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# Research Article
# Fatty Acid Positions in Triacylglycerol of Iridescent Shark Fish Oil (*Pangasius* sp.), Focusing on Omega-3 and Omega-6 Fatty Acids

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## Abstract

**Background and Objective:** Considering the many health benefits of fish oil, the potential of Indonesian fisheries needs to be mapped to find local fish oil sources that have the opportunity to be used as a source of omega-3 and 6. This research aimed to ascertain the glyceride profile of iridescent shark fish oil hydrolyzed by immobilized lipase from *Thermomyces lanuginosus* at the sn-1,3 position and identify the position of omega-3 and omega-6 fatty acids. **Materials and Methods:** To extract the fish oil from the iridescent shark, the soxhletation method was utilized. The analysis of the fatty acid composition that was carried out using gas chromatography (GC) was previously esterified with $BF_3$ before it was carried out to position the fatty acid hydrolysis that was carried out using lipase enzymes to position the fatty acid composition. **Results:** The sample had more unsaturated fatty acids than saturated ones. Omega-3 and omega-6 fatty acids are more concentrated in the fat molecule's sn-2 position than in the sn-1+sn-3 location. Iridescent shark fish oil meets the recommended ratio of omega-3 to omega-6 (1:1) or better (2:1). **Conclusion:** It has been discovered that iridescent shark fish oil is rich in omega-3 and omega-6 fatty acids, especially those in the sn-2 position. This makes it a great food choice for those trying to get more omega-3 unsaturated fatty acids into their diets.

Key words: Fish oil, iridescent sharks, fatty acids, omega-3 and omega-6 fatty acids, food choice, positions

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Omega-3 fatty acids, specifically eicosapentaenoic acid, have many healthy eicosapentaenoic acids (EPA) and docosahexaenoic acid (DHA) are firmly documented existing recommendations for their intake[1]. The potential health benefits of including omega-3 fatty acids in the Greenlandic diet have been the subject of in-depth research and large-scale epidemiological studies involving Eskimos from Canada and Alaska[2]. Large-scale epidemiological studies and research conducted with the participation of Alaskan and Canadian Eskimos have investigated the potential positive health effects of including omega-3 fatty acids in the diet of Greenlanders[3].

Shark fish cannot provide complex unsaturated fatty acids, although, it can still be utilized as such, on average, fresh fish has a lower content of omega-3 fatty acids than fish caught from the ocean. The proportions of different fatty acids and fats in fish can range widely. Some factors that come into play include the type of fish, the time of year, the geographical location, how mature the gonads are and the size of the fish. Shark fish species and harvest time of year affect fat content and fatty acid composition[4].

Nutritional value depends on the glycerol molecule's fatty acid content and structure. Because stereospecific numbering determines the position of fatty acids within fat molecules (sn), precisely the positions of sn-1, 2 and 3 in the fat molecule (triacylglycerol = TAG), This can also have an affectional value of fat, as this position affects the body's metabolic processes. During the proofing of processing fat in the body, the enzyme lipase is essential for hydrolyzing the fatty acids in the TAG structure. Salivary, gastric and pancreatic lipase are the three types of lipase that actively hydrolyze fat in the digestive tract before it is absorbed. The sn-1,3 position is where human lipase enzymes won't hydrolyze acyls at the sn-2 position[5].

Fatty acids on the glycerol molecule determine fat's nutritional value. Iridescent shark fish has the potential to use their oil as a source of omega-3 and unsaturated fatty acids, increasing the fulfilment of people's food and nutrition needs, where the fat content is around 5.75%[6]. This research aimed to determine the glyceride profile and identify the position of omega-3 and 6 fatty acids in the triacylglycerol hydrolysis of iridescent shark fish oil by immobilized lipase from *Thermomyces lanuginosus*. More specifically, the sn-1,3 position was the focus of this investigation.

## MATERIALS AND METHODS

**Materials:** This research was conducted at the Pharmacy Laboratory of the University of North Sumatra. The research began in March, 2018 to June, 2019. The material used was iridescent shark fish purchased from a Medan City, Indonesia market.

