

Comparative Analysis of Extraction Methods for Optimizing Yield and Quality of Tiger Stingray (*Himantura fava*) Liver Oil

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Abstract

This study evaluates the yield, quality, and fatty acid profile of tiger stingray (*Himantura fava*) liver oil extracted using three methods: dry rendering, Soxhlet extraction, and maceration. The choice of extraction method plays a crucial role in determining the quality and composition of the resulting oil. This study assessed quality parameters including free fatty acid content, peroxide value, water content, density, and viscosity, as well as the fatty acid profile characterized by Gas Chromatography-Mass Spectrometry (GC-MS). The results showed that the dry rendering method yielded 32.821±0.050%, which was significantly higher than the yields obtained by Soxhlet extraction (23.246±0.171%) and maceration (23.312±0.056%). Oil extracted by the dry rendering method showed superior quality that met the Indonesian National Standard, as indicated by lower free fatty acid content (0.652±0.031%), peroxide value (2.853±0.022 mEq/kg), and moisture content (0.952±0.018%) than the other methods. GC-MS analysis showed that tiger stingray liver oil contained 61.35% unsaturated fatty acids, including polyunsaturated fatty acids (15.28%), monounsaturated fatty acids (17.20%), omega-3 fatty acids (14.96%), and omega-6 fatty acids (13.91%). Overall, among the extraction methods evaluated, the dry rendering method yielded liver oil with higher yield, better quality, and a more favorable essential fatty acid content under the experimental conditions of this study.

Keywords: tiger stingray liver oil, dry rendering, Soxhlet extraction, maceration, fatty acids

INTRODUCTION

Fish liver oil is a natural source rich in essential fatty acids, particularly omega-3 and omega-6, which play crucial roles in maintaining human health. Omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are known for their benefits in enhancing cognitive function, reducing inflammation, and supporting cardiovascular health (Banaszak *et al.*, 2024; Khan *et al.*, 2021; Welty, 2023). Meanwhile, omega-6 fatty acids, such as linoleic acid, contribute to immune system function and skin health maintenance (Mercola & D'Adamo, 2023; Wang *et al.*, 2025). The presence of these two essential fatty acids underscores that the marine environment offers not only vast biodiversity but also valuable resources that can be harnessed to improve human well-being. In this context, utilizing stingray liver as a potential source of omega-3- and omega-6-rich oil represents a promising strategy to optimize the potential of Indonesia's abundant marine resources.

As the world's largest archipelagic country, Indonesia holds vast potential in marine natural resources, encompassing both biological and non-biological diversity. Among these unique resources are cartilaginous fishes such as sharks and stingrays (Prasetyo *et al.*, 2021). One species of cartilaginous fish frequently caught in Indonesian waters is *Himantura fava*, widely distributed across the Indo-West Pacific region. However, local fisheries primarily utilize only the meat and skin, while internal organs such as the liver are typically discarded as waste. In fact, stingray liver is a valuable and underutilized source of essential fatty acids, particularly omega-3 and omega-6, which are known for their health-promoting properties.

Previous studies have demonstrated considerable variations in the fatty acid composition and physicochemical properties of fish liver oils, reflecting the influence of species, habitat, and extraction methods. Navarro-García *et al.* (2014) investigated the lipid content and fatty acid profiles of liver oils from several stingray species, including *Urotrygon chilensis*, *Urobatis halleri*, *Rhinobatos glaucostigma*, *Rhinoptera steindachneri*, and *Dasyatis dipterura*, collected from the state of Sinaloa, Mexico. Their results revealed relatively high total lipid contents ranging from 30.67% to 46.41%, with *Dasyatis dipterura* exhibiting the highest lipid level. Moreover, these species showed substantial amounts of omega-3 long-chain polyunsaturated fatty acids (LC-PUFAs), particularly EPA and DHA, with concentrations ranging from 4.79 to 14.47 g per 100 g of liver oil. In a separate study, Mititelu *et al.* (2023) reported that the fatty acid profile of stingray (*Dasyatis pastinaca*) liver oil was distinguished by an omega-3 to omega-6 ratio of 2.83, a polyunsaturated to monounsaturated fatty acid ratio of 1.33, an iodine value of 111.85, and a total unsaturated fatty acid content of 68.98%.

The efficiency and quality of extracted fish liver oil are strongly influenced by several critical factors, including the choice of solvent, extraction method, time, and temperature (Dave *et al.*, 2023; Kuepethkaew *et al.*, 2025). Selecting an appropriate solvent is essential as it affects both extraction efficiency and the stability of bioactive compounds in the oil. Optimizing extraction time is equally important to ensure maximum oil yield without compromising its quality; insufficient time may result in low oil recovery, whereas prolonged extraction increases the risk of degradation of sensitive components. Additionally, while higher temperatures can enhance the solubility of target compounds, excessively high temperatures may cause thermal degradation of the oil.

