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Research article

Physicochemical Properties and Antioxidant Activity of Fish Oil from Indian Mackerel (*Rastrelliger kanagurta*)

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Abstract

Fish oil is widely known for its beneficial polyunsaturated fatty acids content, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The Indian mackerel (*Rastrelliger kanagurta*) is an abundant marine fish living in Indonesian waters and offers excellent potential as a fish oil source. However, there have been limited studies on the specific profile of fish oil derived from this species. This study aimed to investigate the physicochemical properties and antioxidant activities of Indian mackerel fish oil. Fish oil was extracted using the wet rendering method, and oil characterization was done using proximate analysis, physicochemical analysis, and FTIR spectroscopy. The antioxidant activity was tested using the DPPH method. The Indian mackerel fish oil extracted had a lipid content of 95.93±0.042%, protein (1.285±0.177%), carbohydrate (1.986±0.143%), moisture (0.775±0.006%), and ash (0.016±0.003%). Physicochemically, this oil had FFA 1.4±0.02%, saponification value 227.46±0.71 mg KOH/g, PV 8.46±0.047 meq/kg, pAV 10.27±0.41, TOTOX 27.19±0.31. The heavy metals Cd and Pb were less than 0.01 ppm, and the Hg content was less than 0.025 ppm. FTIR spectra showed the presence of unsaturated functional groups (=C-H), alkane (-C-H), carbonyl (-C=O), and alkene (-C =C). The antioxidant activity fell into the "very strong" category, with an IC_{50} value of 36.52±1.27 ppm. The overall results demonstrated the fish oil's favorable physicochemical characteristics and bioactivity, providing a foundation for further purification and potential applications.

Keywords: Indian mackerel; fish oil; physicochemical properties; antioxidant activity

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1. Introduction

Fish oil is a popular nutritional supplement with a global market value of USD 2.133 million in 2021, and it is projected to grow annually by 6% and reach up to USD 3.60 billion by 2030 (Research, 2024). Nutraceuticals derived from fish oil comprise various polyphenols and essential omega-3 fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which the human body cannot synthesize (Schuchardt et al., 2024). Fish oil also contains beneficial substances such as vitamins, minerals, monounsaturated fatty acids (MUFAs), saturated fatty acids (SFAs), and carotenoids. Prominent fish species like salmon, sardine, tuna, mackerel, and trout are excellent sources of fish oil. Marine fish production has grown four times over the last fifty years, making fish oil an increasingly crucial dietary supplement (Liu & Dave, 2022).

Indian mackerel (*Rastrelliger kanagurta*) is a widely consumed species in Indonesia and worldwide. This fish is abundant in South Sulawesi, the Flores Sea, and Bone Bay, Indonesia (Zamroni et al, 2016). In 2020, 362,000 tons of Indian mackerel were caught, with production continuing to rise annually (Harahap et al., 2020). Unfortunately, over 70% of harvested fish, including heads, viscera, skin, and scales, are discarded as waste despite these by-products containing valuable fatty acids. Studies have shown that Indian mackerel waste, particularly its skin, has a PUFA content ranging from 20.84% to 34.12% (Widiyastuti et al., 2021; Maharani et al., 2023). PUFAs, along with MUFAs and SFAs, exhibit antioxidant activity that scavenge free radicals in the body. The antioxidant activity plays a role in alleviating diseases such as diabetes, cancer, and cardiovascular disorders (Huang et al., 2022; Pinela et al., 2022). Therefore, utilizing Indian mackerel skin as a source of fish oil holds significant potential for developing functional foods.

This research study fills some of the research gaps in fish oil extraction. Previously, researchers mostly used enzymatic extraction (Gbogouri et al., 2006), solvent extraction (Adeniyi & Bawa, 2006), and supercritical fluid extraction (SFE). These methods, although effective in extracting high-quality oil, face limitations in high capital costs and complex technical parameters that make them difficult to use on an industrial scale (Sahena et al., 2010). This research presents a perspective using a wet rendering method without chemical solvents that is simple, easy to implement on an industrial scale, and economical. The method makes oil extraction more efficient but maintains good oil quality. Physicochemical characteristics, such as solubility, consistency, color, odor, peroxide value, saponification value, free fatty acid content, and heavy metal content, strongly influence the quality of fish oil. Based on previous studies, fatty acid composition, peroxide value (PV), and free fatty acid (FFA) content largely determine the stability and shelf life of fish oil (Azim et al., 2018; Tilinti et al., 2023). Thus, this study closed the gap in previous studies by further investigating fish oil's quality parameters, including proximate tests (fatty acids, proteins, carbohydrates, ash) and physical tests (density, moisture content).

Furthermore, this study confirmed that Indian mackerel is a source of omega-3 fatty acids (EPA and DHA) that are beneficial for health (Sonavane et al., 2017) by confirming the functional groups of fatty acids through FTIR analysis. However, previous studies tended to neglect the exploration of food safety aspects, especially concerning the content of heavy metals and other chemical compounds that may be present in mackerel (Azim et al., 2018). This study not only evaluated the chemical composition of fish oil but also identified the presence of heavy metals (including Cd, Hg, and Pb) and oxidation values (FFA, PV, pAV, and TOTOX), which are critical parameters in the safety and feasibility aspects of fish oil for consumption. In addition to safety and stability aspects, this study also examined the functional potential of fish oil through an antioxidant activity test

using the DPPH method. Research on fish oil as a source of antioxidants remains limited, despite the important role antioxidant compounds play in preventing oxidative stress and various degenerative diseases. Based on this analysis, fish oil from Indian mackerel skin has the potential to be developed into a functional nutraceutical product that offers broader health benefits. By exploring the potential of fish skin as a high-quality oil feedstock, this research contributes to reducing fisheries waste and promoting the circular economy concept. Converting fisheries waste into high-value products can enhance the efficiency of the fish processing industry and support more sustainable and environmentally friendly practices.

