confidence level. The following formula was used to calculate the value parameter:

$$P\left(\bar{x} - \left(1{,}96.\, s/\sqrt{n}\right)\right) \leq \mu \leq \left(\bar{x} + \left(1{,}96.\, s/\sqrt{n}\right)\right) \cong 95\% \quad (1)$$

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

$$S^2 = \frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n} \quad S = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n}}$$

Where:

N : number of panelists;
S^2 : diversity mark quality;
1.96 : coefficient standard deviation at the 95% level;
$\bar{x}$: average quality value;
x_i : quality value from panelist to I;
I : 1,2, 3,…, n;
S : standard deviation mark of quality.

b) Clarity test

The clarity of the extracted fish oil was measured based on a modified AOAC method ([AOAC], 1995; Suseno et al., 2011). The cuvette was cleaned and filled with standard, n-hexane. The needle scale standard was measured until it showed a 100% scale. Then, the cuvette containing the standard was replaced with another cuvette containing oil, and the oil clarity was measured in % Transmission. Ten measurements were made using diluted oil, which was made up of one part oil (1 mL) and nine parts diluent (1 mL). N-hexane was used as the solvent. The wavelengths employed in the measurement included 450, 550, 620, 665, and 700 nm.

$$\text{Clarity (\%T)} = 10^{-A} \times 100\% \quad (2)$$

%T : % Transmission; A: Absorbance of sample

c) Viscosity test

The viscosity of the extracted fish oil was determined with a Brookfield viscometer. A 100 mL beaker was filled with a 15 mL sample. Viscosity was measured using spindle 6 and a speed of 100 rpm. The measurement was performed in three replicates. The needle continued rotating until the desired viscosity of the sample was achieved. Viscosity was displayed on a readable scale in cP (centiPoise) (Obrien, 2009) .

d) Density test

The density of the extracted fish oil was determined with a pycnometer. The pycnometer was washed and dried in the oven. An empty picnometer was weighed and recorded as m_1. Oil was added to the pycnometer and it was kept at 20°C until the capillary tube on the lid was full, preventing the production of air bubbles. Then, it was weighed and recorded as m^2 (Rizal & Murdiya, 2019) . The oil density was calculated using the formula:

$$\text{Density } (\rho) = \frac{w_1 - w_0}{V} \quad (3)$$

Where:

v : pycnometer volume (mL);
w_0 : weight of the empty pycnometer (g);
w_1 : weight of the oil-filled pycnometer (g).

e) Solubility test

Samples of fish oil (0.5 mL) were placed into six distinct reaction tubes. After adding 1 mL of solvent, the mixture was vortexed and agitated until it was homogenous. Oil solubility was determined by observing the characteristic oil solubility of each tube after the fish oil was introduced to ethanol, methanol, n-hexane, diethyl ether, chloroform, and acetone (Yuliana, 2018b) .

Chemical characterization of the purified eel oil

a) Free fatty acid test

A total of 2.5 g of fish oil was added to a 250 mL Erlenmeyer flask. An aliquot of 25 mL of 96% ethanol was added. The sample was heated in a water bath for 10 minutes and 2 mL of phenolphthalein indicator was added. The resulting suspension was titrated with 0.1 N KOH until a soft pink color that did not disappear within 30 seconds was observed ([AOAC], 1995b). The procedure was repeated three times. Percentage free fatty acids was calculated based on the following formula:

$$\text{Free fatty acid content } (\%) = \frac{A \times N \times M}{g} \quad (4)$$

Where:

A : KOH titration volume (mL);
N : Normality of KOH solution;
M : Molecular weight of the dominant fatty acid (EPA = 302, 451 g/mol);
g : Sample weight (g).

b) Peroxide value test

The 250 mL Erlenmeyer flask was filled with 2.5 g of fish oil. An aliquot of 30 mL of a solution containing glacial acetic acid and chloroform (3:2) was added. Then, 0.5 mL of saturated KI solution, 30 mL of distilled water, and 0.5 mL of starch indicator (1%) were added. Subsequently, the suspension was titrated with sodium thiosulfate ($Na_2S_2O_3$) at a concentration of 0.01 N, resulting in the loss of blue colour in the solution ([AOAC], 2005b). The experiment was replicated three times. The peroxide value was calculated using the following formula:

$$\text{Peroxide value (mq/k)} = \frac{(V_1 - V_0) \times M \times 1000}{g} \quad (5)$$

Where:

V_1 : Titrant's volume (mL);
V_0 : Volume of blank titrant (mL);

M : Normality of sodium thiosulfate solution (N);
g : Sample weight (g).

c) P-anisidin value test

Test solution 1 was prepared by dissolving 0.5 g of fish oil sample in 25 mL trimethylpentane. One milliliter of p-anisidine solution (2.5 g/L) was added to 5 mL of test solution 1 to prepare test solution 2, which was then shaken and kept out of the light. The reference solution was made by adding 1 mL of p-anisidine solution (2.5 g/L) to the trimethylpentane solution. The resultant solution was shaken and kept out of direct sunlight. A spectrophotometer was used to measure the absorbance of the solution at a wavelength of 350 nm. Test solution 1 employed trimethylpentane as compensation, while test solution 2 used solution reference as compensation at 350 nm, precisely 10 minutes after the preparation of the solution ([AOCS], 1998). The procedure was repeated three times. The p-anisidine value was calculated with the formula:

$$\text{P-anisidine value (meq/kg)} = \frac{25 \text{ x } (1{,}2\ A1 - A2)}{M} \quad (6)$$

Where:
A1 : Absorbance of test solution 1;
A2 : Absorbance of test solution 2;
M : Mass of sample used in test solution 1.

d) Total oxidation value test

The total oxidation value was obtained by adding 2PV and PAV ([AOCS], 1998).

$$\text{Total oxidation value} = 2PV + PAV \quad (7)$$

Where:
PV : Peroxide value (meq/kg);
PAV : P-anisidine value (meq/kg).

e) Iodine value test

A sample of 0.5 g of fish oil was put into an Erlenmeyer flask. The fish oil sample was then mixed with 25 mL of Wijs solution and 10 mL of chloroform. The suspension was shaken occasionally while being left in the dark for 30 minutes. In addition, 100 mL of distilled water was used to dilute 10 mL of 15% KI. The preparation was titrated with 0.1 N sodium thiosulfate ($Na_2S_2O_3$) to cause a light-yellow color shift. After adding three drops of starch indicator, the mixture was titrated one more time until the blue color disappeared (Digoarachchi et al., 2022). Iodine value was estimated with the following equation:

$$\text{Iodine value} = \frac{(V_B - V_S) \text{ x N x } 12.69}{G} \quad (8)$$

Where:
V_S : Sample volume of titrant (mL);
V_B : Blank volume of titrant (mL);
N : Normality of $Na_2S_2O_3$; 12.69: Equivalent weight of I_2;
G : Sample mass (g).

f) Saponification value test

As much as 1 g of fish oil was weighed and added to a 250 mL Erlenmeyer flask. An aliquot of 50 mL of 0.5 N KOH ethanol was added gradually. The Erlenmeyer flask was connected to a condenser and allowed to boil for 30 minutes. After cooling, 1 mL of phenolphthalein indicator solution was added and titrated with 0.5 N HCl until it turned colorless. Additionally, titration was done using a blank (0.5 N KOH solvent) (Fuadi, 2015) . Saponification value was calculated as follows:

$$\text{Saponification value} = \frac{56.1 \text{ x N x } (V_0 - V_1)}{G} \quad (9)$$

Where:
56.1 : Molecular weight of KOH (mmol/mL);
N : Normality of HCl (mmol/mL);
V_0 : Volume of 0.5 N HCl for titration of blank (mL);
V_1 : Volume of 0.5 N HCl for titration of sample (mL);
G : Sample weight (g).

