Characteristics Squalene of Smallfin Gulper Shark (Centrophorus moluccensis) Livers From Aru Islands, Mollucas, Indonesia

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Abstract. Smallfin gulper sharks (Centrophorus moluccensis) commercially exploited as food ingredient and contain high squalene in their livers. The purpose of this study was to determine the quality and composition of smallfin gulper shark liver oil compounds from the Aru Island waters, Mollucas, Indonesia. Smallfin gulper shark liver oil extraction uses a dry rendering method. The quality was analyzed based on the analysis procedure in SNI 01-2730-1992 regarding the quality standards of fish oil. Meanwhile, to find out the fatty acid content, an analysis was performed using GC-MS. The results showed that the quality of smallfin gulper shark liver oil generally met the quality standard with a yield of 83%, while the compounds composition was dominated by squalene compound of the remaining 95.10% in the form of palmitic acid, α-linolenic acid, EPA and DHA.

1. Introduction
Fish is a water organism that contains protein, fat, vitamins, minerals that are very good and prospective. Fats contained in fish are generally unsaturated fatty acids. The active component that is superior to fish oil is omega-3, omega-6, omega-9. One source of fatty acids in fish is derived from fish oil, which fish oil contains about 25% saturated fatty acids and 75% unsaturated fatty acids [1].

Fish is a water organism that contains protein, fat, vitamins, minerals that are very good and prospective. Fats contained in fish are generally unsaturated fatty acids. The active components that are superior to fish oil are omega-3, omega-6, omega-9 and squalen which are very beneficial for health [2]. Based on the source of raw materials, fish oil is divided into 2 (two), namely fish body oil such as lemuru fish oil, and fish liver oil such as cod liver oil and shark liver oil. Shark liver oil has a high oil content, between 79-88% with the main component of squalene. Squalene is widely used clinically in daily doses such as detoxification, antioxidants, bactericidal and fungicidal agents, antistatic agents and moisturizers in cosmetic and pharmaceutical preparations, and at low temperatures it can function as a lubricant [3]. In addition to squalene there are still other fatty acid compounds in shark liver oil which are beneficial to health including free fatty acids, EPA and DHA [2,4].

Shark liver oil has a good prospect as a squalane producer, the squalene content in shark liver oil reaches a yield of 79.8% at a 100% purity level [5]. Squalene can function as antioxidant in skin...
that experiences oxidative stress due to ultraviolet light. In addition, squalane can also be used as an antitumor material in mouse cells with inhibition values of 54.56% [6].

To get shark liver oil is done by extracting shark liver, the usual extraction method is to use dry rendering method. Dry rendering is the rendering process without the addition of water during the process. Dry rendering is done in the oven. Material that is estimated to contain oil or fat is put into the oven without the addition of water at a certain hot temperature. This heating causes the oil in the oil-containing material to come out of the pores of the material [7,8].

As an island territory dominated by sea waters, Aru Islands Regency, Mollucas has abundant marine resources. The fisheries sector is the backbone of the people's economy which is still very dependent on the supply of natural products. In fishing, non-economical fish are often included, one of the bycatch is the bottle shark. Utilization of sharks in the Aru Islands is limited to taking fins for sale, shark meat and liver are not consumed by the Aru Islands community because they smell of ammonia. Therefore, shark liver must be managed so that it can be utilized maximally, including extracting shark liver.

The purpose of this study was to determine the quality and composition of smallfin gulper shark liver oil compounds originating from the Aru Islands using the dry rendering extraction method.

2. Experimental

2.1. Materials and tools
The raw materials used in this research are the smallfin gulper shark liver (Centrophorus moluccensis) from Aru Island waters, chemicals in the form of anhydride Na2SO4, n-hexane, BF3-methanol, aquades. Equipment used include ovens, analytical scales, hot plates, vacuum pumps, GC-MS Shimadzu QP 2010S.

2.2. Extraction Process
Smallfin gulper shark liver oil extraction is done using the dry rendering method, the extraction process begins with separating liver and body, and then washing the liver. Liver is put into an oven at 60°C for 3 hours to denature protein. Oil which is free from fish liver is collected through a sieve into the dark container that has been provided [4].