### Methods

**General procedure:** This study included eight stages: (1) Removing fish oil or its isolation, (2) Conduct experiments to determine the oil's physicochemical properties, (3) They were manufacturing fatty acid methyl esters at the time, (4) The GC-FID analysis of the fatty acid composition, (5) Assessing the value of fish oil as a source of nutrients, (6) The hydrolysis of fatty acids by the enzyme known as lipase, (7) A study to determine the ratio of omega-3 fatty acids to omega-6 fatty acids at the sn-2 and (8) Calculating the omega-3-to-omega-6 ratio.

This first component begins with a simple finger prick. When the lab receives blood sample, the membranes of the red blood cells was break down to analyze their composition. This is where omega-3s, omega-6s, trans fats and palmitic acid can be measured. Second omega-6: Omega-3 ratio is calculated by dividing the sum of all the omega-6 fatty acids by the sum of all the omega-3 fatty acids. Omega-6 and 3 are two essential fats that are categorized as polyunsaturated fatty acids, or PUFAs for short.

**Extraction fish oil:** Extraction was performed using the Soxhletation technique outlined in SNI 01-2354.3-2006. Five hundred grams of iridescent shark fish fillets were washed and dried in a vacuum oven (Indonesian National Standard) at 70°C for 3 hrs following milling. They were then extracted with n-hexane at 80°C for 50 min. The extracted substance was then subjected to a 60 min distillation at 70°C. In a 50°C oven for 25 min, the extract was dried[7].

Tools used, (a) Electric heaters, supports, condensers and Soxhlet extractors, Specification model number 500, analog heating mantle AHM 500, boiling flash glass 500 mL, condenser glass 225 mm, Soxhlet extractor glass 250 mL, weight 250 kg, (b) 250 mL round smooth flask, specification material glass, outer mouth diameter 3.6 cm, (c) Fat sleeves (extraction thimbles), Specification model AMT-S01, external diameter 17×33 mm, wall thickness 1.5-2.0 mm, packing 25 thimbles/box, (d) Desiccator, Specification model 247826654, DN 250, height 344 mm, tubulature 24/29 NS, volume approx 10500 mL, ID flange 274 mm, weight 10.5 kg, (e) Oven temperature 105°C, Specification model digital laboratory oven type DSO-500D, working temperature 5-200°C, capacity 50 L, chamber dimensions (mm) (W×D×H) 380×365×390, power watts 1000W, overall dimensions (mm) (W×D×H) 480×475×695 and (f) Filter paper, specification pore size: 11 μm, 9 cm diameter qualitative filters. All of this

equipment has been standardized Nationally, method of chemical test in determining fish fat content ICS 67.120.30 National Standardization Agency (BSN), State of Indonesia SNI 01-2354.3-2006.

**Fatty acid esterification:** After adding 1 mL of a 0.5% sodium hydroxide solution dissolved in methanol to a closed test tube, 25 mg of oil are weighed inside the tube. The tube is then shaken for 1 min. After spending 5 min in a water bath heated to 100°C, the tube is cooled to temperatures ranging from 30-40°C. After adding 1 mL of BF3 and resealing the jar, place it in a water bath heated to 100°C for 05 min. After the mixture was cooled to between 30 and 40°C, 1 mL of n-hexane was added and vigorously shaken for 30 sec. Two layers, consisting of water on the bottom and n-hexane on the top, were produced due to the addition of a saturated NaCl solution. The n-hexane layer that forms is separated and the only layer that is left is the water layer. Another millilitre of n-hexane removed the water layer from the sample. The layer of newly formed n-hexane is merged with the layer of n-hexane that was already present. The n-hexane extract underwent a 15 min treatment with 50 mg of anhydrous sodium hydroxide before evaporating. The liquid-free water phase is injected to the maximum extent possible one microlitre to be analyzed using a gas chromatography tool[8].