g) Acid value test

Two grams of sample were weighed and placed in a 250 mL Erlenmeyer flask. Fifty milliliters of 96% ethanol were added. Then, the flask was heated for 10 minutes at 40°C in a water bath. Then, 3-5 drops of phenolphthalein indicator were added and titrated with 0.1 N KOH until there was a color change to pink [30]. The level of acidity was determined from the equation below:

$$\text{Acid value} = \frac{V \text{ x N x } 56{,}1}{G} \quad (10)$$

V : Volume of KOH for titration (mL);
N : KOH normality;
56.1: Molecular weight of KOH (mmol/mL); G: Sample weight (g)

Heavy metal testing of the purified eel oil

a) Lead and cadmium heavy metal testing

Five grams of sample were weighed and placed in a porcelain cup or 100 mL glass beaker. Using a pipette, 10 mL of magnesium nitrate solution in ethanol was added and stirred with a stir bar. The stirring rod was removed and rinsed with 95% ethanol. The ethanol evaporated over a water bath while stirring occasionally. Then, it was heated over an electric bath (covered by a glass beaker with a watch glass). The cup was then placed in a furnace set at 200°C, raised to 500°C progressively over two hours, and left to ash overnight at 450–500°C. The glass cup was removed from the

furnace and left to cool on asbestos. If there was still carbon residue after cooling, 1 mL of water and 2 mL of concentrated HNO_3 were added. A water bath was used to dry it afterward. The dish was reheated for one hour at 500°C. This process was repeated until white ash was obtained. Five milliliters of the mixed solution of HCl and HNO_3 were added to the ash through the walls of the glass cup and heated over a water bath until the ash dissolved. The solution was transferred into a 100 mL measuring flask, and then filled with distilled water. Next, Whatman 40 filter paper was used to filter it. The same reagents were used to assay the blank ([AOAC], 1995b); (Handayani & Yusnimar, 2013); ([AOAC], 2012).

To create a calibration curve, the system safety assessment (SSA) tool was set up and optimized according to the manufacturer's instructions for testing each metal. The standard wavelengths were used to measure the Cd (228.8 nm) and Pb (283.3 nm). Using the capillary tube, the standard solutions were gradually sucked into the SSA device, where they were read and recorded at each intake. If the difference between the measurements was more than 2%, the setting of the equipment was checked and steps 1 and 2 were repeated. However, if the difference was less or equal to 2%, the average of the values was determined. A calibration curve was produced from the data obtained with the Y-axis as the absorbance and the X-axis as the concentration (ppm) and a straight-line equation was determined. Each test sample was prepared individually and then sucked through the capillary tube of the SSA device.

b) Mercury heavy metal testing

One gram of eel oil sample was weighed into a digestion tube. An aliquot of 2 mL of HNO_3 and 1 mL of H_2O were added to it in the digestion tube. The tube was tightly closed, and placed in a microwave oven. The same reagents were used to process the blank as well as the sample. To prepare the standard series, 20 mL of reducing solution was added to the standard series, digestion, and blank solutions. The absorbance of the solutions was measured using a flameless atomic absorption spectrophotometer at a wavelength of 253.7 nm. A calibration curve was produced with the Y-axis as absorbance and the X-axis as concentration (ppm). The Hg content in the sample was determined [20].

To produce a calibration curve, a standard solution was prepared with the following concentrations: 0, 2, 4, 6, 8, and 10 ppm. Each of the standard solutions was diluted with a diluent solution of a mixture of 5% HNO_3 and 5% HCl (1:1). A mercury cathode lamp was installed on the AAS instrument and a hybrid vapor generation tool (VGA-77) was connected to it. The absorption cell was placed on the atomic absorption spectrometer (AAS) burner head. Optimization was carried out according to the manufacturer's instructions. A solution of 0.6% $NaBH_4$ in 0.5% NaOH as a reductant and ion-free water as the acid was prepared. The capillary tube for the sample was dipped into each standard and test sample solution and left for 1 minute. The absorption values of each standard and test sample solution were read and recorded.

c) Asenic heavy metal testing

The standard arsenic solution (10 mg/L) was prepared by pipetting 10 mL of 1,000 mg/L arsenic stock solution into a 1,000 mL volumetric flask. An aliquot of 5 mL of concentrated HCl was added. Some mineral-free water was added and homogenized. To prepare the arsenic working solution (100 µg/L), 10 mL of 10 mg/L of arsenic stock solution was pipetted into a 1,000 mL volumetric flask. An aliquot of 2 – 5 mL of concentrated HNO_3 was added. Then, mineral-free water was added and homogenized.[20] To test for arsenic metal, a 100 mL digestion flask was filled with 50 mL of the test solution or a diluted test sample that fell within the measurement range. Then, 1 mL of 2.5 N H_2SO_4 and 5 mL of 5% $K_2S_2O_8$ were added. Using an electric heater, the suspension was gradually heated to boiling for 30-40 minutes, or until the volume reached 10 mL. After cooling the solution, 50 mL of mineral-free water was added. After that, 5 mL of concentrated HCl was added and mixed thoroughly. Afterward, 5 mL of NaI/KI pre-reductant was added, and the solution was homogenized. The preparation was left for 30 minutes. After aspirating the sample solution, the absorption values were determined. The concentration of arsenic in the test sample was estimated.

Determination of the fatty acid profile of the purified eel oil

The sample was prepared for fatty acid profile determination by adding concentrated 0.5 N NaOH solution containing 20–30 mg of oil with 1 mL of methanol and heated at 65°C for 20 minutes. Afterward, 2 mL of 20% BF_3 was added, and the solution was heated for an additional 20 minutes. Hexane (1 mL) and 2 mL of saturated NaCl were added, and the solution was agitated to produce two layers. After separating the liquid phase, the organic phase was injected into a gas chromatography instrument. Analysis of the fatty acid profile was carried out using the gas-chromatography-flame ionization detector (GC-FID) with an initial temperature of 125°C for 5 minutes. The temperature was steadily raised, first to 185°C for 5 minutes at a flow rate of 10°C/min, then to 205°C for 10 minutes at a flow rate of 5°C/min, and finally to 225°C for 7 minutes at a flow rate of 3°C/min. The injection volume was set at 1 µL under the conditions of an injector split and a 4 mL/min flow rate at 220°C injection temperature. The

detector was set to 240°C, with flow rates of 30 mL/minute for N_2, 30 mL/minute for He, 40 mL/minute for H_2, and 400 mL/minute for air ([AOAC], 1995b).

Microbiological testing of the purified eel oil

a) Total plate count

The pour plate method was employed to determine the *total plate count* (TPC) . An aliquot of 1 mL of eel oil sample was placed into sterile Petri dishes and molten nutrient agar (NA) medium was poured on them. The cultures were swirled gently and then incubated in an incubator at 37°C for 24 hours. Two replicates were made (L. Sari et al., 2022; Dewi et al., 2023).

b) Total yeast and mold count

The sample was diluted and 1 mL of each diluent was then pipetted into Petri dishes. Afterwards, potato dextrose agar (PDA) medium was added. The culture was swirled gently to ensure even distribution. The cultures were incubated at 20-25°C for 5-7 days. After the incubation period, the number of colonies was determined. The procedure was replicated twice (Dewi et al., 2023; Kusuma & Andriani, 2019).

Data analysis

Data analysis was conducted with the statistical package for the social sciences (SPSS). One-way analysis of variance (ANOVA) was employed as the statistical tool. The level of statistical significance was set at $p < 0.05$.

Result and Discussion

Eel fish (*Anguilla marmorata* (Q.) Gaimard) has a high oil content, which contains unsaturated fatty acid compounds. These compounds are beneficial for health; therefore, they can be used for food and pharmaceutical products. The use of eel fish oil for food and pharmaceutical products is still limited due to the low quality of the oil. High-quality fish oil must meet the International Fish Oil Standard (IFOS), which stipulates that FFA cannot exceed 1.5% and peroxide value (PV) cannot exceed 2.75 meq/kg. Additionally, secondary oxidation parameters must be met, such as p-anisidine value (PAV) not exceeding 15.0 meq/kg and total oxidation value (TOV) not exceeding 20.0 meq/kg. Therefore, the present study was conducted to evaluate the effect of adsorbents on the quality of eel fish oil in the silver phase based on chemical, physical, and microbiological characteristics. The eel fish used in this research was *Anguilla marmorata* (Q.) Gaimard, a type of eel fish that is abundant in the water of Central Sulawesi.