In this study, tiger stingray (*Himantura fava*) liver oil was extracted using three different methods: maceration, Soxhlet extraction, and dry rendering. Maceration is a simple extraction method that involves soaking the sample prepared raw material in a coarse or powder form in a solvent of interest at room conditions for at least three days with intermittent agitation Bitwell *et al.* (2023). Dry rendering utilizes heat without solvents, applying pressure and temperature to evaporate moisture and isolate the oil, with the advantage of better preserving unsaturated fatty acids (Kamini *et al.*, 2016). Meanwhile, Soxhlet extraction is a continuous method where oil is separated in a closed circulation system using a solvent (Bitwell *et al.*, 2023). Oils obtained from the three extraction methods were subsequently evaluated their physicochemical properties in accordance with the Indonesian National Standard (SNI) to determine the most effective and efficient extraction technique in terms of yield and oil quality.

MATERIALS AND METHODS

The equipment employed in this study comprised beakers, glass bottles, burettes, desiccators, distillation apparatus, Erlenmeyer flasks, freezers, a GC-MS instrument (Gas Chromatography-Mass Spectrometry, Thermo Scientific Trace 1310), measuring cylinders, a hot plate stirrer (IKA C-MAG HS 7), analytical balances (Mettler Toledo), ovens, pycnometers (Iwaki), dropper pipettes, volumetric pipettes, Soxhlet extractors, spatulas, stopwatches, vacuum dryers, Ostwald viscometers (Iwaki), and water baths (Joanlab).

The raw material utilized was liver tissue from the tiger stingray (*Himantura fava*), previously identified by the Nusantara Fisheries Port, Pemangkat, Sambas Regency, West Kalimantan Province. The chemicals employed included ethanol (C_2H_6O , absolute, Supelco), starch ($C_6H_{10}O_5$), distilled water (H_2O), glacial acetic acid (CH_3COOH , 100%, Merck), phenolphthalein indicator ($C_{20}H_{14}O_4$), hydrochloric acid (0.5 N HCl), potassium hydroxide (0.5 N KOH), fat-free cotton, chloroform ($CHCl_3$, Supelco), saturated potassium iodide solution (KI), sodium thiosulfate (0.05 N $Na_2S_2O_3$), sodium chloride (NaCl), sodium hydroxide (0.1 N NaOH), *n*-hexane, and sodium sulfate (Na_2SO_4 , $\geq 99.0\%$, anhydrous, Sigma Aldrich).

The tiger stingray (*Himantura fava*) liver samples were thoroughly washed with clean water to remove any impurities. After cleaning, the livers were cut into small pieces approximately 1 cm in size. The liver pieces were then placed in a container lined with aluminium foil and stored in a refrigerator

at 4°C to maintain freshness. Throughout the storage process, the liver temperature was kept consistently cold to prevent deterioration.

For the dry rendering method, 150 g of tiger stingray liver was weighed for each extraction batch, and the process was repeated three times. The liver pieces were evenly spread on a baking tray lined with aluminium foil. The samples were then subjected to vacuum drying at a temperature of 50°C and a pressure of 50 kPa for 1 hour. After drying, the apparatus was turned off, and the tray containing the liver samples was carefully removed. The partially dried liver was transferred into a beaker and reheated at the same temperature for an additional hour. This heating process was repeated six times, resulting in a total extraction duration of 6 hours to ensure maximum oil recovery. The oil released during each heating cycle was filtered using filter paper and collected into separate glass bottles lined with aluminium foil to prevent light-induced degradation. Upon completion of the extraction process, the collected tiger stingray liver oil samples were stored in a refrigerator at 4°C until further analysis to assess their physicochemical quality parameters.

A total of 150 grams of tiger stingray (*Himantura fava*) liver sample was placed into a filter paper thimble and covered with fat-free cotton. The thimble was then inserted into a Soxhlet extractor for the extraction process. Subsequently, 500 mL of *n*-hexane was added into the extraction chamber, and the Soxhlet extraction was carried out for 4 hours. A clean and dried Soxhlet flask was used to collect the extracted oil during this process. The entire extraction procedure was performed in triplicate. After extraction, the collected oil was subjected to distillation at 60-70°C for 1 hour to remove the solvent. The purified tiger stingray liver oil was then stored in a freezer at 4°C until further quality analysis.

A total of 150 g of tiger stingray liver was weighed for each batch and placed into three separate Erlenmeyer flasks, with the process performed in triplicate. Extraction was conducted using the maceration method with 500 mL of *n*-hexane as the solvent for a duration of 3 days. Every 24 hours, the solution was filtered, the solvent was replaced with fresh *n*-hexane, and the mixture was stirred to enhance extraction efficiency. After 3 days, the combined extracts were filtered using filter paper, and the solvent was removed through distillation. The resulting tiger stingray liver oil was then cooled and stored in a freezer at 4°C to preserve its quality before subsequent analysis. This method was applied to ensure optimal recovery and maintain the physicochemical properties of the extracted liver oil.

