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Quality and fatty acid profiles of fish oil from tuna by-products extracted using a dry-rendering method

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Abstract. *Djamaludin H, Sulistiyati TD, Chamidah A, Nurashikin P, Roifah M, Notonegoro H, Ferdian PR. 2023. Quality and fatty acid profiles of fish oil from tuna by-products extracted using a dry-rendering method. Biodiversitas 24: 6100-6106.* Tuna is an export commodity with a high fat content, making it a valuable source of fish oil. However, a substantial portion of tuna, including viscera, eyes, and liver, some parts are underutilized, leading to waste. The study aims to determine the impact of extraction temperature (50°C, 60°C, and 70°C) on the yield, peroxide value, free fatty acids (%), p-anisidine value, and total oxidation value of the extracted oil from tuna (*Euthynnus* sp.) by-products. This research used the dry-rendering method to extract crude oil from tuna by-products and analyze quality characteristics and fatty acids. The results from the proximate value indicated that the crude lipid content in tuna by-products ranged from 13.03% to 19.01%. Extraction temperature significantly affected the yield, with the highest yield at 70°C correlating with the highest lipid content in innards. The lowest peroxide value is obtained from an extraction temperature of 50°C from all parts of tuna by-products, ranging from 2.01 to 2.09 meq/kg. Free fatty acid (%) levels in this study met the International Fish Oil Standards, and different temperature treatments had a significant effect ($P<0.05$). The p-anisidine lowest value of 6.11 meq/kg was obtained from an extraction temperature of 50°C for innards samples, and the p-anisidine value increased with temperature, reflecting secondary oxidation products. The total oxidation lowest value was obtained from extraction at 50°C (14.84 meq/kg) from viscera by International Fish Oil Standards (≤20 meq/kg), and it was within acceptable limits. The fatty acid profile of the crude extract of tuna by-product oil resulting from extraction at a temperature of 50°C, where extraction results at 50°C obtained a fatty acid profile of saturated fatty acids>mono-unsaturated fatty acids>poly-unsaturated fatty acids, with significant levels of omega-3 fatty acids such as eicosapentaenoic acid and docosahexaenoic acid.

Keywords: Free fatty acids, p-Anisidine, peroxide value, total oxidation

INTRODUCTION

Tuna (*Euthynnus* sp.) is one of the second largest export commodities in Indonesia after shrimp, which contains 2.34-4.66% lipid (wet weight) (Bahurmiz 2019). Sea fish are rich in omega-3 and omega-6 fatty acids, while freshwater fish contain high omega-9 fatty acids. Tuna is processed earlier than the sale, producing by-products of merchandise up to 70% of the total weight of the fish and containing an excessive part of valuable lipids that can constitute a profitable resource (Garofalo et al. 2023). Fishery by-products are generally only used as fish meal for livestock feed; then, the rest is disposed of in a landfill. Heads and viscera are by-products that need to be appropriately utilized. Even though these by-products contain omega-3 and omega-6 fatty acids (Ahmed et al. 2017), and it can add value to fish oil. By-products of tuna can be used as fish oil containing 37.48%±0.41 of omega-3, 3.74%±0.08 of omega-6 and 8.11%±0.41 of omega-9 (Srichan et al. 2018).

Fish oil is a fat component in fish body tissues that has been extracted as oil. Fish oil is a natural source of poly-unsaturated fatty acids (PUFA) omega-3, especially Eicosapentaenoic acid (EPA) (C20: 5n-3) and docosahexaenoic acid (DHA) (C22: 6n-3) (Vasile et al. 2016). Fish oil generally consists of various types of triacylglycerols in the form of molecules composed of glycerol and fatty acids. The fatty acid chain in fish oil has more than eighteen carbon atoms and five or six double bonds (Mason and Sherratt 2017). The high content of essential fatty acids in fish oil includes linoleic, linolenic, and arachidonic acids. These PUFAs that need to be obtained from foods as they can't be synthesized in the body and are therefore required for health purposes are called essential fatty acids EFA (Kaur et al. 2014).