Eel (*Anguilla marmorata* Q. Gaimard) oil was successfully extracted using the Soxhlet method with diethyl ether as a solvent. Optimal conditions were achieved at 60°C for 5 hours, resulting in a yield of 36.489% (Jamal et al., 2021b). This finding aligns with previous research by Rahman et al (2023) which compared the extraction efficiency of various non-polar solvents (n-hexane, diethyl ether, and chloroform). Diethyl ether consistently demonstrated superior performance, yielding the highest oil content among the solvents tested.

Crude fish oil resulting from extraction processes still contains impurities, which can reduce the quality of fish oil by producing a rancid taste and odor, thereby affecting its stability and shelf life, so a purification process is necessary (Ahmadi & Mushollaeni, 2007). The purpose of purification is to remove unwanted components from oil so that it meets standard oil parameters, recommended by SNI 8467:2018 and IFOS, which makes them safe for consumption and to be used as pharmaceutical raw materials. The purification process consists of 3 stages, namely degumming, neutralization, and bleaching. The degumming process is carried out to separate phospholipid impurities comprising hydratable and non-hydratable phosphatides in the colloidal phase (Zufarov et al., 2008). Oil from the degumming process is then neutralized by the addition of a 9.5% NaOH solution. Neutralization using NaOH can reduce free fatty acids in fish oil and can adsorb dyes and impurities in the form of sap and mucus contained in the oil (Dari et al., 2017). In the bleaching process, three types of adsorbents, which included bentonite, magnesol XL, and zeolite were used. Bentonite is used for bleaching or refining, as bleaching earth, because it contains high levels of montmorillonite. The structure of the bentonite layer consists of silica, alumina, and monovalent components, which are located between the silica and alumina layers. The molecular configuration allows bentonite to be used as an adsorbent, which is quite effective in absorbing impurities in fish oil components (Suseno et al., 2011). Magnesol XL is the best adsorbent compared to other adsorbents, such as activated carbon, bleaching earth, diatomaceous earth, calcium silicate, and silica gel to reduce polar components, so that they can reduce the FFA value contained in cooking oil. Zeolite has high absorption effectiveness and is very efficient as an adsorbent (Hulyadi, 2017). However, the zeolite must be activated first to obtain a zeolite with high adsorption capacity. Zeolite activation is carried out chemically by adding acid (HCl) or base (NaOH) to remove inorganic impurities in the form of alkali or alkaline earth metals and several other types of metals contained in the zeolite framework (Lestari, 2010; Sriatun et al., 2008; Gea et al., 2020). According to Nadhiro et al (2018) bentonite has potential as an adsorbent in the process of refining crude

fish oil. The surface area of magnesol (700 m^2/g) and bentonite (305,957 m^2/gram) is large and they can absorb the maximum impurities contained in the oil (Bardant et al., 2021). In the present study, the refined oil was tested for its physicochemical properties to investigate whether the different adsorbents can improve the quality of eel fish oil based on SNI 8467:2018 and IFOS parameters.

Physical properties of the purified eel oil

Organoleptic (sensory) testing is a test based on the ability of the sensory organs to determine the quality level of a product, such as fish oil (Kusuma et al., 2017). The organoleptic test (aroma/ smell, color, and turbidity) results of the eel fish oil refined with the different adsorbents are presented in Table 1. The eel fish oil purified with 1% bentonite (MISMB) and 3% magnesol (MISMM) had average values of 8.867 and 7.867, respectively, in terms of aroma/smell specifications. Also, the eel fish oil purified with 5% acid-activated zeolite (5% MISZA) and 5% base-activated zeolite (5% MISZB) had average values of 8.133 and 7.5, respectively. Furthermore, the eel fish oil purified with 10% acid-activated zeolite (10% MISZA) and 10% base-activated zeolite (10% MISZB) had average values of 8.733 and 8.600, respectively. The average value of the four samples was more than 7 and close to 9, indicating closeness to the specific odor of fish oil. In terms of color specifications, the MISMB, MISMM, 5% MISZA, 5% MISZB, 10% MISZA, and 10% MISZB samples had average values of 8,600, 8,733, 8.033, 8.067, 7.867, and 7.467, respectively. The four samples had an average value of 7, which revealed that the samples had a golden yellow color. The color of fish oil is a result of color pigments found naturally in the oil during the extraction process, including alpha and beta carotene (carotenoids), which can give a golden yellow color (Eka et al., 2016). Heating process at high temperature (over-heating) can change the color of the oil to red (Wijaya et al., 2019).

Table 1. Organoleptic properties of eel fish (*Anguilla marmorata* (Q.) Gaimard) oil refined with different adsorbents.

Fish oil sample	Test Parameter	Odor	Color	Turbidity	SNI
1% Bentonite	Organoleptic Value Intervals	7.532 ≤ μ ≤ 8.202	8.609 ≤ μ ≤ 8.857	8.578 ≤ μ ≤ 8.885	7
	Mean ± SD (n=30)	8.100 ± 1.8 63	8.600 ± 0.8 14	8.867 ± 0.507	
3% Magnesol	Organoleptic Value Intervals	7.532 ≤ μ ≤ 8.202	8.587 ≤ μ ≤ 8.879	8.577 ≤ μ ≤ 8.889	
	Mean ± SD (n=30)	7.867 ± 1 .8 70	8.733 ± 0.6 91	8.733 ± 0.8 68 _	
5% acid-zeolite	Organoleptic Value Intervals	7.721 ≤ μ ≤ 8.545	7.775 ≤ μ ≤ 8.292	8.253 ≤ μ ≤ 8.614	
	Mean ± SD (n=30)	8.133 ± 1.154	8.033 ± 0.723	8.433 ± 0.504	
5% base-zeolite	Organoleptic Value Intervals	7.073 ≤ μ ≤ 7.927	7.584 ≤ μ ≤ 8.216	7.765 ≤ μ ≤ 8.369	
	Mean ± SD (n=30)	7.5 ± 1.196	8.067 ± 0.868	8.467 ± 0.507	
10% acid-zeolite	Organoleptic Value Intervals	8.486 ≤ μ ≤ 8.980	7.419 ≤ μ ≤ 8.315	8.582 ≤ μ ≤ 9.018	
	Mean ± SD (n=30)	8.733 ± 0.691	7.867 ± 1.252	8.800 ± 0.610	
10% base-zeolite	Organoleptic Value Intervals	8.309 ≤ μ ≤ 8.891	7.060 ≤ μ ≤ 7.874	7.843 ≤ μ ≤ 8.557	
	Mean ± SD (n=30)	8.600 ± 0.814	7.467 ± 1.137	8.200 ± 0.997	

Note:
Odor: Specific fish oil (9); Slightly acidic odor (7); Acidic odor (5); Rancid odor (3); Rancid and rotten odor (1). Color: Light yellow (9); Golden yellow (7); Reddish yellow (5); Brown (3); Brownish red (1). Turbidity: Clear (9); Not clear (7); Slightly cloudy (5); Cloudy (3); Very cloudy (1).

Concerning turbidity parameters, the average values for the MISMB and MISMM samples were 8.100 and 8.733, respectively; the average values for the 5% MISZA and 5% MISZB samples were 8.433 and 8.467, respectively; the average values for the 10% MISZA and 10% MISZB samples were 8,800 and 8,200, respectively. The four samples had an average value of more than 7 and were close to 9, indicating a higher clarity of fish oil samples. The three sensory test results satisfy the minimum score of 7 recommended by the SNI 8467:2018. Several factors influence the turbidity and color of oil, including the free fatty acid content, which easily undergoes primary and secondary oxidation, the amount of adsorbent used in the refining stage, as well as temperature and duration of the extraction process (Putri et al., 2020).