**The detector of Ionization by Flame for Gas Chromatography (GC-FID):** The detector for the gas chromatography, a Shimadzu QP 2010 ULTRA FID, was utilized. The column utilized is DB-23, 30 m in length, with a column temperature of 40-250°C, a rate of increase of temperature of 20°C $min^{-1}$, a temperature of detector 260°C. A flow rate of 37.7 mL $min^{-1}$ and a column rate of 0.72 mL $min^{-1}$ were specified for the nitrogen carrier gas[9].

**Fatty acid hydrolysis by lipase enzymes:** Using a 125 mL Erlenmeyer, 6 grams of oil were measured and weighed. After adding 10 mL of distilled water, 2.5 mL of 0.063M $CaCl_2$, 5 mL of Tris-HCl buffer solution and 100 mg of lipase, incubate the mixture at 37±0.5°C for 10 hrs while shaking it for 10 min every hour. The incubation temperature should be 37±0.5°C. After that, up to 50 mL of ethanol were used to activate them. The mixture is then poured into a separating funnel, shaken and left to sit until two distinct layers' form. The top layer containing the fatty acids is removed, ethanol in increments of up to 50 mL is added and the top layer is removed again. Then evaporate on a water bath in a vaporizer cup already known to weigh. The layer of fatty acids obtained from evaporation is weighed constantly. The hydrolysis reaction is complete when the final weight has stabilized[10].

**To determine the peroxide value of oil:** The traditional method of determining peroxide value (PV) involves a titration of the oil containing potassium iodide in a chloroform-acetic acid mixture. The hydroperoxides oxidize the iodide to iodine, which is determined by titration with sodium thiosulfate.

**Statistical analysis:** No statistical analysis method was used. This study was carried out using a descriptive method, observing the physical and chemical properties and analyzing the fatty acids of shark fish oil using gas chromatography (GC).

**Measured parameter:** Peroxide number was determined by iodometric titration using thiosulfate solution as titration. The principle of this titration is that compounds in fat (oil) will be oxidized by potassium iodide (KI) and the released iodine is then titrated with thiosulfate. Determination of the saponification number using acid-base titration. The working principle of free fatty acid analysis is to heat the sample to which alcohol has been added so that the triglycerides in the sample are hydrolyzed and produce free fatty acids.

## RESULTS

**Physical and chemical constituents of fish oil:** Table 1 shows the chemical composition of the iridescent shark fish oil was determined by examining its cloud point, total solid, peroxide number, saponification number, free fatty acid concentration and iodine number. These characteristics, such as cloud point, total solid, peroxide number, saponification number, free fatty acid content and iodine number, were investigated as part of the analysis to understand the physical and chemical components of the fish oil. The cloudy point test is carried out to identify any foreign material contamination or oil mixing that may be present. The cloudy point and total solid height measurements in Table 1 suggested that the fish oil is not yet in its purest form.

**GC-FID analysis:** The chromatogram of the iridescent shark fish oil that was analyzed using GC-FID can be seen in Fig. 1

Table 1: Physical and chemical characteristics of iridescent shark fish oil

| Characteristics | Unit | Amount |
|---|---|---|
| **Physical properties** | | |
| Cloudy point | °C | 34.50 |
| Total solid | Brix | 52.00 |
| **Chemical properties** | | |
| Peroxide number | meq $kg^{-1}$ | 10.40 |
| Saponification number | mg KOH $g^{-1}$ | 108.84 |
| Free fatty acid | % | 4.70 |
| Iodine number | mg/100 g | 17.87 |