2. Materials and Methods

2.1 Materials

The equipment used in this study included a set of extraction tools, an analytical balance (Mettler Toledo) with a readability of 0.1 mg, a UV-VIS spectrophotometer (Hitachi), a pycnometer, a titration set, and glassware (Pyrex) commonly used in the analytical chemistry laboratory. Indian mackerel skin was obtained locally from Balekambang Market, Surakarta, Central Java. Other reagents used were potassium hydroxide (Merck), sterilized bentonite powder 1000 mesh (Kobayashi Costalc Indonesia), glacial acetic acid (Merck), NaCl (Sigma Aldrich), sodium thiosulfate (Merck), 2,2-diphenyl-1-picrylhydrazyl compound (DPPH) (Sigma Aldrich), potassium iodide (Merck), PP indicator, ethanol 96% (Merck), amylum indicator, starch indicator, ethanol (p.a) (Merck), iodine bromide reagent, chloroform (p.a) (Merck), alpha-tocopherol (Sigma Aldrich), n-hexane (p.a) (Merck), and aquadest.

2.2 Methods

2.2.1 Crude fish oil extraction

The crude fish oil extraction of Indian mackerel skin was done using the wet rendering method with modification (Huli et al., 2014; Iwo, 2019). The fish scales and fins were removed, followed by manually separating the skin from the meat. The fish skin was weighed and placed in a beaker, then added with a 1:1 (w/v) ratio of distilled water (aquadest). The mixture was placed in a water bath at 80°C for 2 h, and then filtered to obtain the filtrate. Next, the filtrate was centrifuged at 15,000 ×g for 20 min at 25°C. After centrifugation, 2.5% NaCl was added to the supernatant in a 1:1 (v/v) ratio, and the mixture was heated at 50°C for another 20 min. This centrifugation step was repeated with the same settings. The resulting supernatant was treated with 3% bentonite as water adsorbent, added at a 1:1 (v/v) ratio. The mixture was homogenized at 29°C for 20 min. The bentonite-treated mixture underwent centrifugation at 10,000 ×g for 20 min at 25°C. The crude fish oil was then stored at -18°C. The yield is calculated using the following formula.

$$\text{Yield (\%)} = \frac{weight\ of\ oil\ produced\ (g)}{wet\ fish\ skin\ weight\ (g)} \times 100\%$$

2.2.2 Proximate analysis of fish oil

1) Lipid content

Two grams of fish oil were homogenized with 300 mL of chloroform in a 2:1 (v/v) ratio at 12,000 rpm for 2-3 min. The mixture was then added with chloroform (100 mL) and distilled water (100 mL). The mixture was then homogenized and filtered (filter paper Whatman No. 1). The residue was added with 100 mL of chloroform, filtered, and added to the previously collected filtrate. The combined filtrates were subjected to phase separation through a separatory funnel. Excess chloroform with lipids from the lower fraction was removed with a rotary vacuum evaporator, and the lipids gained were then weighed (Sündermann et al., 2016). The lipid content obtained was determined based on the following formula.

$$\text{Lipid (\%)} = \frac{weight\ of\ lipid\ (g)}{weight\ sampel\ (g)} \times 100\%$$

2) Protein content

A mixture of 0.2 g sample, H_2SO_4 (20 mL concentrated), and $CuSO_4$ (5 mL, 0.2 N) was added to a conical flask. The digestion process was done for 30 minutes, and a greenish-blue color appeared. Next, the mixture was added with distilled water (10 mL). Boric acid (5 mL, 2%) and 2 drops of phenolphthalein were added to the mixture. Digested material (10 mL) was added with NaOH (20 mL, 40%) (AOAC, 2005; Ndidiamaka & Ifeanyi, 2018). Following this, titration was done with HCl (0.1 N). The protein content in the fish oil was calculated using the formula:

$$\text{Protein (\%)} = \left(\frac{(Volume\ HCl \times normality\ value\ of\ HCl \times 0.014 \times 100)}{Sample\ weight\ (g)} \times 6.25\right)$$

3) Carbohydrate content

The carbohydrate content value was the subtraction of the sum of percent protein, fat, moisture, and ash from 100% (AOAC, 2005).

4) Moisture content

Aluminum dish was filled with samples, and the initial weights (moist weight) were determined. Then, the samples were dried in an oven at 105°C for 3.5 h until the weight was constant. The percentage of moisture content was calculated based on the weight loss before and after drying (AOAC, 2005).

5) Ash content

Empty porcelain cups were dried at 120°C, cooled in a desiccator, and weighed. A 4 g sample was placed in the weighed container. The sample in the porcelain cup was then placed in a muffle furnace at 600°C for 6 h until it turned to ash. The ash was cooled in a desiccator, and the resulting residue was weighed. Total ash content was calculated as a percentage based on dry matter (AOAC, 2005).

2.2.3 FTIR analysis

An oil sample of 0.2 mL was deposited onto the polished salt plate's surface made of potassium bromide (KBr). Another plate was put above the first to create a thin layer of liquid. A thin layer of oil was also rubbed onto the edges of the plate to ensure an even distribution. The measured spectra were noted over a 4000 to 400 cm^{-1} wavelength, with a resolution of 1 cm^{-1}, after applying the KBr sample plate (Schneider et al., 2017).