Viscosity is a measure of fluid's resistance to flow, which is caused by the amount of friction in the fluid (Hastuti & Muthmainnah, 2008). The average viscosity values (Table 2) of MISMB, MISMM, 5% MISZA, 5% MISZB, 10% MISZA, and 10% MISZB were 50, 60, 57, 51.3, 50, and 36.67 cP, respectively. Based on research conducted by Hulu et al (2017) and Suseno et al (2011) they reported the viscosity values of purified fish oil to be 30.89 and 51.13-66.63 cP, respectively. The viscosity value depends on the length of the fatty acid chain, the unsaturation of the fatty acid, temperature, pressure, humidity, and concentration (Aworanti et al., 2012). Sutiah et al (2014) reported that the viscosity value of oil

is influenced by its high density, which increases the friction that occurs between the oil layers. Fish oil purified with 5% acid zeolite had an average density of 0.923 g/mL, while fish oil purified with 5% alkaline zeolite had an average density of 0.966 g/mL as presented in Table 2. Also, the average density value of 0.976 g/mL for oil purified with 10% acid-activated zeolite and 0.865 g/mL for oil purified with 10% base-activated zeolite was achieved. When oil was purified using 3% magnesol XL, the average density value was 0.927; when oil was purified using 1% bentonite, the average density value was 0.924. According to Insani et al (2017) the quality of shark liver oil produced a density value of 0.91 g/cm^3. Meanwhile, pure sardine fish oil had a density value of 0.84 g/mL (Ibrahim et al., 2015). Based on SNI 8467:2018, the maximum density value is 0.91 g/cm^3. Ismaili et al (2015) reported that the density value is related to the weight fraction of the components contained in the oil, and the presence of impurities. The greater the molecular weight of a compound, the greater the density value. According to Handayani & Yusnimar (2013) and Suryani et al (2016) the fish oil refining process at the degumming and bleaching stages can cause a decrease in the density value and also affect the weight of the oil after refining. The degumming process can reduce or eliminate impurities (such as protein, pigment, water, etc.) contained in fish oil, while the bleaching with adsorbents can remove the dye contained therein by adsorbing impurity components (Dari et al., 2017).

Table 2. Viscosity and density of eel fish (*Anguilla marmorata* (Q.) Gaimard) oil refined with different adsorbents.

Parameter	Eel fish oil sample						FI 6th edition, 2020	ANOVA
	1% bentonite	3% magnesol	5% acid-zeolite	5% base-zeolite	10% acid-zeolite	10% base-zeolite		
Viscosity (cP)	60	50	57	51.3	50	36.67	-	0.016 *
Density (g/mL)	0.924 ± 0.006	0.927 ± 0.006	0.923 ± 0.014	0.966 ± 0.025	0.976 ± 0.074	0.865 ± 0.018	0.918 – 0.927	0.065 **

Note: *: Significant difference; **: Non significant difference.

When the eel oil was refined with the different adsorbents, the solubility of the oil is highlighted in Table 3. The solubility of oil purified with 3% magnesol XL, 1% bentonite, and 5% and 10% zeolite activated by acids and bases had the same solubility. They were completely soluble in diethyl ether, n-hexane, and chloroform solvents; partially soluble (turbid) in acetone; and insoluble in methanol and ethanol solvents. According to Faoziyah (2011) eel fish oil was insoluble in non-polar solvents (such as ethanol and methanol), slightly soluble in semi-polar solvents (acetone), and in non-polar solvents (n-hexane, diethyl ether, and chloroform). Eel fish oil dissolves completely. The solubility test results for eel fish oil adhere to the "*like dissolve like*" principle of extraction, which states that nonpolar compounds can only dissolve in nonpolar solvents and polar compounds can only dissolve in polar solvents (Megawati et al., 2020).

Table 3. Solubility of eel fish (*Anguilla marmorata* (Q.) Gaimard) oil refined with different adsorbents.

Fish oil sample	Solvent	Amount (mL)	Amount Solvent (mL)	Information
1% Bentonite	Diethyl ether	0.5	1	Late perfectly
	Ethanol			Insoluble (oil under solvent)
	Methanol			Insoluble (oil under solvent)
	n-Hexane			Late perfectly
	Chloroform			Late perfectly
	Acetone			Late partial (cloudy)
3% Magnesol	Diethyl ether	0.5	1	Late perfectly
	Ethanol			Insoluble (oil under solvent)
	Methanol			Insoluble (oil under solvent)
	n-Hexane			Late perfectly
	Chloroform			Late perfectly
	Acetone			Late partial (cloudy)
5% Acid-Zeolite	Diethyl ether	0.5	1	Late perfectly
	Ethanol			Insoluble (oil under solvent)
	Methanol			Insoluble (oil under solvent)
	n-Hexane			Late perfectly
	Chloroform			Late perfectly
	Acetone			Late partial (cloudy)
5% Base-Zeolite	Diethyl ether	0.5	1	Late perfectly
	Ethanol			Insoluble (oil under solvent)

Fish oil sample	Solvent	Amount (mL)	Amount Solvent (mL)	Information
10% Acid-Zeolite	Methanol	0.5	1	Insoluble (oil under solvent)
	n-Hexane			Late perfectly
	Chloroform			Late perfectly
	Acetone			Late partial (cloudy)
	Diethyl ether			Late perfectly
	Ethanol			Insoluble (oil under solvent)
10% Base-Zeolite	Methanol	0.5	1	Insoluble (oil under solvent)
	n-Hexane			Late perfectly
	Chloroform			Late perfectly
	Acetone			Late partial (cloudy)
	Diethyl ether			Late perfectly
	Ethanol			Insoluble (oil under solvent)
	Methanol			Insoluble (oil under solvent)
	n-Hexane			Late perfectly
	Chloroform			Late perfectly
	Acetone			Late partial (cloudy)

Chemical characteristics of the purified eel oil

The results of the FFA, PV, PAV, and TOV tests of eel oil refined with the different adsorbents are displayed in Table 4. Free fatty acids are one of the primary oxidation parameters in determining the quality of oil. A high value of FFA will cause the oil to have a poor taste and aroma (Megawati et al., 2020). Table 4 shows that the percentage of free fatty acids in oil purified with 1% bentonite was 0.831%, whereas the percentage of FFAs in oil purified with 3% magnesol was 0.503%. The average percentage of FFAs in oil purified with zeolite with 5% acid activation and base activation was 0.778 and 0.898%, respectively, and the average percentage of FFA in oil purified with zeolite with 10% acid activation and base activation was 0.463 and 0.252%, respectively. These results indicate that the oil samples are of good quality and can be used as raw materials because they meet the INS (<1%) and IFOS (<1.5%) standards. The ability of magnesol and bentonite to absorb FFAs and pigments depends on the silanol group. According to Kusumastuti (2004) the use of 10% zeolite can reduce the acid value from 4.63 to 4.31 mg KOH/g oil or a 7% reduction from the original. In the treatment with zeolite, there was no significant increase in the amount of FFAs. This is very beneficial because treatment with zeolite does not cause hydrolysis of fish oil. Hydrolysis of fish oil (triglycerides) causes fatty acids to be released from their bonds with glycerol so that the amount of free acid will increase. According to Raharjo (2018) triglycerides in meat or fish (animal products) can hydrolyze into diglycerides, and monoglycerides, and will form FFAs. Increasing hydrolysis of oil will increase the amount of free fatty acids produced. Increasing the amount of FFAs reduces oil quality and increases the potential for oil spoilage. Oil spoilage can affect the aroma so that the oil smells rancid (Ahmadi & Mushollaeni, 2007).