All extraction experiments were performed in triplicate and the results are presented as mean $\pm$ standard deviation (SD). Statistical analysis was carried out using IBM SPSS Statistics version XX. Data normality and homogeneity of variance were evaluated prior to analysis. Differences among extraction methods were analysed using one-way analysis of variance (ANOVA), followed by the Least Significant Difference (LSD) post hoc test to identify significant differences between treatment means. Differences were considered statistically significant at $p < 0.05$.

A total of 1 gram of tiger stingray (*Himantura fava*) liver oil was accurately weighed and transferred into an Erlenmeyer flask. Then, 50 mL of preheated 95% neutral ethanol and 2 drops of phenolphthalein indicator were added to the flask. The mixture was titrated with 0.1 N NaOH standard solution until a persistent pink colour appeared and remained for at least 30 seconds. This procedure was performed in triplicate to ensure accuracy. The free fatty acid (FFA) content was calculated using the following formula:

$$\%FFA = \frac{Mr \times V \times N}{M \times 1000} \times 100\%$$

where *Mr* is molecular weight of dominant fatty acids, *V* is volume of NaOH (mL), *N* is normality of NaOH (N), and *M* is mass of sample (g).

A total of 1 gram of tiger stingray (*Himantura fava*) liver oil was accurately weighed and transferred into an Erlenmeyer flask. Then, 15 mL of a glacial acetic acid:chloroform mixture (3:2 v/v)

was added to the flask, followed by the addition of 0.5 mL of saturated potassium iodide (KI) solution. The flask was tightly closed and shaken gently. Afterward, 15 mL of distilled water and 0.5 mL of 1% starch indicator solution were added. A stable blue colour appeared and persisted for at least 30 seconds after shaking. The mixture was titrated with 0.05 N sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) solution until the blue colour disappeared, indicating the endpoint (returning to the original oil colour), and the colour remained stable for 30 seconds. This determination was repeated three times for accuracy. The same procedure was performed for the blank solution as a control. The peroxide value was calculated using the following formula:

$$\text{Peroxide value} = \frac{N \times V \times 8}{M \times 1000}$$

where N is normality of $\text{Na}_2\text{S}_2\text{O}_3$ (N), V is volume of $\text{Na}_2\text{S}_2\text{O}_3$ (mL), and M is mass of oil (g).

A total of 1 gram of tiger stingray (*Himantura fava*) liver oil was accurately weighed into a small beaker glass. The sample was then heated in an oven at 105°C for 1 hour. After heating, the sample was placed in a desiccator for 15 minutes to cool and prevent moisture absorption from the environment. The sample was then reweighed. This heating, cooling, and weighing process was repeated until a constant weight was obtained, indicating that all moisture had been removed. The determination was carried out in triplicate to ensure accuracy. The water content was calculated using the following formula:

$$\text{Water content (\%)} = \frac{M_1 - M_2}{M} \times 100\%$$

where M1 is the mass of oil before heating (g), M2 is the mass of oil after heating (g), and M is the mass of oil sample (g).

Prior to use, the pycnometer must be thoroughly cleaned and completely dried. The volume of the pycnometer is recorded. The empty, dry pycnometer is then weighed, and its mass is noted. Next, the pycnometer is filled with the oil sample until full. The filled pycnometer is carefully closed, wiped clean with tissue to remove any excess oil, and weighed again. The mass of the pycnometer containing the sample is recorded, and the difference between the full and empty pycnometer is calculated to determine the mass of the oil sample. This procedure is repeated three times to ensure accuracy. The same procedure is also performed using distilled water as a reference. The density of the sample is calculated using the following formula:

$$\text{Density} = \frac{M_1 - M_2}{V}$$

where M1 is the mass of the oil-filled pycnometer (g), M2 is the mass of the empty pycnometer (g), and V is the volume of the pycnometer (mL).

The instrument used for the viscosity test was an Ostwald viscometer, which was cleaned and dried thoroughly before use. The oil sample was introduced into the Ostwald viscometer, and the liquid was drawn up using a pipette bulb until it reached the calibration mark. The time required for the oil to flow from the upper mark to the lower mark was measured and recorded using a stopwatch. This measurement was repeated three times to ensure accuracy. The same procedure was performed using distilled water as a reference liquid. The viscosity was calculated using the following formula:

$$\eta = \eta_0 \times \frac{t \times \rho}{t_0 \times \rho_0}$$

where η is the viscosity of the sample (cP), η_0 is the viscosity of the reference fluid (cP), t is the flow time of the sample fluid (seconds), t_0 is the flow time of the reference fluid (seconds), ρ is the density of the sample fluid (g/mL), and ρ_0 is the density of the reference fluid (g/mL).