Fish parts that can be used as a source of fish oil are divided into two groups: fish liver oil and fish body oil. Fish liver oil contains several vitamins A and D. Ferdosh et al. (2015) and Šimat et al. (2020) research shows that fish oil can also be extracted from fish by-products, including tuna liver. Fish oil may be processed into supplements, meals, feed combos, and uncooked materials for non-meals industries. The content of fish oil in omega-3 fatty acids has a critical function in the fitness quarter because omega-

3s contain EPA and DHA, which help for boost health, including anti-inflammatory and anti-cancer (Troesch et al. 2020).

Fish oil production is generally carried out in three ways: rendering, mechanical press, and solvent. An important factor determining the quality of fish oil is in the extraction process. Several things play a role, such as method, pressure, temperature, and length of extraction time. The fish oil extraction method by rendering is divided into dry-rendering and wet-rendering. Between dry and wet-rendering methods, fish oil extraction using the wet-rendering method has a weakness. The wet-rendering extraction method can reduce PUFA levels in the presence of high temperatures (Pudtikajorn and Benjakul 2020). This is because the dry-rendering process does not use water as a carrier and uses inappropriate temperatures and length of time. The temperature in the fish oil production process also affects the DHA content. Ferdosh et al. (2015) stated that the DHA content of cooked and uncooked tuna head extraction showed significant differences. Thus, temperature affects the results of the content and quality of fish oil.

However, the method of dry-rendering extraction has not been studied in the extraction of oil from by-products of tuna. To our knowledge, very few studies have been conducted concerning the extraction of oil from by-products by using the dry-rendering method. The study of analyzing the quality of crude oil extract from by-products of tuna is still relatively limited. Hence, the research aims to analyze the quality characteristics of crude oil extract by-products i.e. viscera, eyes, and liver tuna extracted using the dry-rendering method.

MATERIALS AND METHODS

Study area

The research was conducted from March to June 2023 in the Fish Product Technology Laboratory, Faculty of Fisheries and Marines Science, Universitas Brawijaya, Malang City, East Java Province, Indonesia. The samples were seawater fish, namely tuna (*Euthynnus* sp.), obtained from the fish landing site in Sendang Biru, South Malang, Malang Regency. The samples were then prepared to separate the viscera, liver and fish eyes. The viscera, liver, and eyes of the fish that have been separated were then weighed using a scale, and the yield value was calculated.

Procedures

Extraction

The tuna viscera were ground using a blender, then weighed, placed in a filter container, and heated in the oven on the top shelf at varying temperatures of 50°C, 60°C, and 70°C for 60 minutes. The extracted oil was collected in an aluminum container placed on the bottom shelf of the oven, filtered and placed in a dark glass bottle, then characterized, which included measuring the yield, analysis of peroxide value, free fatty acids, p-anisidine value, and total oxidation value. Oxidation parameters were analyzed: peroxide value and free fatty acids were using the titration method, p-anisidine value was using spectrophotometry with a UV-Vis spectrophotometer at 350 nm, and total oxidation was calculated from the sum of 2 times the peroxide value and the p-anisidine value (AOAC 2005). The same procedure replaced the viscera samples with tuna eyes and liver.

Analysis procedure

The test procedure includes proximate analysis using the AOAC (2005) to determine the content of water and lipids. Analysis of the water content by heating the sample in the oven until all the water was evaporated and the fat content by extracting fat using a Soxhlet tool.

Analysis of oil oxidation parameters based on AOAC (2005), which includes analysis of peroxide number by titration using saturated KI and starch, free fatty acids using phenolphthalein (PP) indicator and titration with 0.5 N KOH, p-anisidine analysis to see the hydroperoxide decomposition process using a UV-VIS spectrophotometer at a wavelength of 350 nm, Total Oxidation analysis (TOTOX) by calculating twice the peroxide value with the p-anisidine value. Fatty acids profile analysis using the GC-MS method with the Agilent Technologies 7890 Gas Chromatograph with Auto Sampler (Hurria et al. 2023).