The peroxide value is a primary product of the oxidation process, which indicates the level of damage in the form of hydroperoxide content in the oil (Bija et al., 2017). The average peroxide values of 1,811, 3,424, 2,7132, 2,5839, 1,680, and 1,119 meq/kg were obtained for the MISMM, MISMB, 5% MISZA, 5% MISZB, 10% MISZA, and 10% MISZB, respectively. The results satisfy both the INS of <3 meq/kg and the IFOS of <3.75 meq/kg, indicating that the samples are of good quality for usage as a raw material. The peroxide value in the zeolite treatment with acid activation reached a maximum of 10% (102 meq/kg) and then continued to decrease. This observation is because the hydrogen peroxide resulting from oil oxidation is unstable and is then further degraded to form secondary oxidation products. This change causes the detected peroxide to decrease so that the peroxide value in turn decreases (Ketaren, 2012). The peroxide value in the oil purified with 3% magnesol XL was lower than the oil purified with 1% bentonite. The low peroxide value is due to the saponification reaction in the neutralization and bleaching processes. The use of magnesol XL in the bleaching process can adsorb organic compounds, including peroxide compounds due to the presence of silanol groups on the surface of magnesol (Yuliana et al., 2005).

The p-anisidine value is a measure of the secondary oxidation product formed due to primary oxidation, which produces non-volatile carbonyl side products [18]. In the present study, the average p-anisidine values of 2.667 meq/kg (MISMM), 3,993 meq/kg (MISMB), 12.5972 meq/kg (5% MISZA), 11.5370 meq/kg (5% MISZB), 8.103 meq/kg (10% MISZA), and 6,213 meq/kg (10% MISZB) were recorded, as presented in Table 4. These values meet both the IFOS (<15 meq/kg) and INS (<20 meq/kg) standards, which indicate that the oil samples are of good quality and can

be used as raw materials for pharmaceutical preparations. In addition to the inherent antioxidants in fish oil, storage time also contributes to the production of p-anisidine molecules, which can lower the p-anisidine value from 16.73 to 4.72 meq/kg, according to research by Rozi (2018). The high p-anisidine value is due to the presence of impurities in the oil, which can further decompose into aldehydic compounds. Aldehydic compounds and their derivatives are formed from intermediate reactions of PUFA with oxygen and high temperature to form aldehydic compounds, ketones, and their derivatives. Oil purified with 3% magnesol XL had a lower p-anisidine value compared to oil purified with 1% bentonite. The low p-anisidine value indicates that the added bentonite and magnesol XL can reduce fat oxidation products, such as peroxides, aldehydes, and ketones (Haryati et al., 2017).

The total oxidation value is related to the peroxide and p-anisidine values, which are obtained by adding these two values and are often used to determine the degree of oxidation of oil (Estiasih, 2009). Based on the results highlighted in Table 4, the total oxidation value at MISMM was 4.478 meq/kg, at MISMB was 7.417 meq/kg, at 5% MISZA was 18.0236 meq/kg, at 5% MISZB was 16.7049 meq/kg, at 10% MISZA was 9,408 meq/kg, and at 10% MISZB was 6,959 meq/kg. The total oxidation values for all oils met the standards of IFOS (< 20 meq/kg) and INS (< 26 meq/kg), which signifies good quality oil that can serve as raw materials. Fish oil purified with magnesol XL had the least total oxidation value. According to Bhattacharya et al (2008) magnesol XL is an adsorbent that can significantly reduce the levels of FFAs as well as primary and secondary oxidation products. Refining using magnesol XL can improve oil quality with a TOV of 76.41% (Rozi, 2018). The low TOV is caused by the ability of the adsorbent to reduce the oxidation parameters of fish oil because it is related to the adsorption capacity of the adsorbent. Also, factors that influence the adsorption capacity of the adsorbent, such as surface area, pore size, adsorbate solubility, pH, and temperature can reduce TOV (Haryati et al., 2017). Total oxidation value is a parameter for analyzing primary and secondary oxidation of fish oil. If the primary (FFA and PV) and secondary (PAV) oxidation parameters increase, the TOV produced will also be higher. The primary and secondary parameter values will increase with more purification steps, raising the TOV in the process (Dari et al., 2017).

Table 4. Chemical characteristics of eel fish (*Anguilla marmorata* (Q.) Gaimard) oil refined with different adsorbents.

Parameter	Purified Eel Fish Oil Sample						IFOS	SNI	ANOVA
	1% bentonite	3% magnesol	5% acid-zeolite	5% base-zeolite	10% acid-zeolite	10% base-zeolite			
FFA (%)	0.831 ± 0.068	0.503 ± 0.124	0.778 ± 0.106	0.898 ± 0.144	0.463 ± 0.193	0.252 ± 0.127	<1.5 %	<1 %	0.190 **
PV (meq /kg)	3.424 ± 0.886	1.811 ± 0.263	2.713 ± 0.387	2.584 ± 0.592	1.680 ± 0.646	1.119 ± 0.323	<3.75 meq/kg	<3 meq /kg	0.251 **
PAV (meq/kg)	3.993 ± 0.687	2.667 ± 0.037	12.597 ± 0.657	11.537 ± 0.166	8.103 ± 2.701	6,213 ± 2.590	<15 meq /kg	<20 meq/kg	0.431 **
TOV (meq/kg)	7.417 ± 1.565	4.478 ± 0.242	18.024 ± 0.693	16.705 ± 1.038	9.408 ± 2.642	6.959 ± 2.502	<20 meq /kg	<20 meq /kg	0.308 **

Note: *: Significant difference; **: Non significant difference.

The iodine value can be expressed as the degree of unsaturation of oil or fat, where the greater the iodine value, the higher the degree of unsaturation. Unsaturated fatty acids in oil or fat can absorb a certain amount of iodine and form saturated compounds (Pradhana, 2020). Iodine, saponification, and acid values, as well as clarity in fish oil refined with different adsorbents, are presented in Table 5. The average iodine value from oil purified with 3% magnesol XL was 67,206 m/yod, and oil purified with 1% bentonite was 69,943 m/yod. The average value of iodine obtained from oil purified with 5% acid-activated zeolite was 84.1397 m/yod and oil purified with base-activated 5% zeolite was 79.9488 m/yod. Furthermore, the average iodine value obtained from oil purified with 10% acid-activated zeolite was 72.872 m/yod and the iodine value of oil purified with base-activated 10% zeolite was 67.839 m/yod. The iodine values did not meet the SNI 8467:2018 (>120 m/yod) standard. The high amount of iodine absorbed indicates the number of double bonds or unsaturated bonds. The double bonds in oil can be damaged by heat and oxidation processes. Therefore, the low iodine value in refined oil can be caused by damage to double bonds during the refining process (Ibrahim et al., 2015; Kurniasih & Pardi, 2020).

The saponification value indicates the high level of free fatty acids in the oil, where the higher the saponification value, the more alkali needed to saponify the oil. Furthermore, the molecular weight of oil affects the saponification value, indicating that an oil with a lower molecular weight will have a larger saponification value than one with a higher molecular weight

(Kurniasih & Pardi, 2020; Tarigan, 2019). The results of the saponification value test are depicted in Table 5. Oil refined with 1% bentonite had an average saponification value of 164.983 mg KOH/g. The average saponification value obtained from oil purified with 3% magnesol XL was 155.033 mg KOH/g. The value obtained from oil purified with 5% acid-activated zeolite was 36.2682 mg KOH/g and oil purified with 5% base-activated zeolite was 42.7702 mg KOH/g. Furthermore, oil purified with 10% acid-activated zeolite had an average saponification value of 137.936 mg KOH/g, and oil purified with 10% base-activated zeolite had an average saponification value of 116.928 mg KOH/g. Based on the results obtained, the oil purified with bentonite and magnesol XL is composed of FFAs, which have a relatively small molecular weight compared to the oil purified with zeolite.

The acid value is a measure of the amount of FFAs contained in the oil (Sukeksi et al., 2017). The average acid value (Table 5) obtained from the oil purified with 5% acid-activated zeolite was 1.4075 mg KOH/g and the oil purified with 5% base-activated zeolite was 2.5023 mg KOH/g. The average acid value obtained for oil purified with 10% acid-activated zeolite was 0.489 mg KOH/g and the acid value for oil purified with 10% base-activated zeolite was 0.677 mg KOH/g. The average acid value in oil purified with 3% magnesol XL was 0.603 mg KOH/g and the acid value in oil purified with 1% bentonite was 1.475 mg KOH/g. These values met the IFOS and SNI standards, which is <3 mg KOH/g. The acid value shows the amount of FFAs contained in the oil or fat, which is usually associated with the hydrolysis process of the oil or fat. Hydrolysis of oil or fat by water with an enzyme catalyst or heat on triglyceride ester bonds will produce FFAs. The presence of FFAs in oil is usually used as an initial indicator that the oil is damaged due to the hydrolysis process (Pakiding et al., 2014).