The determination of fatty acid components in tiger stingray (*Himantura fava*) liver oil began by converting fatty acids and triglycerides present in the extract into their corresponding methyl

esters using sodium methoxide and boron trifluoride methanol complex ($\text{CH}_3\text{BF}_3\text{O}$) as catalysts. Upon completion of the reaction, the resulting fatty acid methyl esters (FAMES) were analysed using Gas Chromatography-Mass Spectrometry (GC-MS) equipped with an HP-5MS UI capillary column (30 meters in length, 0.25 mm internal diameter, and 0.25 μm film thickness). Helium gas was used as the carrier phase at a flow rate of 1.0 mL/min, and the column temperature was maintained between 325-350 °C to ensure optimal separation. The separation results were presented as chromatograms and mass spectra, which were used to identify and determine the fatty acid composition in the tiger stingray liver oil samples.

RESULT AND DISCUSSION

Yield is a crucial parameter in evaluating the efficiency of oil extraction methods. As shown in Tab. 1, the dry rendering method produced the highest yield, reaching $32.821 \pm 0.050\%$, indicating that this method was more effective in extracting oil compared to the other techniques. In contrast, the soxhlet extraction and maceration methods yielded nearly similar results, at $23.246 \pm 0.171\%$ and $23.312 \pm 0.056\%$, respectively. The results of the Least Significant Difference (LSD) test showed that the extraction method significantly affected the oil yield obtained. Dry rendering produced a significantly higher yield than the Soxhlet and maceration methods ($p < 0.001$). The average difference in yield between dry rendering and Soxhlet was 9.68%, while between dry rendering and maceration it was 9.61%. Conversely, there was no significant difference between the Soxhlet and maceration methods ($p = 0.484$), with an average difference of only 0.07%. This indicates that both methods have relatively equivalent extraction capabilities under the conditions of this study.

The lower yield obtained from the Soxhlet extraction and maceration methods suggests that these techniques were less efficient in recovering oil from tiger stingray liver. The suboptimal result of the soxhlet extraction method may be attributed to the use of high temperatures, which can lead to thermal degradation of the oil. Meanwhile, the maceration method involves a prolonged extraction time, potentially increasing the risk of oxidative degradation of the oil components, thereby affecting both yield and quality. These findings indicate that the dry rendering method is superior in maximizing oil recovery while maintaining its stability during the extraction process.

The dry rendering method offers a major advantage through the use of low temperature and pressure conditions (Vega-Gálvez *et al.*, 2022), resulting in oil with superior quality and better-preserved nutritional content. The process begins with a preheating stage, during which low temperatures are applied to reduce moisture content without damaging the lipid structure. In the subsequent stage of cell disruption and oil release, the combination of low temperature and pressure gently breaks down the liver tissue, allowing the oil to be extracted without causing fatty acid degradation. The low temperature plays a crucial role in maintaining oil stability, preventing oxidation, and reducing the risk of peroxide formation that can deteriorate oil quality. Meanwhile, the low-pressure environment ensures that the oil is released gradually, preserving the integrity of the lipid structure (Pasten *et al.*, 2024).

In terms of extraction stability, the dry rendering method showed the smallest standard deviation (± 0.050), indicating more consistent results compared to the other two methods. Soxhlet extraction exhibited the standard deviation (± 0.171), suggesting greater variability in the obtained yield, possibly due to factors such as differences in sample conditions or variations in solvent efficiency. Maceration had a standard deviation of ± 0.056 , which was more stable than Soxhlet extraction but still higher than dry rendering. Based on these results, the dry rendering method can be considered the most superior technique in terms of both extraction efficiency and consistency.

The quality of the extracted oil was assessed based on several physicochemical parameters, including free fatty acid content, peroxide value, water content, density, and viscosity. As presented in Tab. 2, notable differences were observed in the oil characteristics resulting from the three extraction methods: dry rendering, Soxhlet extraction, and maceration. These variations are largely influenced by the distinct extraction principles employed in each method, which affect the oil's composition and its overall physicochemical properties.

The analysis results showed that the maceration method produced the highest free fatty acid (FFA) content at $1.116 \pm 0.012\%$, indicating that the oil was more susceptible to degradation due to oxidation or hydrolysis during the extraction process. The Soxhlet extraction method resulted in an FFA value of $0.898 \pm 0.021\%$, which was still higher compared to the dry rendering method. Meanwhile, the dry rendering method yielded the lowest FFA content of $0.652 \pm 0.031\%$, demonstrating its superior effectiveness in maintaining oil stability. According to the Indonesian National Standard SNI 8467:2018 for refined fish oil which specifies a maximum FFA limit of $\leq 1\%$ only the maceration method failed to meet the quality standard, as its FFA level exceeded the allowable threshold. In contrast, both the Soxhlet extraction and dry rendering methods produced oils within the acceptable range, with dry rendering recognized as the most effective method for preserving oil stability and minimizing the risk of rancidity.