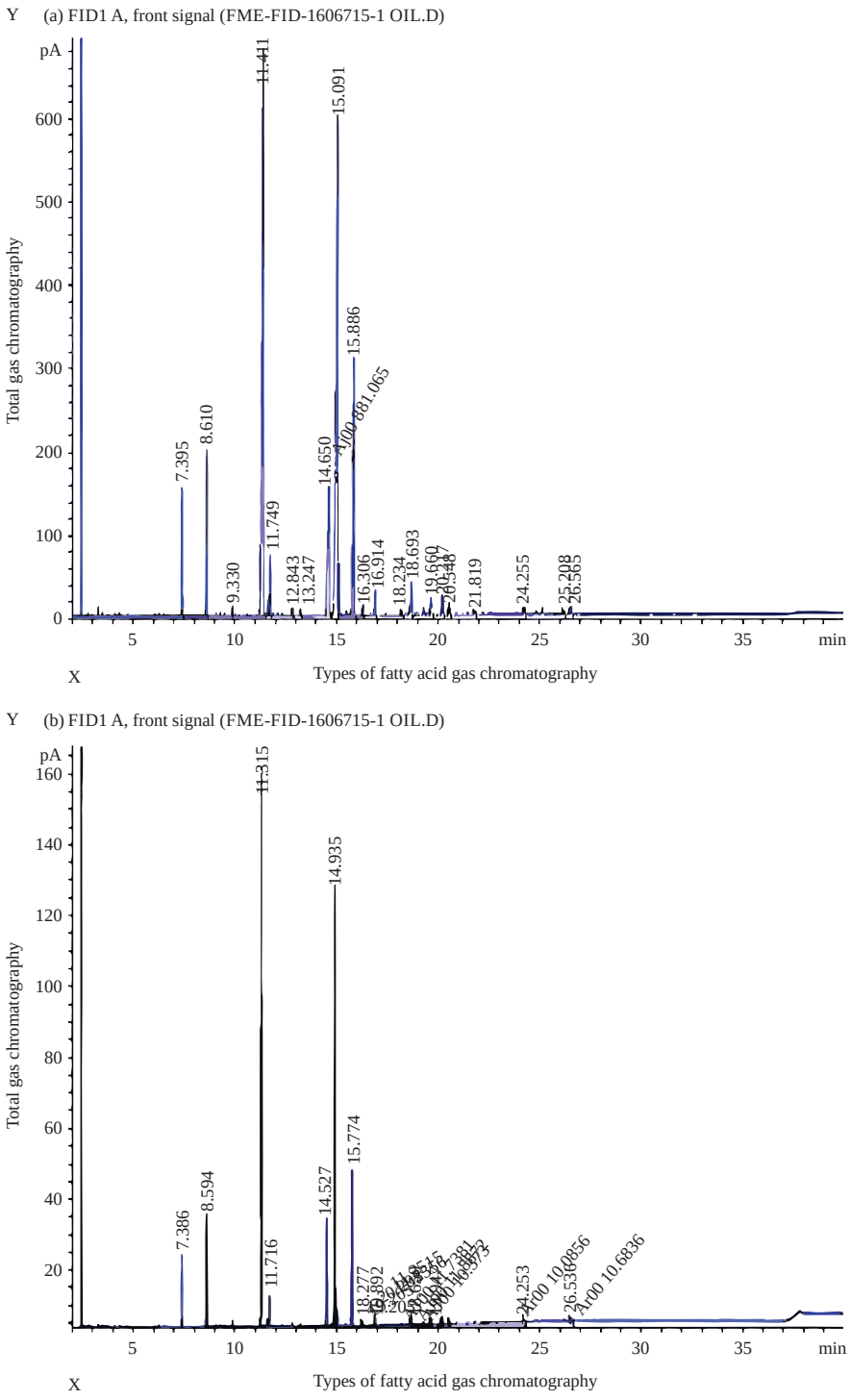


Fig. 1(a-b): Iridescent shark fish oil chromatogram, (a) Before hydrolysis and (b) After hydrolysis

(Iridescent shark fish oil chromatogram (a) Before hydrolysis and (b) After hydrolysis). The fatty acids in iridescent shark fish oil were outlined in Table 2. Figure 1 and Table 2 indicated that the iridescent shark fish oil, both before and after the hydric process, contains moderated fatty acids (MUFA and PUFA) than saturated fatty acids. Palmitic acid can be found in the greatest quantities of all saturated fatty acids. Omega-3, omega-6 and omega-9 fatty acids are needed by the body (oleic acid and eicosenoic acid).