2.3. Process of Making Test Samples
One gram of oil was added with 10 ml of 10% BF3 methanol then stirred and refluxed on a hot plate at 50°C for 1 hour. The reflux product is cooled and then put into a separating funnel and washed with 30 ml of distilled water. Add 20 ml of n-hexane let stand to form 2 layers, the lower layer contains glycerol while the upper layer contains methyl esters. The methyl ester layer was separated and then extracted with 10 ml of n-hexane 2 times. After that add 10 g of anhydrite Na2SO4, strain. The solvent was evaporated using a vacuum pump [9].

2.4. Sample Analysis
The quality of smallfin gulper shark liver oil was analyzed based on the analysis procedure in SNI 01-2730-1992 regarding the quality standards of fish oil [10]. Whereas to find out the fatty acid content, an analysis was carried out using GC-MS under methyl ester conditions with the following operational conditions:
Column type: Rtx - 5 MS, Column length: 30 cm, ID: 0.25 mm, Detector type: FTD, Column temperature: 150 °C, Injector temperature: 300 °C, Injector model: split, Pressure: 35.6 kPa, Total flow: 60 ml/minute, Oven temperature: 150-280 °C with a rate of 5 °C.
3. Results and Discussion

3.1. Yield

The yield of smallfin gulper shark liver oil is calculated by comparing the weight of smallfin gulper shark liver oil after extraction with the initial weight of smallfin gulper shark liver before being extracted. The yield produced from the extraction of 500 grams of smallfin gulper shark liver is around 83%. This result is very high when compared to the yield of silky shark liver oil using the dry rendering method, about 22.8% [11]. Cooking can cause protein to coagulate thereby facilitating the separation of solid and liquid (oil) fractions [12]. Higher extraction temperatures produce higher yields too, but extraction temperatures that are too high cause fatty acids to break the carbon chain [13].

3.2. Quality of smallfin gulper shark liver oil

The quality of fish oil is influenced by several factors. The quality of fish oil is highly dependent on the temperature at the time of extraction, the lower the temperature at the time of extraction, the better the quality of oil [14]. Fish oil damage is caused by light, heat, fat peroxide, heavy metals, hemoglobin, myoglobin, chlorophyll and the lipooksidase enzyme. The dark color of fish oil is caused by the oxidation process of vitamin E and also caused by heating temperatures that are too high so that the oil is oxidized [7]. The quality of smallfin gulper shark liver oil extracted using the dry rendering method can be seen in Table 1.

Table 1. Comparison of smallfin gulper shark liver oil quality and quality standards of fish oil

| Parameter                | Smallfin gulper shark liver oil | SNI 01-2730-1992 |
|--------------------------|---------------------------------|------------------|
| Color                    | Yellowish white                 | Colorless        |
| Odor                     | Smell fishy                     | Smells a little salty fish and not rancid |
| Boiling point (°C)       | 210                             | >230             |
| Specific weight (20°C)   | 0.9135                          | 0.85-0.92        |
| Refractive index (20°C)  | 1.49                            | 1.485-1.492      |
| Iod number (mg/100g)     | 136.34                          | <360             |
| Acid number (mg KOH/g)   | 1.25                            | 3                |
| Saponification number (mg KOH/g) | 184          | -                |

Table 1 can be seen that in general smallfin gulper shark liver oil has met the quality requirements except for the boiling point and color parameters. The boiling point on smallfin gulper shark liver oil is 210 °C while the quality requirements are based on SNI 01-2730-1992 which is >230 °C. High and low boiling point depends on the short length of the carbon chain in oil. Extraction using a temperature of 60 °C, high extraction temperatures cause the breaking of the carbon chain so that the boiling point on smallfin gulper shark liver oil is low. The results of iodine analysis showed a value of 136 mg/100g, this value still meets the specified quality standards of <360 mg/100g. The results showed that iodine number of catfish oil was 102.96 mg/100g using the dry rendering method [15]. Iod numbers showed how much unsaturated fatty acids contained in smallfin gulper shark liver oil. High iodine numbers indicate that the oil contains a lot of unsaturated fatty acids. Oils that contain lots of unsaturated fatty acids, will bind large amounts of iodine.