2.2.4 Antioxidant test by DPPH method

In this procedure, an equal amount (500 µL) of DPPH solution (Sigma Aldrich, St. Louis, MO, USA) was mixed with the sample, including fish oil and ascorbic acid. The resulting mixture was then agitated with a vortex and incubated at room temperature without the presence of light for 30 min. After incubation, absorbance at λ = 517 nm was measured in triplicate using a UV-VIS spectrophotometer (Hitachi, Japan). Eight different concentration variants were used in all experiments. Antioxidant activity was expressed as the IC_{50} value, representing the test sample concentration needed to inhibit 50% of free radicals. This value was determined using a linear regression equation based on the percentage of inhibition (Herawati et al., 2022). The percentage of inhibition for the antioxidant tests conducted using the DPPH method was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{(control\ absorbance - sample\ absorbance)}{control\ absorbance} \times 100\%$$

2.2.5 Characterization of crude fish oil

1) pH value

The pH was measured without diluting the samples and directly inserting the pH meter electrodes into fish oil at 25°C. The pH reading was done in triplicate (AOAC, 2005).

2) Density

A pycnometer was weighed in an empty state (W1). The fish oil was put in the pycnometer and then incubated in a bath for 30 min. After incubation, the pycnometer was opened and cleaned with filter paper. The pycnometer was left at room temperature, dried, and weighed (W2). The procedure was repeated with aqueous blanks. The following equation was used to calculate the density value (AOAC, 2005).

$$\rho\ (g/mL) = \frac{(W2-W1)}{V}$$

Described as, W1, W2 (weight in grams); V (pycnometer volume in mL)

3) Free fatty acid (FFA)

A total of 2-5 g of oil mixed with 25 mL of 95% alcohol, and heated in a water bath for 10 min. The mixture was dripped with PP indicator as much as 2 drops. After that, the mixture was shaken and titrated with 0.1 N KOH until a pink color appeared that did not disappear

within 10 s. The percentage of FFA was calculated based on the following equation (AOAC, 2005).

$$\text{FFA (\%)} = \frac{(A \times N \times M)}{G}$$

Described as, A (amount of KOH titration in mL); N (normality of KOH); G (gram sample); M (molecular weight of dominant fatty acid).

4) Saponification

The saponification value is the amount of potassium hydroxide (KOH) needed to turn oil (1 g) into soap. One gram of fish oil was added with KOH-ethanol (50 mL) in an Erlenmeyer flask and dissolved homogeneously. Then, it was subjected to heat at 80-85°C for 30 min. The cooled mixture was added with phenolphthalein (1 mL). The mixture was subjected to titration with 0.5 N HCl until the mixture was no longer pink-colored. The saponification value was calculated using the following equation (AOAC, 2005).

$$\text{Saponification value (mg KOH/g)} = \frac{(Volume\ of\ HCl - Volume\ of\ blank) \times Normality\ of\ HCl \times 56.1}{mass\ of\ sample\ (g)}$$

5) Peroxide number

The titrant was a thiosulfate solution, and the indicator was a starch solution. This method can detect substances that oxidize potassium iodide under acidic conditions. A sample of 2.5 g of fish oil was put in an Erlenmeyer flask, mixed homogeneously with potassium iodide (0.5 mL), and added with chloroform and acetic acid and solution in a ratio of 2:3. The mixture was slowly shaken, and 30 mL of aquadest was added. The titration was done with 0.01 N sodium thiosulfate ($Na_2S_2O_3$) until a yellow color appeared. A 1% starch indicator solution of 0.5 mL was added to the mixture to change the color to blue. The titration was continued by shaking until the solution changed color to light blue. This color change indicated iodine release from the chloroform layer. The following equation calculated the peroxide value (AOAC, 2005)

$$\text{Peroxide Value (meq/kg)} = \frac{(Volume\ of\ sample - Volume\ of\ blank) \times N \times 1000}{weight\ of\ sample}$$

Described as below:
V sample: Volume of $Na_2S_2O_3$ titrant used for the sample (mL)
V blank: Volume of $Na_2S_2O_3$ titrant used for blank (mL)
N: Normality of $Na_2S_2O_3$ solution
Weight of sample in grams

6) p-anisidine

The p-anisidine value was determined by dissolving 0.5 g of fish oil into 25 mL trimethylpentane as test solution 1. Further, test solution 2 was made by adding 1 mL of p-anisidine solution (2.5 g/L) into 5 mL of test solution 1, then homogenized in a dark room. A blank solution was made by adding 1 mL of p-anisidine solution into 5 mL of trimethylpentane solution and homogenized. After incubation, absorbance at λ = 350 nm

was measured in triplicate using a UV-VIS spectrophotometer (Hitachi, Japan). The anisidine value was determined by the following equation (Watson, 1994; Delfanian et al., 2018).

$$\text{pAV} = \left(25 \times \frac{(1.2 \times A2) - A1}{M}\right)$$

Described as, A1 (absorbance of solution before reaction); A2 (absorbance of solution after reaction with p-anisidine); M (mass of sample in grams)

7) TOTOX

Determination of the total oxidation value (TOTOX) was calculated by the equation below

$$\text{TOTOX} = 2\text{PV} + \text{pAV}$$

Where PV stands for peroxide number value and pAV stands for anisidine value. An empirical parameter known as the TOTOX value sums together two values with different components (Koohikamali & Alam, 2019).

8) Lead content

The fish oil (2 mL) was added to a porcelain cup with 4 mL of nitric acid (HNO_3). The sample was then digested and cooled to room temperature. After cooling, distilled water (50 mL) was added and filtered using filter paper. The sample and the standard solution were analyzed using an atomic absorption spectrophotometer (AAS) (Thermo Scientific ICE 3500) at 283.3 nm wavelength (Schneider et al., 2017).