Table 2: Composition of fatty acids contained in shark fish oil before hydrolysis and after hydrolysis

| | | | Amount (%) | |
|---|---|---|---|---|
| Type of fatty acids | Carbon | Name of fatty acids | Before | After |
| Saturated fatty acids | C:14-0 | Myristic acid | 4.373 | 3.616 |
| | C:15-0 | Pentadecanoic acid | 0.464 | 0.378 |
| | C:16-0 | Palmitic acid | 33.34 | 31.33 |
| | C:17-0 | Heptadecanoic acid | 0.558 | 0.466 |
| | C:18-0 | Stearic acid | 8.809 | 8.329 |
| | C:20-0 | Arachidonic acid | 0.509 | 0.509 |
| | C:21-0 | Heneicosanoic acid | 0.800 | 0.695 |
| | C:24-0 | Lignoceric acid | 0.491 | 0.428 |
| | | ΣSFA | 49.344 | 45.751 |
| Unsaturated fatty acids | C:16-1 | Palmitoleic acid | 1.599 | 1.497 |
| | C:17-1 | Cis-10-Heptadecanoic acid | 0.523 | 0.425 |
| | C:18-1 | Oleic acid$^{ω-9}$ | 35.810 | 33.350 |
| | C:20-1 | Eicosenoic acid$^{ω-9}$ | 1.169 | 0.960 |
| | | ΣMUFA | 39.101 | 36.304 |
| | C:18-2 | Linoleic acid$^{ω-6}$ | 11.580 | 11.110 |
| | C:18-3 | γ-Linolenic acid$^{ω-6}$ | 0.503 | 0.388 |
| | C:18-3 | Linolenic acid$^{ω-3}$ | 0.828 | 0.819 |
| | C:20-2 | Eicosadienoic acid | 0.786 | 0.780 |
| | C:20-3 | Eicosatrienoic acid$^{ω-3}$ | 0.617 | 0.482 |
| | C:20-5 | Eicosapentaenoic acid$^{ω-3}$ | 0.556 | 0.434 |
| | C:22-6 | Docosahexaenoic acid$^{ω-3}$ | 0.553 | 0.376 |
| | | ΣPUFA | 15.423 | 14.389 |

SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acid and PUFA: Polyunsaturated fatty acid

Table 3: Distribution positions of unsaturated fatty acid (%) on TAG molecules of iridescent shark fish oil

| | Amount (distribution positions %) | | |
|---|---|---|---|
| Name of fatty acids | TAG | sn-2 | sn-1+sn-3 |
| Palmitoleic acid | 1.599 | 1.497 (96.621) | 0.102 (3.379) |
| Cis-10-Heptadecanoic acid | 0.523 | 0.425 (81.262) | 0.098 (18.738) |
| Oleic acid$^{ω-9}$ | 35.810 | 33.350 (93.130) | 2.460 (6.870) |
| Linoleic acid$^{ω-6}$ | 11.580 | 11.110 (95.941) | 0.470 (4.059) |
| γ-Linolenic acid$^{ω-6}$ | 0.503 | 0.388 (77.137) | 0.115 (22.863) |
| Linolenic acid$^{ω-3}$ | 0.828 | 0.819 (98.913) | 0.009 (1.087) |
| Eicosenoic acid$^{ω-9}$ | 1.169 | 0.960 (82.121) | 0.209 (17.879) |
| Eicosadienoic acid | 0.786 | 0.780 (99.237) | 0.006 (0.763) |
| Eicosatrienoic acid$^{ω-3}$ | 0.617 | 0.482 (78.120) | 0.135 (21.880) |
| Eicosapentaenoic acid$^{ω-3}$ | 0.556 | 0.434 (78.058) | 0.122 (21.942) |
| Docosahexaenoic acid$^{ω-3}$ | 0.553 | 0.376 (67.870) | 0.177 (32.130) |
| Omega-3 | 2.554 | 2.111 | 0.443 |
| Omega-6 | 12.083 | 11.498 | 0.585 |
| Omega-9 | 36.979 | 34.310 | 2.669 |

**Distribution position of omega-3 and 6 in the TAG molecule:** Table 3 illustrated where the unsaturated fatty acids were located within the triacylglycerol of the iridescent shark fish oil.