The acid number is 1.25 mg KOH/g, this value meets the established quality standards of a maximum of 3 mg KOH/g. Free fatty acids contained are shown in the form of acid numbers. Free fatty acids can be formed because of the presence of water which might speed up the hydrolysis process. In addition, free fatty acids can also be formed if the temperature is increased so that the oil will split into glycerol and free fatty acids. Large acid numbers indicate the formation of large amounts of free fatty acids. The greater the acid number, the lower the oil quality.
The saponification number 184 mg KOH/g, the value of this saponification number is almost the same as the lemuru fish, which is 187.4 mg KOH/g [16]. The higher the saponification of oil, the better the quality of the oil is because the shorter the carbon chain, the fatty acids will be easier to metabolize the body, conversely if the long carbon chains of fatty acids can only be digested by the lipase enzyme with the help of bile acids [16].

3.3. Compounds composition of smallfin gulper shark liver oil
Squalene is the main hydrocarbon found in smallfin gulper shark liver oil. Squalene mostly used in the pharmaceutical industry in the health sector and in cosmetics. Squalene compound is one of the constituents of non-soapy substances found in smallfin gulper shark liver oil. Identification of squalene compounds contained in fish oil was tested semi-quantitatively using Gas Chromatography-Mass Spectrometer (GC-MS). This analysis was conducted to determine the components of the compound contained in smallfin gulper shark liver oil (Fig. 1). GC-MS identification results indicate that there are 4 (four) peak chromatograms which are constituent compounds in smallfin gulper shark liver oil.

![Figure 1. Chromatogram of smallfin gulper shark liver oil transesterification](image)

| Peak No. | Ret. Time | Percent Relative | Formula | Molecular Weight | Compound                      |
|---------|-----------|------------------|---------|------------------|-------------------------------|
| 1       | 32.594    | 95.10            | C₃₀H₅₀  | 410              | Squalene                     |
| 2       | 35.092    | 0.93             | C₁₅H₂₆O | 222              | Fernasol                     |
| 3       | 35.349    | 1.06             | C₁₅H₂₆O | 222              | Fernasol                     |
| 4       | 35.474    | 2.91             | C₂₀H₃₄O | 290              | Geranyl linalool             |
|         |           |                  | C₃₀H₅₁BRO| 506              | 6,10,14,18-tetracosapentaen-2-ol |
|         |           |                  | C₂₇H₄₄O | 384              | 2,6,10,14,18-Eicasapentaena   |
|         |           |                  | C₁₅H₂₆O | 222              | Fernasol                     |

From the results of the chromatogram, a bibliographic approach to each chromatogram peak was obtained for 7 (seven) types of compounds that can be identified in smallfin gulper shark liver oil (Table 2). The peak of chromatogram number 1 with a retention time of 32.594 minutes is a squalene compound (C₃₀H₅₀) with an area percentage of 95.10% which is the main constituent of smallfin gulper shark liver oil with molecular weight 410 and mass spectrum M/Z = 41 (base peak),
55, 69, 81, 95, 109, 121, 136, 149, 161, 175, 191, 203, 217, 243, 257, 273, 285, 299, 329, 341, 367, 395 and 410 (Fig. 2).

**Figure 2.** Mass spectrum of squalen compound (C\textsubscript{30}H\textsubscript{50}) smallfin gulper shark liver oil

At the peak of chromatogram number 2 with a retention time of 35.092 minutes and an area percentage of 0.93% were fernasol compounds (C\textsubscript{15}H\textsubscript{26}O), with molecular weights 222 and mass spectrum M/Z = 41 (base peak), 55, 69, 81, 93, 109, 123, 136, 161, 179, 191 and 222 (Fig. 3).

**Figure 3.** Mass spectrum of fernasol compound (C\textsubscript{15}H\textsubscript{26}O) smallfin gulper shark liver oil

While at the peak of chromatogram number 3 with a retention time of 35.349 minutes and an area percentage of 1.06% there were Fernasol compounds (C\textsubscript{17}H\textsubscript{28}O) and compounds 4,8,12-Tetradecatrienal (C\textsubscript{17}H\textsubscript{30}O). The molecular weight of the C\textsubscript{17}H\textsubscript{28}O compound is 248 and its mass spectrum M/Z = 41 (base peak), 55, 69, 81, 93, 107, 123 and 136 (Fig. 4).