9) Cadmium content

A 0.5 mL sample of oil was mixed with 5 mL of 37% HNO_3 and 3 mL of 30% hydrogen peroxide (H_2O_2) in a tightly closed vessel. The solution was subjected to high temperatures until it became clear. Then, the mixture was diluted with aquadest to reach 15 mL. This dilution was performed once the temperature had decreased. The concentration of cadmium was determined using high-resolution continuum source graphite furnace atomic absorption spectroscopy (HR-CS GF AAS) (Analytik Jena) at 228.8 nm (Schneider et al., 2017).

10) Mercury content

Fish oil (1 mL) was mixed with nitric acid (HNO_3, 2.5 mL), sulfuric acid (H_2SO_4, 5 mL), and 5% potassium permanganate ($KMnO_4$, 15 mL). The mixture was allowed to sit for 15 min. Following this, a 5% potassium persulfate ($K_2S_2O_8$) solution was added. The mixture was placed in a 95°C water bath for 2 h. After the mixture was cooled to room temperature, the excess $KMnO_4$ was reduced by adding a 10% hydroxylamine-hydrochloride solution, and the sample was filtered. The filtrate was added with 5 mL of a 10% Tin (II) chloride ($SnCl_2$) solution. The mercury (Hg) content was subsequently measured using a Shimadzu AA-7000 atomic absorption spectrophotometer (SSA) at 253.7 nm (Schneider et al., 2017).

2.2.6 Data analysis

The data obtained in this study were collected in 3 repetitions and tabulated, and the mean and standard error for each test parameter were calculated. The concentration values for the antioxidant activity parameter, converted into linear regression equation curves and represented as y = a + bx, were used to determine the IC_{50} value and the percentage of inhibition.

3. Results and Discussion

3.1 Proximate analysis

Extraction of fish oil from Indian mackerel skin using the wet rendering method resulted in a lipid content of 95.93±0.042%, indicating that this method successfully targeted the lipid component of the crude oil. To the best of our knowledge, this study provides the first information on the proximate content of fish oil from this species. Other than that, fish oil from yellowfin tuna (*Thunnus albacares*) head extracted using enzymatic hydrolysis after a single ultra-high pressure pre-treatment (EHSUP) yielded lipids of 67.97% (Zhang et al., 2021). The high lipid content extracted using wet rendering with distilled water solvent indicated that Indian mackerel had great potential in producing oil rich in fat. Although this study did not test the fatty acid profile, previous findings by Sahena et al. (2010) showed Indian mackerel skin to be a potentially high source of essential fatty acids such as EPA and DHA. Thus, the wet rendering technique may produce high-quality oils with an essential fatty acid profile similar to that found in SFE extraction. However, it should also be noted that the wet rendering method faces the challenge of oxidation levels due to the heat treatment process (Djamaludin et al., 2023). Therefore, the temperature range used in the extraction process must be considered when attempting to produce oil of good quality, especially lipid content.

The remaining composition was protein (1.285±0.177%), carbohydrate (1.986± 0.143%), moisture (0.775±0.006%), and ash (0.016±0.003%). When comparing carbohydrate content between different fish oils, we observed variations influenced by species and habitat. The low carbohydrate content in Indian mackerel oil (1.98±0.143%) may reflect a less rigorous extraction process or specific properties of the fish species. While this carbohydrate amount is not significant enough to drastically impact the oil's nutritional profile, it does provide a minor energy contribution and may affect the oil's physical behavior during storage. On the contrary, Atlantic mackerel (*Scomber scombrus*) oil typically has a higher carbohydrate content (4.00%) (Fuadi et al., 2014), while African catfish (*Clarias gariepinus*) oil showed quite similar carbohydrate content (1.77%) (Ndidiamaka & Ifeanyi, 2018). These differences may arise due to variations in the living environments of these species.

Moisture in oil acts as a pro-oxidant and is undesirable, as it needs to be removed during the oil refining process. In this study, the moisture content (0.775%±0.006) was notably lower than that found in sardine oil (2%), catfish oil (0.933%), and carp oil (0.89%) but higher than tuna oil (0.29%) (Zhang et al., 2021; Lakmini et al., 2022; Tilinti et al., 2023). Higher rendering temperatures lower moisture levels. The high moisture level in fish oil can promote hydrolysis, increasing free fatty acids and rancidity. The Indian mackerel oil in this study contained a much lower ash (0.016±0.003%) than tuna oil (5.20±0.12%) (Lakmini et al., 2022) and the typical range for fish oil (0.21-1.18%) (Tilinti et al., 2023), indicating a high level of purity. Lower ash content signifies fewer inorganic substances, enhancing the

fish oil's overall quality. However, the measured protein content (1.285%±0.177) was lower than the recommended value of FAO (19.8%) and other studies (6.7-19.4%) (Shahi et al., 2018). The result indicates that the Indian mackerel fish oil can be considered a high-quality product with low moisture and ash levels. However, it would benefit from an additional refining process to remove any remaining undesirable components. Differences in the proximate composition of the same and different species from different parts of the world can be caused by physiological factors (migration or spawning), and natural factors (diet), as these affect the chemical composition, especially deviations in the high lipid fraction of the fish (Pradhan et al., 2014; Lakmini et al., 2022).

3.2 FTIR spectra analysis

FTIR can detect the presence of fatty acids by detecting specific functional groups in the fish oil. In this study, FTIR analysis was done in 4000 cm^{-1} to 400 cm^{-1} wavelengths. The results of FTIR analysis of Indian mackerel fish oil (Figure 1, Table 1) revealed that several functional groups were present, including unsaturated groups (=C-H), alkanes (C-H), alkenes (-C=C), carbonyls (-C=O), esters (-C-O), and methyls ($-CH_2$). Identifying functional groups was done by observing the absorption value at a certain wavelength.