Clarity has been used as an index of oil quality. High clarity in oil shows that there are fewer impurities in the oil (Pakiding et al., 2014). The clarity test results are presented in Table 5. A distinct percentage transmittance was observed in the clarity test results for oil that was purified with 10% acid-base activated zeolite. A wavelength of 450 nm wavelength was used for the measurement because it is the wavelength that has maximum light absorption, while wavelengths of 550, 620, 665, and 700 nm were used as a comparison (Rochyatun & Rozak, 2010). Oil purified with 1% bentonite had an average % transmittance of 78.524%. An average % transmittance of 88.233% was obtained for oil purified with 3% magnesol XL. Fish oil purified with 5% activated acid had an average % transmittance of 75.761%, while fish oil purified with 5% base activated zeolite had 72.130%. Furthermore, oil purified with 10% acid-activated zeolite and 10% base-activated zeolite had average % transmittance of 76.996% and 71.236%, respectively. According to Insani et al (2017) a high transmittance value and even close to 100% indicates that fish oil has a good level of clarity. Oil purified with 3% magnesol XL and 1% bentonite had higher clarity values than oil purified with 10% zeolite. Estiasih (2009) reported that primary and secondary oxidation products tend to affect the clarity of fish oil. Fish oil with a higher concentration of primary and secondary oxidation products will have a darker colour and less clarity.

Table 5. Iodine, saponification, and acid values, and clarity of eel fish (*Anguilla marmorata* (Q.) Gaimard) oil refined with different adsorbents.

Parameter	Purified eel fish oil sample						IFOS	SNI	ANOVA
	1% Bentonite	3% Magnesol	5% Acid-Zeolite	5% Base-Zeolite	10% Acid-Zeolite	10% Base-Zeolite			
Iodine Value (m/iod)	69.943 ± 0.000	67.206 ± 0.901	84.139± 1.992	79.948± 0.756	72.822± 1.972	67.839± 2.775	-	>120g/100 g	0.064 **
Saponification Value (mg KOH/g)	164.983± 0.833	155.033± 0.827	36.268± 3.893	42.770± 5.945	137.93± 6.895	116.92± 5.646	-	-	0.015 *
Acid Value (mg KOH/g)	1.475 ± 0.306	0.603 ± 0.200	1.407 ± 0.296	2.502 ± 0.251	0.489 ± 0.106	0.677 ± 0.221	< 3	< 3	0.254 **
Clarity (%)	87.167 ± 0.416	88.233 ± 0.503	75.761 ± 2.107	72.130 ± 2.061	76.996 ± 5.759	71.236 ± 4.887	-	-	0.047 *

Note: *: Significant difference; **: Non significant difference.

Heavy metal content of the purified eel fish oil

Heavy metals are dangerous substances if consumed above the threshold because they can damage or reduce the function of the central nervous system, and damage the composition of the blood, lungs, kidneys, and other vital organs (Rochyatun & Rozak, 2010). The levels of heavy metal contamination in eel oil purified with different adsorbents are presented in Table 6. The use of 3% magnesol XL and 1% bentonite met the minimum standards for mercury and arsenic metal levels with both values being less than 0.005. The amount of heavy metal content in oil refined with 5%

acid-activated zeolite met the minimal mercury content level with a value of <0.005, while the amount of arsenic and mercury metals in the 10% acid-activated zeolite treatment met the standards with <0.08 and <0.005, respectively. These results indicated that the arsenic value in eel fish oil purified with 10% zeolite was smaller than that obtained in eel fish oil purified with 5% zeolite. The results for Pb were high in all of the adsorbents used. Because eel fish migrate from sea waters to land waterways and vice versa, this is predicted. This migratory behavior allows fish to consume a variety of foods which increases the risk of heavy metal bioaccumulation (Digoarachchi et al., 2022).

Table 6. Heavy metal contamination of eel fish (*Anguilla marmorata* (Q.) Gaimard) oil refined with different adsorbents.

Eel fish oil sample	Metal	Results	Concentration (mg/Kg)	IFOS (mg/Kg)	BPOM 2019 (mg/Kg)
3% Magnezol	Cd (Cadmium)	Qualify	< 0.1	< 0.1	≤ 0.3
	Pb (Lead)	Not eligible	< 0.5		10.0
	Hg (Mercury)	Qualify	< 0.005		≤ 0.5
	As (Arsenic)	Qualify	< 0.005		≤ 5.0
1% Bentonite	Cd (Cadmium)	Qualify	< 0.1	< 0.1	≤ 0.3
	Pb (Lead)	Not eligible	< 0.5		10.0
	Hg (Mercury)	Qualify	< 0.005		≤ 0.5
	As (Arsenic)	Qualify	< 0.005		≤ 5.0
5% Acid-Zeolite	Cd (Cadmium)	Not eligible	< 0.25	< 0.1	≤ 0.3
	Pb (Lead)	Not eligible	< 0.75		10.0
	Hg (Mercury)	Qualify	< 0.005		≤ 0.5
	As (Arsenic)	Not eligible	0.18		≤ 5.0
10% Base-Zeolite	Cd (Cadmium)	Not eligible	< 0.25	< 0.1	≤ 0.3
	Pb (Lead)	Not eligible	< 0.75		10.0
	Hg (Mercury)	Qualify	< 0.005		≤ 0.5
	As (Arsenic)	Qualify	0.08		≤ 5.0

Fatty acid profile of the purified eel fish oil

Fatty acid profile analysis was conducted to determine the fatty acid content in eel fish oil. Determination of the fatty acid profile in purified eel fish oil was carried out using a GC-FID. This instrument is a gas chromatography device with a flame ionization detector. Gas chromatography was used for the analysis because oil has a fairly high vapor point due to its composition of triacylglycerol. The oil sample was first transesterified using a base catalyst and boron trifluoride (BF_3) to produce fatty acid methyl ester (FAME) before it was examined (Sasongko et al., 2022). Figures 1-4 show the fatty acid profiles of fish oil refined with different adsorbents, including 1% bentonite (MISMB), 3% magnesol XL (MISMM), 5% zeolite (MISMZ 5%), and 10% zeolite (MISMZ 10%). Monounsaturated fatty acids, both MUFA and PUFA, predominate in pure eel fish oil. MISMM contains unsaturated fatty acids consisting of 9 types of MUFA amounting to 34.34% (w/w), and 10 types of PUFA amounting to 9.95% (w/w). MMISB consists of 9 types of MUFA amounting to 36.27% (w/w), and 10 types of PUFA amounting to 11.71% (w/w). Eight MUFA types totaling 22.07% (w/w) and 10 types of PUFA totaling 12.63% (w/w) were found in 5% MISMZ. Meanwhile, 8 MUFA types totaling 23.96% (w/w) and 10 PUFA types totaling 11.45% (w/w) were observed in 10% MISMZ. According to (Gea et al., 2020) the highest MUFA (10.10%; w/w) and PUFA (2.56%; w/w) contents were obtained in the crude fish oil from Marble eel. Furthermore, eel fish oil that was purified with 3% magnesol XL and 1% bentonite had a higher MUFA and PUFA contents because the purification process could remove dirt, water content, and other components (Jamaluddin et al., 2019). The results of the present study showed that MISMZ had a lower unsaturated fatty acid composition compared to MISMM and MISMB. The low composition of unsaturated fatty acids in oil is linked to increased PV, PAV, and TOV, which indicates oxidation in fish oil (Dari et al., 2017). MMISM had lower MUFA and PUFA contents. According to Insani et al (2017) the oxidation process in fish oil containing fatty acids can be triggered by the presence of oxygen, peroxidase enzymes, radiation (light), and the presence of pollen metal ions. Apart from that, differences in fatty acid composition are also related to the type of feed consumed, environmental conditions, age of gonad, and species (Ayu et al., 2019).