**Figure 4.** Mass spectrum of C\textsubscript{17}H\textsubscript{28}O compound of smallfin gulper shark liver oil

At the peak of chromatogram number 4 with a retention time of 35.474 minutes and an area percentage of 2.91% there were several compounds including: C\textsubscript{20}H\textsubscript{34}O (geranyl linalool) with a molecular weight of 290 and mass spectrum M/Z = 41 (base peak), 55, 69, 81, 93, 109, 121, 137, 163 and 177 (Fig. 5); C\textsubscript{30}H\textsubscript{51}BRO (6,10,14,18,22-Tetracosapentae-2-ol) with a molecular weight of 506 and mass spectrum M/Z = 41 (base peak), 43, 69, 81, 93, 107, 121, 135, 147, 161, 189 and 203 (Fig. 6); C\textsubscript{25}H\textsubscript{42} (2,6,10,14,18-Eicasapentaena) with a molecular weight of 342 and spectrum of mass M/Z = 41 (base peak), 69, 81, 93, 109, 121, 135, 149, 161, 175, 191, 203 and 217 (Fig. 7); C\textsubscript{27}H\textsubscript{44}O (Docosa-2, 6, 10, 14, 18-pentaen-22-al) with a molecular weight of 384 and mass spectrum M/Z = 41 (base peak), 55, 69, 81, 95, 109, 121, 137, 149, 163, 189, 205 and 273 (Fig. 8); and fernasol (C\textsubscript{15}H\textsubscript{26}O) (Fig. 2).
The results of identification of smallfin gulper shark liver oil showed that the squalene compound (C$_{30}$H$_{50}$) was the largest component, amounting to 95.10% while the rest (<5%) was unsaturated fatty acids that had undergone decomposition or breaking the atomic chain carbon such as palmitic acid (C$_{16}$H$_{32}$O) which is a decomposition of fernasol (C$_{15}$H$_{26}$O) with its isomer 2,6,10-dodecatrien-1-ol and 3,7,11-trimethyl. Then there are also compounds 4,8,12-tetracatrienal (C$_{17}$H$_{28}$O) which decomposes from α-linolenic acid (C$_{18}$: 3). Likewise with the compound C$_{27}$H$_{44}$O (Decosa-2,6,10,14,18-pentaen-22-al which has been decomposed from decosahexanoic acid (DHA).

However, the compound C$_{20}$H$_{34}$O (geranyl linalool) with its isomer hexadeca-2,6,10,14-tetraen-1-ol and 3,7,11,16-trimethyl which is a derivative of eicosapentonoic acid (EPA) does not experience decomposition, only on compound C$_{25}$H$_{42}$ (2,6,10,14,18-Eicasapentaena) has undergone a decomposition of the C atom chain from eicosapentonoic acid (EPA). The breakdown of the atomic chain C in the fatty acids mentioned above is caused because at the time of oil extraction from shark liver, the extraction temperature used is too high (± 60°C). So that this causes the breaking or breaking down of the C atomic chain bonds in essential fatty acids (omega-3) like, α-linolenic, eicosapentonoic (EPA) and decosahexanoic (DHA). The use of temperatures that are too
The process of isolating fatty acids will cause plural unsaturated fatty acids (PUFA) such as essential fatty acids (omega-3) namely α-linolenic, eikosapentonoat and decosahexanoic) it will be easy to experience breaking or breaking the carbon atomic chain bonds [13]. Therefore, if the purpose of smallfin gulper shark liver oil extraction is to use its omega-3, the extraction temperature must be below 50°C. Or more effective is extraction by pressing and using solvent.

The fatty acids contained in banuna cone liver oil were palmitic acid 12.59%, oleic acid 17.86%, EPA 1.50% and DHA 14.35% with a total SFA of 18.59%, total MUFA 24.54 and total PUFA 19.11% [17]. Several deep sea fish species contained PUFA (4.11–99.63 mg/g), MUFA (66.17467–22 mg/g) and SFA (13.11–486.55 mg/g) ) with the concentration of dominant fatty acids namely oleic and palmitic fatty acids. The difference in the dominant fatty acids in each oil is caused by the different fish habitat and the food consumed by each fish. Fatty acids in each species will vary, which is influenced by several factors such as season, temperature, place of development, fish species, age, sex, and feeding habits [18].

4. Conclusion
In general, smallfin gulper shark liver oil has met the quality standard with a yield of 83%, while the compound composition is dominated by squalene compounds of the remaining 95.10%, the form of palmitic acid, α-linolenic acid, EPA and DHA.

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