The absorption values were determined from the peaks in the spectrum, indicating a low transmittance region. Low percent transmittance indicates a higher absorbance, reflecting the presence of a specific molecular group. The spectral analysis of Indian mackerel fish oil showed a strong absorption at 3011.01 cm^{-1}, corresponding to an unsaturated group (=C-H), and a weak absorption at 1653.07 cm^{-1}, indicating the presence of a double bond (C=C). These results confirm that unsaturated fatty acids were present in the Indian mackerel fish oil.

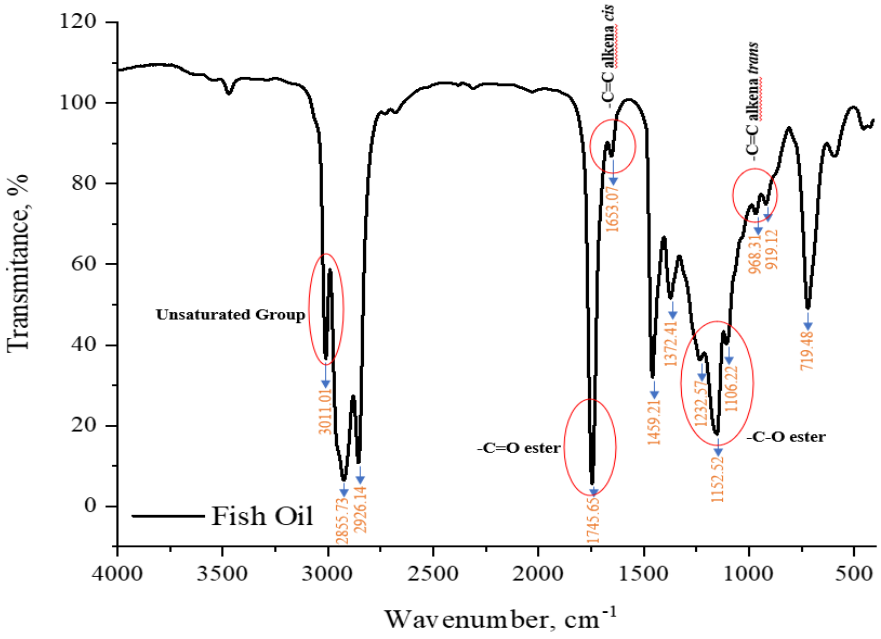

Figure 1. The functional group spectra of Indian mackerel fish oil

Table 1. Spectra and functional groups of Indian mackerel fish oil (according to Loughrill et al., 2019; Megawati et al., 2020)

Wavelengths (cm^{-1})	References (cm^{-1})	Functional Group
3011.01	3010 - 30002	=C-H (unsaturated group)
2855.73 and 2926.14	3000 - 28501	-C-H alkane
1653.07	1680 - 16002	-C=C alkene *cis*
968.31	968 - 9662	-C=C alkene *trans*
1745.65	1725 - 17001	-C=O carbonyl
1106.22; 1152.52; 1232.57	1300 - 10001	-C-O ester
1459.21	14652	$-CH_2-$

It is noteworthy to highlight the finding of a carbonyl group (-C=O) in the Indian mackerel fish oil sample, indicated by a strong absorption at 1745.65 cm^{-1} and weak absorption between 3500-3025 cm^{-1}. The result indicates the stretching vibration of the carbonyl group. The sharp absorption at 1152.52 cm^{-1} further confirmed that this carbonyl group belongs to an ester group. There were also strong absorption peaks at 2926.14 cm^{-1} and 2855.73 cm^{-1}, suggesting the presence of methyl groups (CH_3) from alkyl (Tilinti et al., 2023). This finding was supported by the bending vibration observed at 1459.21 cm^{-1}, which corresponded to the methylene group (CH_2) (Megawati et al., 2020). The methyl group is a typical structure found at the end of a fatty acid. In conclusion, the FTIR analysis showed the presence of several important functional groups in Indian mackerel fish oil, such as unsaturated fatty acids, carbonyl, ester, and methyl groups, which are key indicators of oil composition and quality.

3.3 Antioxidant activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method, a cornerstone in antioxidant testing, is widely used due to its simplicity, speed, and sensitivity to samples with low concentrations. The principle is based on the ability of antioxidant compounds to donate hydrogen atoms or electrons to reduce DPPH free radicals, leading to a change in the absorbance spectrum, marked by a color change from purple to yellow (Antolovich et al., 2002). The inhibitory concentration (IC_{50}) is the sample concentration required to inhibit 50% of DPPH radicals, where the lower the IC_{50} value, the higher the antioxidant activity. This method is a powerful test in our quest to understand the antioxidant properties of fish oils.

In this study, Indian mackerel oil showed an IC_{50} value of 36.52±1.27 ppm (Table 2, Figure 2), indicating very strong antioxidant activity based on Agatonovic-Kustrin's (2014) classification (<50 ppm). This result was better than oil from *Odonus niger* fish liver (53.26 ppm) and catfish (40.88±0.18 ppm) but less effective than tindarung fish bone oil (20.958±2.7 ppm) (Rumalutur et al., 2024). This difference is thought to be influenced by factors such as the extracted fish parts, extraction method, and fatty acid composition. For example, tindarung bone oil contains extracted fatty acids that enhance its radical-scavenging ability, while liver oil tends to contain more saturated fat, which might reduce its antioxidant effectiveness.