The high FFA value observed in the Soxhlet extraction method is likely due to the elevated temperature applied during extraction, which accelerates the breakdown of triglycerides into free fatty acids and promotes oil oxidation as a result of prolonged heat exposure. In contrast, the maceration method showed the highest FFA value because of the longer extraction time and the presence of water in the sample, which can trigger hydrolysis of lipids. Free fatty acids are formed when triglycerides react with water, especially in the presence of lipase enzymes or under elevated temperatures that accelerate this process, thus increasing the oil's acidity (Soenarno *et al.*, 2024; Menalla *et al.*, 2024). Additionally, high temperatures can enhance lipid oxidation, further contributing to the deterioration of oil quality (Wang *et al.*, 2023; Zhuang *et al.*, 2022). Unlike these two methods, the dry rendering method resulted in a lower FFA value because the heating process under atmospheric pressure facilitates the evaporation of water from the sample, thereby preventing hydrolysis reactions that could lead to increased FFA levels. Therefore, the dry rendering method is more effective in maintaining oil stability and minimizing the risk of degradation compared to the other extraction techniques (Kamini *et al.*, 2016).

Peroxide value is used to measure the degree of primary oxidation in oils, where a higher value indicates a greater potential for rancidity (Bustani & Soni, 2023). The analysis results showed that the maceration method produced the highest peroxide value at 8.585 ± 0.048 mEq/kg. In comparison, the Soxhlet extraction and dry rendering methods yielded significantly lower peroxide values of 2.789 ± 0.057 mEq/kg and 2.853 ± 0.022 mEq/kg, respectively. According to the Indonesian National Standard SNI 8467:2018 for refined fish oil, which sets a maximum peroxide value limit of ≤ 3 mEq/kg, only the oil extracted using the maceration method failed to meet the required standard, as its peroxide value exceeded the permissible limit. Conversely, the oils extracted by Soxhlet extraction and dry rendering remained within the safe and acceptable range. The high peroxide value obtained from the maceration method is likely due to the prolonged extraction time, which increases the oil's exposure to oxygen, thus promoting oxidative reactions. Additionally, the presence of moisture in the sample during maceration can trigger the hydrolysis of triglycerides into free fatty acids (Pambayun & Broto, 2023), making the oil more prone to degradation compared to the Soxhlet extraction and dry rendering methods.

Table 1 The yield of oil extracted using different methods varied depending on the technique applied

Extraction method	Yield of oil extracted (%)
Dry rendering	32.821 ± 0.050^a
Soxhlet extraction	23.246 ± 0.171^b
Maceration	23.312 ± 0.056^b

ANOVA (p-value) < 0.001

Table 2 The quality of oil extracted using different extraction methods

Parameter	Dry rendering	Soxhlet extraction	Maceration
Free fatty acid (%)	0.652 ± 0.031	0.898 ± 0.021	1.116 ± 0.012
Peroxide value (mEq/kg)	2.853 ± 0.022	2.789 ± 0.057	8.585 ± 0.048
Water content (%)	0.952 ± 0.018	1.106 ± 0.013	1.830 ± 0.050
Density (g/mL)	0.964 ± 0.013	0.956 ± 0.002	0.972 ± 0.007
Viscosity (cP)	33.068 ± 1.058	33.171 ± 0.813	32.681 ± 0.801

Moisture content in oil greatly affects its stability and shelf life, as high water levels can trigger hydrolysis reactions that degrade oil quality (Pambayun & Broto, 2023). The analysis results showed that the maceration method produced oil with the highest moisture content ($1.830 \pm 0.050\%$), followed by the Soxhlet extraction method ($1.106 \pm 0.013\%$). Meanwhile, oil extracted using the dry rendering method exhibited the lowest moisture content ($0.952 \pm 0.018\%$), indicating that this method was the most effective in reducing moisture levels compared to the other techniques.

The high moisture content in the oil obtained through maceration is likely due to the absence of a heating process, which prevents the evaporation of water from the sample. In contrast, the dry rendering method resulted in lower moisture content because the direct heating process under atmospheric pressure allowed for maximum water evaporation during extraction. This differs from the Soxhlet extraction method, which, although involving heating, also uses solvents that can retain small amounts of water in the oil.

According to the IFOMA (International Fish Oil Manufacturers Association) 1998 standard, which specifies an acceptable moisture content range of 0.5% to a maximum of 1% for fish oil, only the oil extracted by the dry rendering method met the quality standard. In contrast, the moisture content of oils obtained by maceration and soxhlet extraction exceeded the maximum allowable limit. Therefore, although the moisture content of the dry rendering method was close to the upper standard limit, this method remains the most effective in reducing moisture content compared to the other extraction techniques.