The position of the omega-3 distribution in iridescent shark fish oil, specifically linolenic acid, eicosatrienoic acid, EPA and DHA, is more dominant in the lowest sn-2 position, with 67.870% than it is in the highest sn-2 position, with 98.913%. This is because the lowest sn-2 position contains 67.870% of the total. Linoleic acid and -linolenic acid are found in the sn-2 position of omega-6, ranging from 77.137-95.941%. It is reasonable to conclude, based on the position of the distribution of omega-3 and 6 fatty acids in fish oil triacylglycerol, that omega-3 and omega-6 fatty acids are found in higher concentrations at the sn-2 position compared to the sn-1 and sn-3 positions. This is because the position of the distribution of omega-3 and 6 fatty acids in fish oil triacylglycerol was shown in Fig. 1. This is because the omega-3 and omega-6 fatty acids have a longer chain length in the sn-2 position than they do in the sn-1 and sn locations, respectively.

**Comparison of omega-3 with omega-6 iridescent shark fish oil:** The results of a gas chromatography analysis were presented in Table 4, a comparison of the omega-3 and omega-6 fatty acids present in iridescent shark fish oil. Omega-3 consumption in excess can negatively affect enzyme activity and membrane permeability.

Table 4: Analysis of the differences between omega-3 and omega-6 fatty acids

| Distribution | ώ-3 (%) | ώ-6 (%) | ώ-3/ώ-6 |
|---|---|---|---|
| TAG 2.554 | 12.083 | (1:4.7) | |
| sn-2 2.111 | 11.498 | (1:5.4) | |
| sn-1 and sn-3 | 0.443 | 2.669 | (1:6) |

Table 5: Iridescent shark fish oil and the possible benefits it could have on one's health

| | Fatty acid composition (deviation) | | | |
|---|---|---|---|---|
| Sample | SFA (%) | MUFA (%) | PUFA (%) | Deviation (%) |
| Ideal composition | 33.33 (0.00) | 33.33 (0.00) | 33.33 (0.00) | 0.00 |
| Iridescent shark fish oil | 49.344 (16.01) | 39.101 (5.77) | 15.423 (17.90) | 39.68 |

SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acid and PUFA: Polyunsaturated fatty acid

**Nutritional value of iridescent shark fish oil:** The nutritional value of iridescent shark fish oil was presented in Table 5 and is analyzed according to how closely it comes to the ideal composition. According to this data, Iridescent shark fish oil does not have a nutritional value that satisfies the criteria for an optimum composition. The proportion of each of the three fatty acids does not correspond to the ideal of 33.33% and the departure from this ideal is significant altogether. Fish oil is abundant in unsaturated fatty acids (MUFA and PUFA), which outnumber saturated fatty acids (SFA).

## DISCUSSION

When analyzing the iridescent shark fish oil, variety various and chemical properties were observed, including cloud point, total solid, iodine number, peroxide number, saponification number and free fatty acid content (Table 1). The benefits of shark oil are very different according to the different species of fish[11]. If the peroxide number is high, the fish oil is of poor quality, if it is low, it is of high quality. Foaming in iridescent shark fish oil is far below the SNI recommended level of 196-200 mg KOH $g^{-1}$. The low value of lathering indicates the formation of longer-chained fatty acids within the oil, resulting in the oil possessing a high molecular weight and a low number of saponifications.

Iridescent shark fish oil maintains a balance of omega-3 to omega-6 fatty acids that fall within the desirable range (1:1) or at least (2:1), which is the optimal ratio[9,11]. The highest yield of shark liver oil was obtained from extraction at 60°C (49.4%)[12]. The acid number is proportionally higher when the oil's quality is lower. According to SNI 04-7182-2006, the iodine number of iridescent shark fish oil is lower than the standard iodine number, which is 45-46 mg/100g. This is the range that the standard iodine number falls into. It is possible to conclude that the oil has a low iodine number and does not contain many unsaturated fatty acids[13].