Table 2. Antioxidant activity of Indian mackerel fish oil

Sample	Antioxidant Activity (IC_{50}±SD)	References
Ascorbid acid	6.24±2.8 ppm	
Indian mackerel skin	36.52±1.27 ppm	
Tindarung fish bones	20.958±2.7 ppm	Rumalutur et al., 2024
Odonus niger liver	53.26 ppm	Giruba et al., 2022
Patin fish oil	40.88±0.18 ppm	Putri et al., 2020

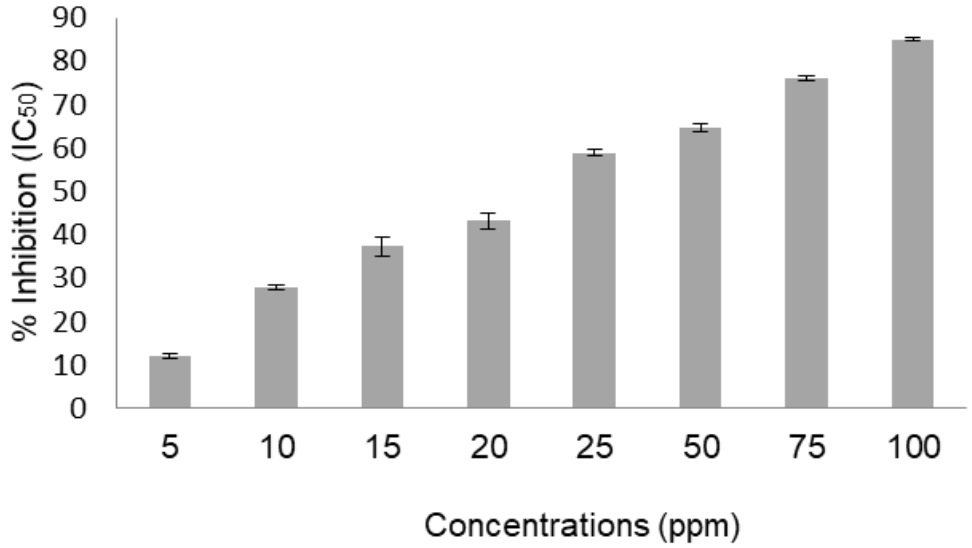

Figure 2. Free radical inhibition of different Indian mackerel fish oil concentrations. The data are the average of three replicates±standard error mean (SEM).

The omega-3 fatty acid (DHA and EPA) content of fish also plays a crucial role. These compounds have stronger antioxidant effects than other fatty acids, such as oleic and stearic, due to their ability to donate more reactive electrons (Haq & Chun, 2018). In comparison, Atlantic salmon belly oil extracted using hexane solvent showed an IC_{50} of 100 ppm, a much higher value in the moderate category (100-150 ppm), indicating that the extraction method significantly affects antioxidant activity (Agatonovic-Kustrin, 2014). It should be noted that the classification of IC_{50} values in the range of 151-200 ppm is included in the weak category, and >200 ppm is included in the very weak category (Agatonovic-Kustrin, 2014). Ascorbic acid as a positive control produced an IC_{50} value of 6.24±2.8 ppm, which is still higher than all fish oil samples. The variation in antioxidant activity among fish oils underscores the importance of optimizing the extraction process, such as maceration, steaming, or Soxhlet, and selecting fish parts rich in bioactive compounds. This study highlights the promising potential of fish oils as nutraceutical products, and further research is needed to identify the fatty acids responsible for antioxidant activity to fully realize this potential.

3.4 Physicochemical properties of Indian mackerel fish oil

The extracted oil was a yellow liquid with a mild, distinctive fish odor. The oil had a slightly acidic pH of 4.84±0.11, which is considered the recommended range for oil acidification (4.0-6.9) (Shahidi & Ambigaipalan, 2018; Ahmed et al., 2020). The oil yield through extraction was 21.33±0.17% from a 150-gram portion of skin (Table 3). This yield was higher than some studies with the same method, such as the extraction of *Priacanthus tayenus* skin oil, which yielded 1.23% (Huli et al., 2014), the yield of *Pangasius hypophthalmus* oil (14.37%) (Nurjanah et al., 2014), and mackerel scad (*Decapterus macarellus*) oil (0.9%) (Suseno et al., 2020). Further, comparing the oil yield from waste products of Aji-aji fish (*Seriola nigrofasciata)* extracted using the Soxhlet method, a yield of 18.3% was obtained. In contrast, the oil yield from Indian mackerel remained higher, indicating that Indian mackerel was a more efficient source for oil extraction (Shamsudin & Salimon, 2006). The oil yield of Indian mackerel was compared to the SFE method found in previous research. In 2010, Sahena et al. found that the cosolvent technique with SFE at 35 MPa pressure and 75°C temperature gave the highest oil yield of 53.2%. SFE is a more sophisticated technique than the one we applied in our study, making it more efficient in extracting the oil.

In addition, the amount of raw material used in our study was 150 g, so the ratio of ingredients could affect the extraction efficiency. The quality of the fish skin used and extraction conditions, such as temperature and pressure, could also play an important role in influencing the results. The variations in yield might be due to the different fatty acid composition of the species, rendering temperature and time. Higher temperatures can increase oil yield by breaking down cell walls more efficiently and thus facilitating oil extraction. Overall, this analysis reflects that choosing the right extraction technique and setting the process conditions can greatly influence the oil yield obtained from fish skin.

Table 3. Physicochemical properties of Indian mackerel fish oil compared to oil from sardine and tuna

Parameter	Indian Mackerel (mean±SD)	Sardine[a,b] (mean±SD)	Tuna[c,d] (mean±SD)
Total yield (%)	6.40±0.22	9.2	26.1
pH	4.84±0.11	-	-
Density (g/mL)	1.06±0.06	0.79 0.004	0.90
FFA (%)	1.4±0.02	5.19±0.05	0.99±0.01
Saponification (mg KOH/g)	227.46±0.71	174±0.2	175±0.6
Peroxide (meq/kg)	8.46±0.47	6.27±0.27	14.54±0.02
p-anisidine	10.27±0.41	16.34±1.39	46.81±0.24
TOTOX	27.19±0.31	28.88±0.85	75.89±0.28
Pb (mg/kg)	<0.01	Not detected	0.08
Cd (mg/kg)	<0.01	0.01	0.02
Hg (mg/kg)	<0.025	0.03	Not detected