Density is a parameter that indicates the compactness of a liquid, including extracted oils (Sahasrabudhe *et al.*, 2017). According to Insani *et al.* (2017), the density of shark liver oil was recorded at 0.91 g/cm^3 , whereas tiger stingray liver oil exhibited a higher density, with values of $0.964 \pm 0.013 \text{ g/mL}$ for the dry rendering method, $0.972 \pm 0.007 \text{ g/mL}$ for the maceration method, and $0.956 \pm 0.002 \text{ g/mL}$ for the Soxhlet extraction method. Density is influenced by several factors, including the fatty acid composition, triglyceride profile, temperature, and the presence of minor constituents such as phospholipids and free fatty acids. The slight differences observed among extraction methods may therefore reflect variations in the chemical composition of the extracted oil. Therefore, the higher density observed in the dry-rendered oil may indicate differences in the composition of the extracted lipid fraction.

Viscosity is a physical property of oil that reflects its resistance to flow (Sahasrabudhe *et al.*, 2017). According to Insani *et al.* (2017), the viscosity of shark liver oil was reported to be 38.18 cPs, whereas tiger stingray liver oil exhibited a lower viscosity, measured at $33.068 \pm 1.058 \text{ cPs}$ using the dry rendering method, $33.171 \pm 0.813 \text{ cPs}$ using the Soxhlet extraction method, and $32.681 \pm 0.801 \text{ cPs}$ using the maceration method. These differences in viscosity are influenced by density, as oils with higher density tend to exhibit more stable viscosity. This is due to the denser molecular structure, which increases intermolecular friction and slows down the flow of the oil. Therefore, the dry rendering method can be considered the most effective extraction technique, as it produced oil with an optimal viscosity profile.

Overall, the dry rendering method demonstrated superior performance in maintaining oil stability, as evidenced by its lower moisture content, free fatty acid levels, and better-controlled

oxidation rate. In contrast, the maceration method produced oil with higher moisture content, peroxide value, and free fatty acid levels, indicating a greater potential for degradation. The Soxhlet extraction method showed intermediate results between these two methods but exhibited greater variability, particularly in overall stability. Therefore, the selection of an extraction method should carefully consider the balance between extraction efficiency and the quality of the resulting oil.

The GC-MS analysis identified 18 methyl ester fatty acid compounds with Similarity Index (SI) values ranging from 674 to 937. Most of the compounds exhibited SI values above 850, indicating a high level of identification accuracy. The compound with the highest SI was methyl Docosahexaenoate (DHA) at 937, followed by methyl arachidonate (AA) and methyl adrenate with SI values of 931 and 932, respectively. The compound with the lowest SI was methyl elaidate at 674, reflecting a lower level of matching accuracy compared to the other identified compounds.

Based on Tab. 3, the GC-MS identification results also revealed that the sample contained various types of unsaturated fatty acids, including polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs), omega-3, and omega-6 fatty acids. The identified fatty acids included linoleic acid, arachidonic acid (AA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA). In addition, monounsaturated fatty acids such as oleic acid, eicosenoic acid, and palmitoleic acid were also detected. The data indicate that polyunsaturated fatty acids (PUFAs) were present in relatively high proportions, while monounsaturated fatty acids (MUFAs) were also detected in significant amounts. The diversity of fatty acids identified in tiger stingray (*Himantura fava*) liver oil is summarized in Tab. 4.

Table 3. Fatty Acid Profile of Tiger Stingray (*Himantura fava*) Liver Oil

Identified Compounds Based on GC-MS Analysis	Chemical Formula	Retention time (minutes)	Relative Peak Area (%)	SI
Methyl Linoleate	C ₁₉ H ₃₄ O ₂	37.32	3.55	815
Methyl Docosadienoate	C ₂₃ H ₄₂ O ₂	40.58	3.11	871
Methyl Docosadienoate (duplicate, possibly isomer)	C ₂₃ H ₄₂ O ₂	40.64	1.98	887
Methyl Eicosapentaenoate (EPA)	C ₂₁ H ₃₂ O ₂	37.14	5.78	886
Methyl Docosapentaenoate (DPA)	C ₂₃ H ₃₆ O ₂	40.42	6.43	874
Methyl Docosahexaenoate (DHA)	C ₂₃ H ₃₄ O ₂	40.16	2.75	937
Methyl Eicosenoate	C ₂₁ H ₄₀ O ₂	37.69	6.01	906
Methyl Oleate	C ₁₉ H ₃₆ O ₂	34.21	3.51	919
Methyl Palmitoleate	C ₁₇ H ₃₂ O ₂	30.48	3.09	906
Methyl Erucate	C ₂₃ H ₄₄ O ₂	40.97	1.80	889
Methyl Elaidate	C ₁₉ H ₃₆ O ₂	34.26	1.44	674
Methyl cis-11-Eicosenoate	C ₂₁ H ₄₀ O ₂	37.83	1.35	905
Methyl Eicosapentaenoate (EPA) (duplicate)	C ₂₁ H ₃₂ O ₂	37.14	5.78	886
Methyl Docosapentaenoate (DPA) (duplicate)	C ₂₃ H ₃₆ O ₂	40.42	6.43	874
Methyl Docosahexaenoate (DHA) (duplicate)	C ₂₃ H ₃₄ O ₂	40.16	2.75	937
Methyl Arachidonate (AA)	C ₂₁ H ₃₄ O ₂	37.00	7.38	931
Methyl Adrenate	C ₂₄ H ₄₀ O ₂	40.29	5.41	932
Methyl Eicosadienoate	C ₂₁ H ₃₈ O ₂	37.62	1.12	929