As can be seen in Fig. 1(a-b) and Table 2, palmitic acid is the primary contributor to the presence of saturated fatty acids in the human diet. This is the case even if palmitoleic acid is also present. Particular omega-3 fatty acids, such as linolenic acid, eicosatrienoic acid, docosahexaenoic acid and docosahexaenoic acid, particular omega-6 fatty acids, such as linoleic acid and -linoleic acid and particular omega-9 fatty acids, such as oleic and eicosapentaenoic acid, have been identified. The prevalence of omega-9 fatty acids is higher than omega-6 and omega-3. This is to the statement that fish oil is known to contain omega-9 greater than omega-6 and 3[14].

From Table 3, it can be seen that the position of the omega-3 distribution of iridescent shark fish oil, namely linolenic acid, eicosatrienoic acid, EPA and DHA, is more dominant in the lowest sn-2 position of 67.870% and the highest is 98.913%. This is by the statement that there is more fish oil. Omega-3 and omega-6 fatty acids in fish oil occupy the sn-2 position of the triacylglycerol molecule, as opposed to the more prevalent sn-1,3 position[15,16]. The omega-3 fatty acids in marine fish oil are primarily derived from shark liver. Omega-3 fatty acids are abundant in this oil[17].

In Table 4, the findings of a gas chromatographic analysis comparing omega-3 and omega-6 fatty acids found in iridescent shark fish oil are presented next to one another. Omega-6 intakes above the 20:1 ratio/ratio (n-6: n-3) have been linked to inflammation, cancer, vision loss, autoimmune diseases and neurodegenerative disorders[18,19]. A population with inadequate omega-3 PUFA intakes in Spain necessitates an immediate increase in consumption and consideration of the necessity of supplementation[20].

In Table 5, the proportion of the three different kinds of fatty acids does not correspond to 33.33 percent and the overall deviation is very high. Total unsaturated MUFA and PUFA fatty acids are greater than saturated fatty acids[21]. According to Paszczyk *et al.*[22], quantities of Polyunsaturated and Monounsaturated Fatty Acids (MUFA and PUFA) in unsalted cheese substitutes were found to be the highest (39.29±1.49 and 9.13±0.33%, respectively). According to the research findings, the omega-3 and omega-6 fatty acid content of colourful shark oil helps the body better absorb fat and speed up its metabolism.

The implication of the composition of unsaturated fatty acids is greater than that of saturated fatty acids. Distribution positions of omega-3 and 6 fatty acids in colorful shark oil fat molecules were found to be more dominant at the sn-2 position than at the sn-1+sn-3 position.

Hence, only gas chromatography (GC) was carried out for observing physical and chemical properties and analyzing shark oil fatty. No further technique was used for this purpose which is a limitation of this study. Future recommendations for the fatty acid content of colorful shark oil contain omega-3 and omega-6 fatty acids and the proportion of omega-3 and omega-6 fatty acids at the sn-2 position is higher so that the fat content is better.

## CONCLUSION

This study concluded that the composition of unsaturated fatty acids is greater than that of saturated fatty acids. Recommendations for the future are that the fatty acid content of colorful shark oil contains omega-3 and omega-6 fatty acids and the percentage of omega-3 and omega-6 fatty acids at the sn-2 position is higher so that it is better in the fat absorption process.

## SIGNIFICANCE STATEMENT

This study is important because it aims to determine the glyceride profile of colored shark oil hydrolyzed by lipase immobilized from *Thermomyces lanuginosus* at the sn-1,3 position and identify the positions of omega-3 and omega-6 fatty acids. The results showed that the samples had more unsaturated fatty acids than saturated ones. Omega-3 and omega-6 fatty acids are more concentrated at the sn-2 position of the fat molecule than at the sn-1+sn-3 location. Colorful shark oil meets the recommended ratio of omega-3 to omega-6 (1:1) or better (2:1).

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