([a]: Dari et al., 2017; [b]: Haryati, 2024; [c]: Apituley et al., 2020; [d]: Trilaksani et al., 2023)

The density value is an important parameter when assessing the quality of fish oil, as it indicates the purity and composition of the oil. A low value of density indicates that the fish oil is not mixed with impurities and thus reflects better quality. In this study, the density of Indian mackerel oil was measured at 1.06±0.06 g/mL, which was higher than that of sardine (*Sardinella* sp.) oil, which had a density of 0.63 g/mL and tuna bone oil, which stood at 0.90 g/mL (Dari et al., 2017; Apituley et al., 2020). The density value according to the standard for fish oils (CXS 329-2017) is ≤0.86 g/mL, which regulates animal and vegetable fats and oils (Codex Alimentarius Commission, 2017). However, in some studies, the density value was influenced by various factors, including the refining process applied, the type of adsorbent used during extraction, and the type of fish (Ayeloja et al., 2024).

Changes in density during storage may indicate the presence of oxidation or degradation processes in the oil, which directly impact the quality and shelf life of the product. Fish oils rich in essential fatty acids, especially omega-3 fatty acids such as EPA and DHA, tend to have lower density values than oils that contain more saturated fatty acids. This is due to the chemical structure of unsaturated fatty acids with double bonds, making their molecules more flexible and less dense than saturated fatty acids with rigid and denser carbon chains (Kazuo, 2019). As a result, fish oils rich in unsaturated fatty acids usually exhibit lower densities than those dominated by saturated fatty acids (Arab-Tehrany et al., 2012). Further research is needed to explore the relationship between fatty acid composition, extraction method, and other physical characteristics of Indian mackerel oil to improve its quality.

The analysis of free fatty acid is a validation test to show the level of oil deterioration due to triglyceride hydrolysis reactions that cause fatty acid and glycerol bond breaks. In addition, free fatty acid values also result from the breaking and oxidation of acidic double bonds (Uçar et al., 2024). Higher free fatty acid values are related to the quality of fish oil (Vicentini-Polette et al., 2021). The results showed that the free fatty acid content in Indian mackerel oil reached 1.4±0.02%. This result met the requirements according to the standard for fish oils (CXS 329-2017), the maximum allowable limit for free fatty acids (FFA) in fish oil is ≤1.50% (Codex Alimentarius Commission, 2017). The free fatty acid content in this study was higher than that of *Pangasius hypophthalmus,* which reached 0.85±0.03%; and *Thunnus* sp. which reached 0.99±0.03% (Sembiring et al., 2018; Suseno et al., 2021). However, the results of this study were lower than those of sardine oil, which was 5.19±0.05% (Dari et al., 2018). Fish oil impurities, such as protein, water, carbohydrates, and color associated with more FFA, contribute to the high acid number. Since the adsorbent used was capable of adsorbing FFA, a low acid number was achieved (Wang et al., 2023).

The saponification value is an important parameter that reflects the oil's average molecular mass of fatty acids. It indicates the number of milligrams of potassium hydroxide (KOH) required to saponify 1 gram of oil, so the higher the saponification value, the lower the average molecular mass of the constituent fatty acids (Bako et al., 2017). In this study, Indian mackerel oil had a saponification value of 227.46 mg KOH/g, which was higher than *Sardinella* sp. (174±0.2 mg KOH/g), *Thunnus* sp. (175±0.62 mg KOH/g), but lower than eel oil (252.01 mg KOH/g) (Dari et al., 2017; Apituley et al., 2020; Hidayah et al., 2022). However, the results did not reflect compromise fish quality as the standard for fish oils (CXS 329-2017) does not explicitly regulate the saponification value but focuses on parameters such as FFA (≤1.5%) (Codex Alimentarius Commission, 2017). The higher the FFA, the more alkali (KOH) is required to neutralize the fatty acids during the saponification reaction (Ayeloja et al., 2024).

Indian mackerel oil was known to contain higher levels of FFA than *Thunnus* sp., thus increasing its saponification value. This was in line with previous studies showing that

refining fish oil (such as neutralization and degumming) can reduce FFA and lower the saponification value (Suseno et al., 2014). The high saponification value of Indian mackerel (227.46 mg KOH/g) indicates the potential of this oil for use in the soap or cosmetics industry, where efficient saponification reactions are required. However, it also underscores the critical need for strict control of FFA levels to prevent rancidity during storage. This is a key aspect of quality control in the production and use of Indian mackerel oil. The variation in saponification values between fish species is influenced by fatty acid composition, extraction method, and refining level. This study confirmed that Indian mackerel oil has unique characteristics, making it a potential feedstock for industrial applications. However, optimization of the refining process is required to reduce FFA and improve oxidative stability as per standard for fish oils (CXS 329-2017) (Codex Alimentarius Commission, 2017).