Table 4. Classification of Identified Polyunsaturated Fatty Acids (PUFAs) in Tiger Stingray (*Himantura fava*) Liver Oil Based on Omega Group

Category	Compound	Omega group	Molecular weight (g/mol)
Polyunsaturated Fatty Acid (PUFA)	Linoleic Acid	Omega-6	280
	Eicosapentaenoic Acid (EPA)	Omega-3	302
	Docosapentaenoic Acid (DPA)	Omega-3	330
	Docosahexaenoic Acid (DHA)	Omega-3	328
	Arachidonic Acid (AA)	Omega-6	304
	Adrenic Acid	Omega-6	350
	Eicosadienoic Acid	Omega-6	308
Monounsaturated Fatty Acid (MUFA)	Eicosenoic Acid	Omega-9	310
	Oleic Acid	Omega-9	282
	Palmitoleic Acid	Omega-7	254
	Erucic Acid	Omega-9	338
	Elaidic Acid	Omega-9	282
	Gondoic Acid	Omega-9	310

In the liver oil of tiger stingray (*Himantura fava*), polyunsaturated fatty acids (PUFAs) were detected at a total of 15.28%, comprising methyl linoleate (3.55%), methyl docosadienoate (3.11% and 1.98%), methyl stearate (2.17%), methyl heptadecanoate (2.66%), and methyl palmitate (1.81%). In contrast, according to Widiyanto (2015), the liver oil of mondol stingray contained a higher PUFA content of 33.63%, including methyl linoleate (7.12%), methyl docosadienoate (5.74%), methyl stearate (4.01%), methyl heptadecanoate (8.22%), and methyl palmitate (8.54%). The differences in PUFA content are likely influenced by fish species, habitat, and dietary sources consumed during growth (Guil-Guerrero *et al.*, 2011; Wu *et al.*, 2024). PUFAs are known to contribute to brain health, improve immune function, and exhibit anti-inflammatory properties beneficial to the body (Mititelu *et al.*, 2023; Oppedisano *et al.*, 2020).

Monounsaturated fatty acids (MUFAs) in tiger stingray liver oil were present at 17.20%, consisting of methyl eicosenoate (6.01%), methyl oleate (3.51%), methyl palmitoleate (3.09%), methyl erucate (1.80%), methyl elaidate (1.44%), and methyl gondoate (1.35%). In comparison, Widiyanto (2015) reported a MUFA content of 24.84% in mondol stingray liver oil, with methyl eicosenoate (8.33%), methyl oleate (9.76%), methyl palmitoleate (4.21%), methyl erucate (1.87%), methyl elaidate (2.01%), and methyl gondoate (1.66%). These differences may be attributed to variations in lipid metabolism among fish species, as those inhabiting deeper waters generally possess higher levels of unsaturated fatty acids. MUFAs play an essential role in lipid balance and cardiovascular health support (Kharazmi-Khorassani *et al.*, 2021).

The total omega-3 content in tiger stingray liver oil was 14.96%, comprising methyl EPA (5.78%), methyl DPA (6.43%), and methyl DHA (2.75%). According to Widyanto *et al.* (2015), mondol stingray liver oil exhibited a higher omega-3 content of 23.44%, consisting of methyl EPA (8.94%), methyl DPA (9.32%), and methyl DHA (5.18%). These differences can be influenced by migratory patterns and biological activities of each fish species, as those living in colder, deeper waters and consuming plankton- or algae-rich diets may accumulate more omega-3 fatty acids. Omega-3 fatty acids are essential for brain function, inflammation reduction, and cardiovascular health (Dighriri *et al.*, 2022; Sherratt *et al.*, 2023).

The omega-6 content in tiger stingray liver oil was 13.91%, including methyl arachidonate (7.38%), methyl adrenate (5.41%), and methyl eicosadienoate (1.12%). In comparison, Widyanto *et al.* (2015) reported an omega-6 content of 10.19% in mondol stingray liver oil, composed of methyl

arachidonate (5.94%), methyl adrenate (3.67%), and methyl eicosadienoate (0.58%). Omega-6 fatty acids contribute to cell growth, immune function, and metabolic regulation (Djuricic & Calder, 2021); however, maintaining a balanced omega-3 to omega-6 ratio is crucial to avoid promoting inflammatory responses in the body (DiNicolantonio & O'Keefe, 2021).