Data analysis

Fish oil data from dry-rendering extraction (yield, peroxide number, free fatty acids, p-anisidine, TOTOX) were analyzed using a completely randomized design with one factor, namely temperature. The average value data is processed into residual values to analyze the assumptions of normality and homogeneity and obtained ($P>0.05$), which indicates that it meets the assumptions of normality and homogeneity so that ANOVA one-way parametric analysis can be used. The data was then tested by ANOVA using Minitab 17.0 software. If there is a significant difference ($P<0.05$), continue Duncan's Multiple Range Test (DMRT) as post hoc test.

RESULTS AND DISCUSSION

Proximate content of tuna by-products: Viscera, eyes, and liver

Proximate analysis, including lipid content, was performed on the by-products of tuna. This lipid content information can describe the lipid content that can be extracted into oil from the tuna by-products used. The crude lipid content of the tuna by-products is listed in Table 1.

Table 1. Proximate content of tuna by-products: viscera, eyes, and liver

Proximate	By-products		
	Viscera	Eyes	Liver
Lipid (%)	19.01±0.11	13.05±0.01	17.03±0.21
Water (%)	9.23±0.33	7.11±0.10	8.13±0.15

The lipid content, expressed on a wet-weight basis, varied from 13.03 to 19.01%. By and large, fish can be classified into four categories based on their fat content: as lean (up to 2% fat), medium fat (2-7% fat), fatty (7-15% fat), and very fatty (over 15% fat) (Pyz-Łukasik 2020). The data obtained depicts that tuna is classified as a high-fat fish.

Several previous studies received information about the lipid content in seawater fish less than 4% (for economically important fishes from the eastern central Pacific) (Murillo et al. 2014), and other studies reported ranging from 0.25% up to 3.09% (fish species from the Brazilian Northeastern coast) (Gonçalves et al. 2021), 0.90-5.94% (fish species caught in the Northeastern Mediterranean coast) (Durmus 2019), marine fish species from South China Sea had a low to moderate lipid content 0.51-7.35% (Zhang et al. 2020), and total lipid content of fish from Java Sea ranged from 1.73% (Tetraodontidae) to 9.82% *(Leiognathus equulus* Forsskål 1775) (Priatni et al. 2018). The findings of this study showed that the highest percentage of lipid content was found in the viscera, which was 19.01%. The rate of lipid content contained in each part of tuna by-products can influence the amount of oil produced. The high fat content in fish will also cause it to have more fish oil. Differences affect the variation in the percentage of lipid content in the type of fish and food. Increasing the lipid content in fish food and the amount of fish consumed will increase the lipid content contained in fish. Although the lipid content reported herein could be mainly attributed to differences in the part of tuna by-products, the lipid content might also be influenced by multiple factors, including geographical origin, catch season, diet, reproductive stage, and age variation.

Yield of crude oil extract of by-products of tuna: viscera, eyes, and liver

Yield shows the percentage of raw materials that can be made into the end product. The yield of crude oil extract from tuna by-products i.e. viscera, eyes, and liver, was calculated by comparing the weight of the crude fish oil extract obtained with the weight of the main raw material. The results of calculating the yield of crude oil extract for each part of the tuna by-products obtained can be seen in Figure 1.