Peroxide number (PV) is the amount of peroxide in milli-equivalents of oxygen contained in 1,000 g of compound (Suseno et al., 2014). Peroxides are compounds that arise from the oxidation of oil by free air. Oxidation occurs when oxygen binds to unsaturated fatty acids during processing and storage (Phung et al., 2020). The iodometry method uses the peroxide value to determine the degree of deterioration in the oil. The peroxide value of Indian mackerel oil was 8.46±0.47 meqO_2/kg, showing a moderate degree of primary oxidation. The higher the peroxide number, the higher the level of oil rancidity is. This is inversely proportional to the status of lipid stability in oil (Yulianto et al., 2022). According to Bimbo (1998), fish oil is considered to have a good PV quality if it fulfils the following range of 3-20 meq/kg but has not reached the standard for fish oils (CXS 329-2017) of ≤5 meq/kg (Codex Alimentarius Commission, 2017). The results of this study were higher than sardine oil (6.27±0.27 meq/kg) and lower than *Thunnus* sp. (14.54 ±0.02 meq/kg) (Dari et al., 2017; Apituley et al.,2020). One-time refined fish oil is thought to have increased PV compared to crude fish oil. There are influencing factors such as heat, light, fatty acid composition, oxygen, unwanted minor compounds such as free fatty acids, metals, pigments, phospholipids, monoglycerols and diacylglycerols, and thermally oxidized compounds, and antioxidants (Choe & Min, 2006; Padial-Domínguez et al., 2023). Enzymatically and non-enzymatically formed lipid peroxides can also be a factor in increasing the peroxide value of oil (Oenel et al., 2017). Based on various factors, PV, and established standards, Indian mackerel oil is still considered safe for consumption.

The quality of fish oil during storage is associated with the anisidine value (pAV) (Padial-Domínguez et al., 2023). pAV is a measurement of secondary fat oxidation products, determining the number of aldehydes. Table 3 shows the pAV of Indian mackerel oil with one-time refining using 3% bentonite, which was 10.27±0.41. The pAV results obtained were lower than those of *Sardinella* sp. and *Thunnus* sp. (Dari et al., 2017; Apituley et al., 2020). The maximum value of pAV for the standard for fish oils (CXS 329-2017) is ≤ 20 (Codex Alimentarius Commission, 2017). Our results were consistent with those of Purnamayati et al. (2023), who reported that hydrolysis reactions can occur in oil because of impurities in components. Oxygen can increase pAV so that the degradation of peroxides into aldehydes occurs, which causes the pAV value to increase, thus the oil quality decreases (Domínguez et al., 2019). In addition, other factors affect the pAV value, such as the content of unsaturated fatty acids in high concentrations, which increases pAV and cause oxidative damage to occur (Damerau et al., 2020). Although the fatty acid profile of Indian mackerel oil is not yet known, the presence of unsaturated fatty acid content may react more easily with oxygen, light, and heat during refining and storage, forming aldehyde and ketone compounds (Domínguez et al., 2019).

TOTOX is another parameter used to analyze the primary and secondary oxidation of fish oil. The TOTOX value is generated from the sum of pAV and PV values. If the

primary (FFA and PV) and secondary pAV oxidation parameters are higher, it is directly proportional to the TOTOX value (Lamas et al., 2022). The TOTOX value of Indian mackerel was 27.19±0.31, which is in a suitable category compared to the other two fish species, as shown in Table 3 (Dari et al., 2017; Apituley et al., 2020). The standard value for total oxidation (TOTOX) according to standard for fish oils (CXS 329-2017) is ≤26 (Codex Alimentarius Submission, 2017). If more refining is done, the fish oil will often be exposed to oxygen and temperature variations, increasing pAV and TOTOX parameters. Thus, to produce parameters that meet fish oil standards, refining should be done a maximum of 3 times without the alkali neutralization stage as done in this study (Damerau et al., 2020).

Heavy metal analysis was done to ensure that the Indian mackerel fish oil was safe for consumption. The lead (Pb) content in the oil was <0.01 mg/kg, significantly lower than in tuna fish oil (≤0.5 mg/kg) and well below the maximum limit of ≤0.04 mg/kg according to Codex Alimentarius (Codex Alimentarius Commission, 2017; Yulianto et al., 2022). The cadmium (Cd) content was also <0.01 mg/kg, similar to other studies, such as 0.03 mg/kgin sardine oil and well below the 0.1 mg/kg maximum limit for marine products according to Codex Alimentarius (Huli et al., 2014; Codex Alimentarius Commission, 2017; Huli et al., 2022). Meanwhile, mercury (Hg) levels in Indian mackerel fish oil were <0.025 mg/kg, lower than the range reported for cod liver oil (0.030-0.207 mg/kg) and within the safety standard of ≤0.5 mg/kg set by Codex Alimentarius (CXS 329-2017). Environmental conditions often influence mercury levels in fish oils (Huli et al., 2022; Brodziak-Dopierała et al., 2023). All heavy metal levels in this study complied with the safety standards of the Codex Alimentarius Commission, confirming that Indian mackerel fish oil is safe and high-quality for consumption.

In summary, the oil extracted from Indian mackerel skin exhibited favorable physicochemical characteristics, including acceptable density, FFA, PV, pAV, and TOTOX values. Furthermore, the oil meets safety standards for heavy metal content, so it can be safely consumed. However, additional fatty acid composition in Indian mackerel fish oil will be needed for a more complete analysis.

4. Conclusions

The wet rendering method, an effective and environmentally friendly technique, yielded an appreciable oil extraction rate from the skin of Indian mackerel. This method produced a yield of 21.33±0.17%, which was a significant output achieved without the need for hazardous chemical solvents. The fish oil results, including proximate value, FTIR analysis, antioxidant activity, and characteristic properties, met the expected standards for fish oil. Notably, the oil had a strong antioxidant activity of 36.52±1.27 ppm for scavenging free radicals, indicating its potential applications in functional food oils. Future investigations should focus on refining processes to reduce the peroxide value, improving the oxidative stability. Moreover, further analysis of the skin oil fatty acid content, and feasibility assessment of the oil to be utilized as raw materials in the fish oil industry should be explored.

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6. Authors' Contribution

EH and PA contributed to the conception of the work. PA, EH, and UHP were involved in data acquisition, analysis, and interpretation. The initial draft of the manuscript was prepared by PA and EH. All authors (EH, PA, UHP, and DP) reviewed and provided critical revisions to the manuscript.

7. Conflicts of Interest

The authors declare no conflict of interest.

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