The omega-3 acids in this study consisted of linolenic acid, eicosatrieonic acid, EPA, and DHA. Three omega-3 fatty acids (linolenic acid, EPA, and DHA) are known to play a very important role in human health.

The presence of omega-3 fatty acids such as EPA and DHA show that eel fish oil nutrition is of high quality. In this study, omega-6 was composed of linoleic, gamma-linolenic, eicosadienoic, arachidonic, and docosadienoic acids. Arachidonic acid is the highest fatty acid in omega-6 and has an important role in brain growth and development, and skin firmness (Almatsier, 2002). In the fish oils employed in this research, there are 2 essential fatty acids, namely linolenic acid (omega-3) and linoleic acid (omega-6). These fatty acids are unsaturated and are required by the human body to maintain health, but the human body does not have the enzymes to produce them. Essential fatty acids in the body contribute to the growth of the body's hair and skin, as well as maintaining bone health, metabolism, reproductive health, preventing heart disease, and contributing to brain function (Obrien, 2009; Febrianta & Rawendra, 2019). The omega-9 fatty acid in this study is composed of linolelaidic acid, elaidic acid, oleic acid, and erucic acid methyl ester. The most abundant fatty acid in omega-9 is oleic acid, which makes up 27.92% of MISMM, 27.31% of MISMB, 19.40% of 5% MISMZ, and 21.38% of 10% MISMZ. Oleic acid is the most common type of unsaturated fatty acid and is the precursor for producing most PUFA. It plays an important role in the body. Compared to PUFA, oleic acid is a MUFA that is more stable and plays a superior role (Sartika & Firdauzy, 2008). Meanwhile, MUFA can lower K-LDL but also raise K-HDL, PUFA can lower LDL cholesterol but also have an impact on lowering HDL (Febrianta & Rawendra, 2019). Based on the results of the analysis of the eel fish oil's quality and the fatty acids' pharmacological activity, it has been shown to add value to the refined oil so that it can be used as a raw material for pharmaceutical compositions.

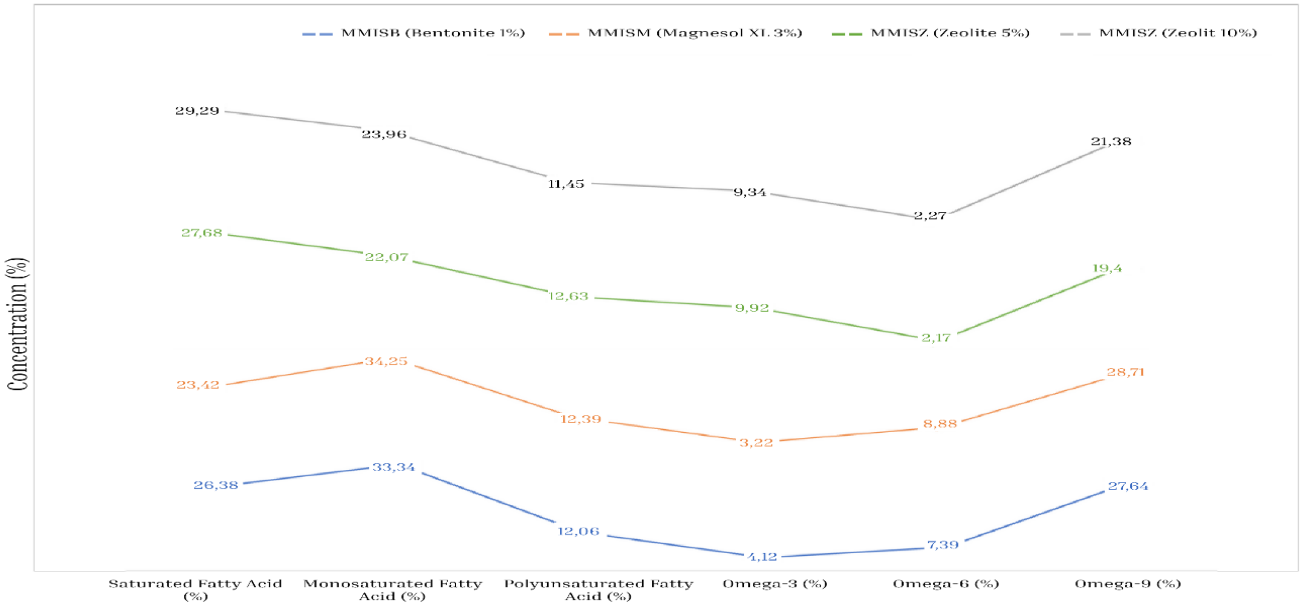

Figure 1. Fatty acid contents of eel fish (*Anguilla marmorata* (Q.) Gaimard) oil refined with different adsorbents.

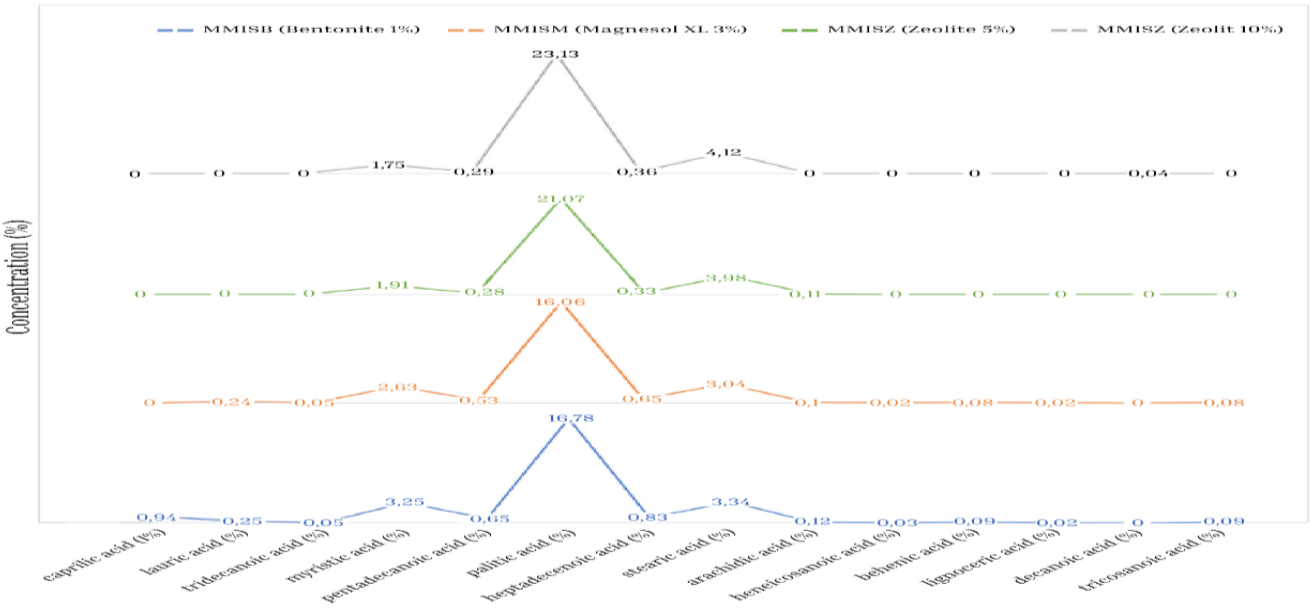

Figure 2. Saturated fatty acid content of eel fish (*Anguilla marmorata* (Q.) Gaimard) oil refined with different adsorbents.