Extraction temperature treatment significantly affected the yield of oil produced (P-Value 0.000<0.05). The amount of yield increased with increasing extraction temperature (Figure 1). According to research by Adeoti et al. (2014) that temperature affects the breakdown of the matrix wall in the cell membrane of the lipid. Niu and Xiang (2018) also stated that through a holistic effect involving changes in lipid composition and interactions between lipids and specific membrane proteins, elevated temperatures can directly and effectively change the properties of these membranes, including their fluidity and permeability. Thus, the higher the extraction temperature, the greater the yield of oil produced. The highest yield was obtained at 70°C, ranging from 30% to 48%, and oil yield from tuna viscera has the highest yield. It is in line with the results of lipid content analysis (Table 1), showing that the highest lipid content was shown in viscera at 19.01%. These results prove that more fish oil will be produced from the high-fat content in fish. This result is higher than the research conducted by Suseno et al. (2021), which used the dry-rendering method on the viscera of the tilapia (*Oreochromis* sp.), which obtained the highest yield at 50°C for one hour of 14.91±0.16%.

Quality of fish oil from by-products of tuna

The quality of the crude extract oil of tuna by-products can be determined by testing the oxidation parameters of the crude extract oil produced. Oxidation parameters that need to be observed include peroxide value (PV), free fatty acids (FFA), p-anisidine number, and total oxidation (TOTOX).

Peroxide number value

The measurement of the peroxide number aims to determine the hydroperoxide compounds' content in the oil extract. The peroxide value is defined as milliequivalent of active oxygen per kilogram of oil meq O_2/Kg of oil (Grossi et al. 2015). The oxidation value is significant as a determining indicator of the quality of fish oil produced. Higher peroxide values lead to changes in rancidity, whereas lower peroxide values imply good quality of the oil (Bustani and Soni 2023). The results of testing the peroxide number value of crude extract oil tuna by-products can be seen in Figure 2.

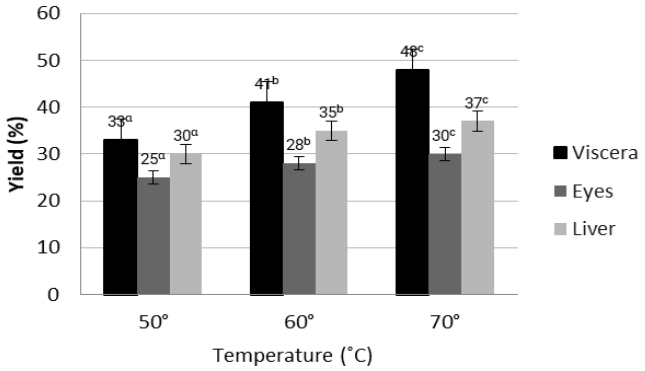

Figure 1. Yield of crude oil extract of by-products of tuna. Different superscript letters in the charts show significant differences (P<0.05) at the 5% test level (significance test with Duncan test)

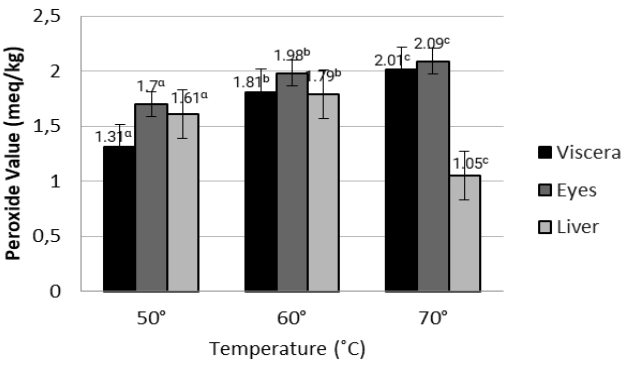

Figure 2. Peroxide value of crude oil extract of by-products of tuna. Different superscript letters in the charts show significant differences (P<0.05) at the 5% test level (significance test with Duncan test)

Peroxide number increased significantly (P-Value 0.000<0.05) with increasing extraction temperature (Figure 2). The lowest value is obtained from an extraction temperature of 50°C from all parts of tuna by-products, ranging from 2.01 to 2.09 meq/kg and was significantly different from other extraction temperatures. The peroxide value of this product meets fish oil standards (IFOS) (2011) is ≤3.75 meq/kg. Based on research by Deepika et al. (2014), extraction temperatures of 30 and 40°C with time-different PV values obtained from 0.28 to 2.65 meq/kg while at a temperature of 90°C, the PV value of 5.26 meq/kg. PV levels increase with the highest temperatures (80°C). Huli et al. (2014) reported that in extracting *swangi* (*Priacanthus tayenus* Richardson, 1846) fish skin using the wet-rendering method, the peroxide value increases with increasing temperature and decreases because there is no more dissolved oxygen. Dias et al. (2022) also reported that dry-rendering induced slight oxidation of the extracted fat but with high yields. In addition, the difference in peroxide value levels in the crude fish oil extract is caused by the content of unsaturated fatty acids found in each type of fish.