According to Tab. 3, arachidonic acid (AA) was the predominant fatty acid, showing the largest peak area. Therefore, a fragmentation pattern analysis was performed to elucidate its molecular structure using mass spectrometry fragment ions. This fragmentation profile aids in confirming the presence of arachidonic acid and differentiating it from other compounds with similar molecular weights.

The fragmentation of methyl arachidonate in mass spectrometry begins with the molecular ion at m/z 318, which undergoes cleavage at the long carbon chain, producing a fragment ion at m/z 150 and releasing a fragment at m/z 168. This cleavage occurs near the middle of the carbon chain, forming more stable ions. The m/z 150 fragment further breaks down to m/z 120 by losing an ethane molecule (C_2H_6 , m/z 30). The m/z 120 ion then loses a methyl group ($-CH_3$, m/z 15), producing the m/z 105 ion. Subsequent cleavage of a methylene group ($-CH_2$, m/z 14) from m/z 105 yields the m/z 91 ion, which finally loses another methyl group to form the stable m/z 77 ion.

Fragmentation proceeds with the cleavage of a methylene group ($-CH_2$, m/z 14) from the ion at m/z 107, resulting in the formation of an ion at m/z 93. This is followed by the loss of a methyl group ($-CH_3$, m/z 13), producing a stable ion at m/z 80. This fragmentation pattern illustrates that methyl arachidonate undergoes stepwise decomposition through the sequential release of small groups such as ethane, methyl, and methylene. The presence of double bonds in the structure contributes to the stabilization of the resulting fragment ions. This analysis is valuable for elucidating the molecular structure of fatty acids present in tiger ray liver oil. The detailed fragmentation pathway of methyl arachidonate is presented in Fig. 1.

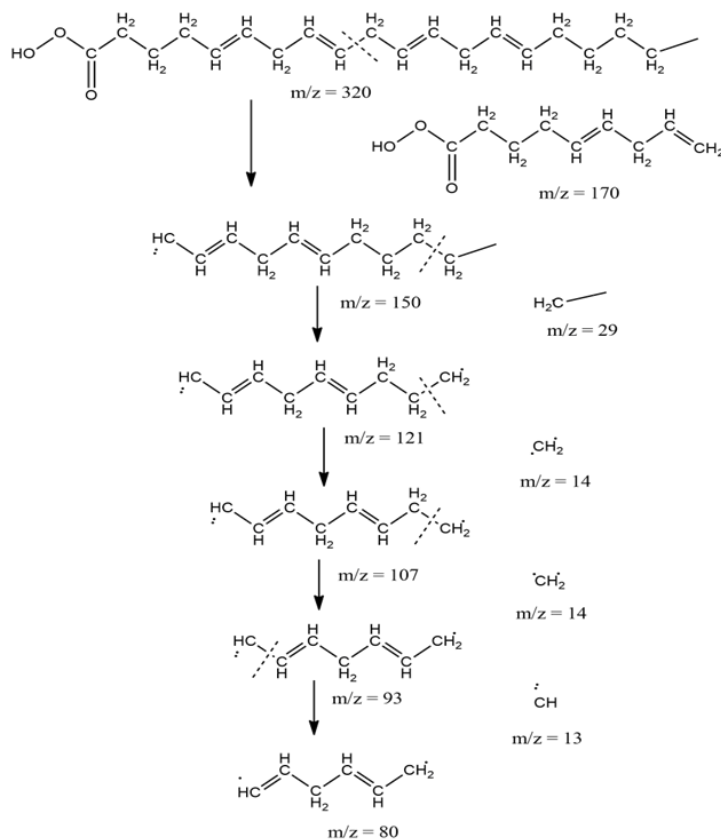


Figure 1. Arachidonic Acid Fragmentation Pattern

CONCLUSION

Based on the findings of this study, it can be concluded that the dry rendering method is the most efficient technique for extracting tiger stingray liver oil, providing the highest yield at $32.821 \pm 0.050\%$ and producing superior oil quality compared to the soxhlet extraction and maceration methods. The superior quality of the extracted oil was demonstrated by its low free fatty acid content, peroxide value, and moisture content, indicating excellent stability against oxidative and hydrolytic degradation. Moreover, the extracted stingray liver oil exhibits a high proportion of unsaturated fatty acids, accounting for 61.35% of the total relative area, comprising polyunsaturated fatty acids (PUFA) at 15.28%, monounsaturated fatty acids (MUFA) at 17.20%, as well as omega-3 (14.96%) and omega-6 (13.91%) fatty acids. This favourable fatty acid composition underscores the potential of tiger stingray liver oil as a promising natural source of essential nutrients beneficial to human health.

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