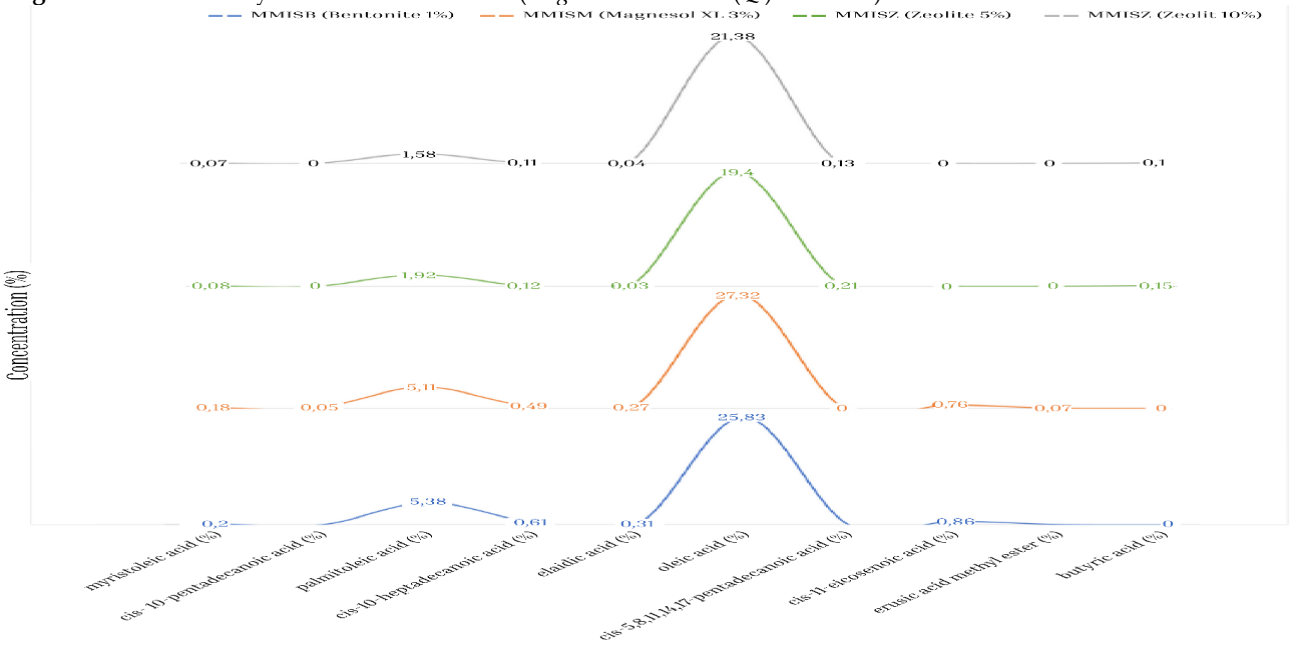

Figure 3. Monounsaturated fatty acid content of eel fish (*Anguilla marmorata* (Q.) Gaimard) oil refined with different adsorbents.

Figure 4. Polyunsaturated fatty acid content **of** eel fish (*Anguilla marmorata* (Q.) Gaimard) oil refined with different adsorbents.

Microbiological properties of the purified eel fish oil

To ensure the quality and safety of the refined eel fish oil, microbiological analysis was carried out using the TPC and TYMC to determine the quantity of microbial growth in the oil. Microbiological contamination is one of the test parameters for pure fish oil, following the SNI (2018) quality standard. When the techniques of TPC and TYMC were used to evaluate the microbial contamination of eel oil purified with the different adsorbents, the results obtained are shown in Table 7. An average value of 5.7x10^3 colonies/g was obtained from the use of zeolite adsorbent (MMISZ), while there was no TPC value recorded for the use of magnesol XL adsorbent. The cultures that failed to exhibit colony growth may have resulted from the media being overheated during incubation. Temperature is one of the factors that can influence microbial growth, where generally microbes can only grow optimally at human body temperature (Yusmaniar et al., 2017) The range of 25–250 colonies, which was used to calculate the TPC value, did not include the number of colonies obtained. Accordingly, based on (PPOMN, 2006) the number of colonies is determined by multiplying the average number of colonies by the dilution factor if the average number of colonies in each Petri dish is not between 25 and 250 colonies. Therefore, the average value of TPC from the two replicates was 5.7x10^3 colony/g. Fishery products are susceptible to contamination by microbes, especially bacteria because the condition of the fish supports their growth, which can cause damage to the fishery products (Akbar et al., 2022). When microbial contamination rises over defined limits, it can harm consumers by lowering the quality of fish-based raw materials and increasing the risk of infections and digestive disorders (Liang et al., 2022).

Table 7. Microbiological analysis of eel fish (*Anguilla marmorata* (Q.) Gaimard) oil refined with different adsorbents.

Eel fish oil sample	Replicate	Dilution	No. of Colony	Total plate count (colonies/g)	Total yeast and mold count (colonies/g)
5% Acid-Zeolite	1	10^{-1}	5	5.8 x 10^3	-
		10^{-2}	3		
		10^{-3}	3		
		10^{-4}	2		
		10^{-5}	0		
	2	10^{-1}	5	5.6 x 10^3	-
		10^{-2}	3		
		10^{-3}	2		
		10^{-4}	2		

		10^{-5}	0		
			Average	5.7×10^{3}	-
		10^{-1}	10		
		10^{-2}	7		
	1	10^{-3}	7		10×10^{1}
		10^{-4}	6		
5% Acid-Zeolite		10^{-5}	3		
		10^{-1}	8		
		10^{-2}	5		
	2	10^{-3}	7		8×10^{1}
		10^{-4}	3		
		10^{-5}	0		
			Average		9×10^{1}
		10^{-1}	0		
		10^{-2}	0		
	1	10^{-3}	0	0	
		10^{-4}	0		
3% Magnesol XL		10^{-5}	0		
		10^{-1}	0		
		10^{-2}	0		
	2	10^{-3}	0	0	
		10^{-4}	0		
		10^{-5}	0		
			Average	0	
		10^{-1}	0		
		10^{-2}	0		
	1	10^{-3}	0		0
		10^{-4}	0		
3% Magnesol XL		10^{-5}	0		
		10^{-1}	0		
		10^{-2}	0		
	2	10^{-3}	0		0
		10^{-4}	0		
		10^{-5}	0		
				Average	0

An average TYMC value was recorded for MMISZ ($9x10^{1}$ colonies/g), but there was no value recorded for MMISM. The range of 10-150 colonies, which was used to calculate the TYMC value, did not include the number of colonies obtained at dilution 10^{-1} - 10^{-5} for the second replicates. According to (PPOMN, 2006) the actual number of the lowest dilution level was noted and computed as an estimated mold/yeast number if none of the Petri dishes displayed a number between 10-150 colonies. Thus, 9×10^{1} colony/g was the average TYMC number obtained from the two replicates. The impact of consuming food contaminated with mold, which continuously produces aflatoxin can damage liver function and reduce the body's immune system (Amascual et al., 2023).

Conclusion

The present study underscores the efficacy of selected adsorbents in refining eel fish oil to meet the stringent quality standards outlined by SNI 8467:2018 and IFOS. The incorporation of magnesol XL 3%, bentonite 1%, zeolite 5%, and zeolite 10% during the purification process resulted in a marked improvement in the overall quality of the fish oil, encompassing physical, chemical, and microbiological attributes. The observed enhancements can be attributed to the adsorbents' capacity to effectively remove impurities such as free fatty acids, peroxides, and heavy metals, thereby contributing to a more refined and stable fish oil product. These findings highlight the potential of adsorbent-based refining techniques in optimizing the quality of eel fish oil and ensuring its suitability for various applications.

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Author Contributions
Conceptualization, J.M., Y.L; methodology, J.M., W.O.M., Y.Y., S.S.; validation, J.M., P.A., A.M., .; formal analysis, J.M., Y.L., G.P., M.F.H.,; investigation, W.O.M., S.L., N.A.F., S.B.A., S.A.; resources, J.M., A.H., N.S.; data curation, J.M.: writing—original draft preparation, J.M., Y.L.,; writing—review and editing, J.M., A.M., N..S; visualization, J.M., dan Y.Y.

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Conflicts of Interest
The authors declare no conflicts of interest.

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