Free Fatty Acids (FFA) value

FFA is a fatty acid that is free and not bound as triglycerides. The hydrolysis of oils and fats produces FFA. The degree of FFA relies upon time, temperature, and dampness content because the oils and fats are presented with different conditions like capacity, handling, warming or searing (Pietro et al. 2020). The FFA value in the crude extract oil of tuna by-products was analyzed based on IFOS (2011). The results of testing the FFA value of the crude extract oil of viscera, eyes, and liver of tuna can be seen in Figure 3.

Different temperature treatments had a significant effect (P-Value 0.000<0.05) on the FFA value (Figure 3). FFA levels in this study met the IFOS standard, namely <1.13%. The free fatty acids formed in tuna by-product oil extract are due to the hydrolysis of triglycerides so that the fatty acids are released from the bonds with glycerol and are also caused by splitting and oxidation of the double bonds of fatty acids (Deepika et al. 2014). During oil hydrolysis, triglycerides are broken down into fatty acids, which produce compounds with bad smells (rancid), such as aldehydes, ketones and acids, increasing the acid content (Yuan et al. 2022). The low FFA value in this study is thought to be because the most significant fatty acid profile composition of tuna fish oil is oleic acid (C18:1 Ω-9), a mono-unsaturated fatty acid (MUFA), so the oil is more stable. Based on research by Ali et al. (2013), saturated fatty acid (SFA), such as palmitic and stearic acid and also oleic acid is not damaged by heating.

p-Anisidine value

To evaluate the second oxidation of fatty acids and their correlation with the presence of rancid aldehydes and ketones, which cause smells and taste, p-anisidine is considered an appropriate value. Thus, the p-anisidine value shows the "oxidative history" of a specific oil or fat (Alsufiani and Ashour 2021). This assay aims to determine the presence of aldehyde compounds in tuna viscera, eyes, and liver oil extracts. The results of testing the p-anisidine value of crude oil extract from tuna by-products can be seen in Figure 4.

Different temperature treatments had a significant effect (P-Value 0.000<0.05) on the p-anisidine value (Figure 4). The higher the extraction temperature, the greater the p-anisidine value. The p-anisidine value indicates secondary oxidation, so the higher the peroxide value produced from the primary oxidation process, the faster it will experience decomposition into secondary oxidation products (Deepika et al. 2014). The lowest value of 6.11 meq/kg was obtained from an extraction temperature of 50°C for viscera samples. Surprisingly, the lowest PV value is the PV value of the viscera sample with a temperature of 50°C. The p-anisidine value of this study was lower than the study by Suseno et al. (2021), which obtained p-anisidine values from tilapia processing with the dry-rendering method for one and two hours, 8.07 meq/kg and 13.17 meq/kg, respectively. The p-anisidine value of all treatments met IFOS, namely <15 meq/kg.

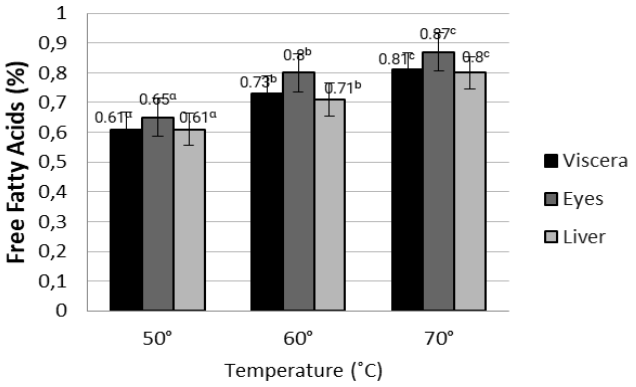

Figure 3. Free fatty acids value of crude oil extract of by-products of tuna. Different superscript letters in the charts show significant differences (P<0.05) at the 5% test level (significance test with Duncan test)

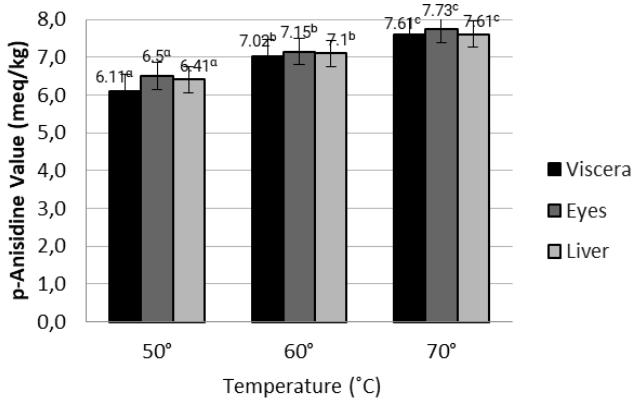

Figure 4. p-Anisidine value of crude oil extract of by-products of tuna. Different superscript letters in the charts show significant differences (P<0.05) at the 5% test level (significance test with Duncan test)

Total Oxidation (TOTOX) value

The total oxidation value is used to estimate lipid oxidative damage by adding twice the peroxide value to the p-anisidine value. Based on Figure 5, the extraction temperature significantly affects the total oxidation value of tuna by-products fish oil. The lowest value was obtained from extraction at 50°C (14.84 meq/kg) from viscera samples and was by IFOS (≤20 meq/kg). The total oxidation value in this study was much lower than the research results of Suseno et al. (2021) in extracting fish oil from tilapia processing with dry-rendering method, which was 20.59 meq/kg.

Different temperature treatments had a significant effect (P-Value 0.000<0.05) on the TOTOX value (Figure 5). Analysis of the quality of tuna oil by-products resulting from dry-rendering extraction shows that extraction using a temperature of 50°C is the best treatment considering that all oxidation parameters, both primary and secondary, comply with IFOS and the total oxidation value reaches the lowest value. The best-extracted crude oil extract was then analyzed for its fatty acid profile by using GC-MS.

Fatty acids profile

Analysis by GC-MS has high specificity when analyzing the fatty acid components contained in fats and oils and the molecular weight of each fatty acid (Djamaludin and Chamidah 2021). Detailed dry-rendering extraction and GC-MS chromatograms for the fatty acid content analysis of crude oil extract from by-products tuna are shown in Table 2 and Figure 6.

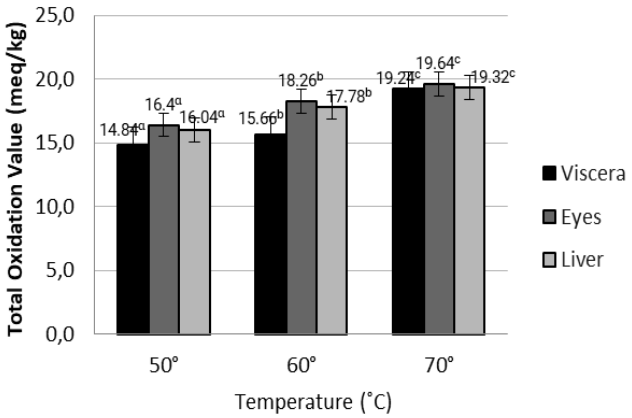

Figure 5. TOTOX value of crude oil extract of by-products of tuna. Different superscript letters in the charts show significant differences (P<0.05) at the 5% test level (significance test with Duncan test)

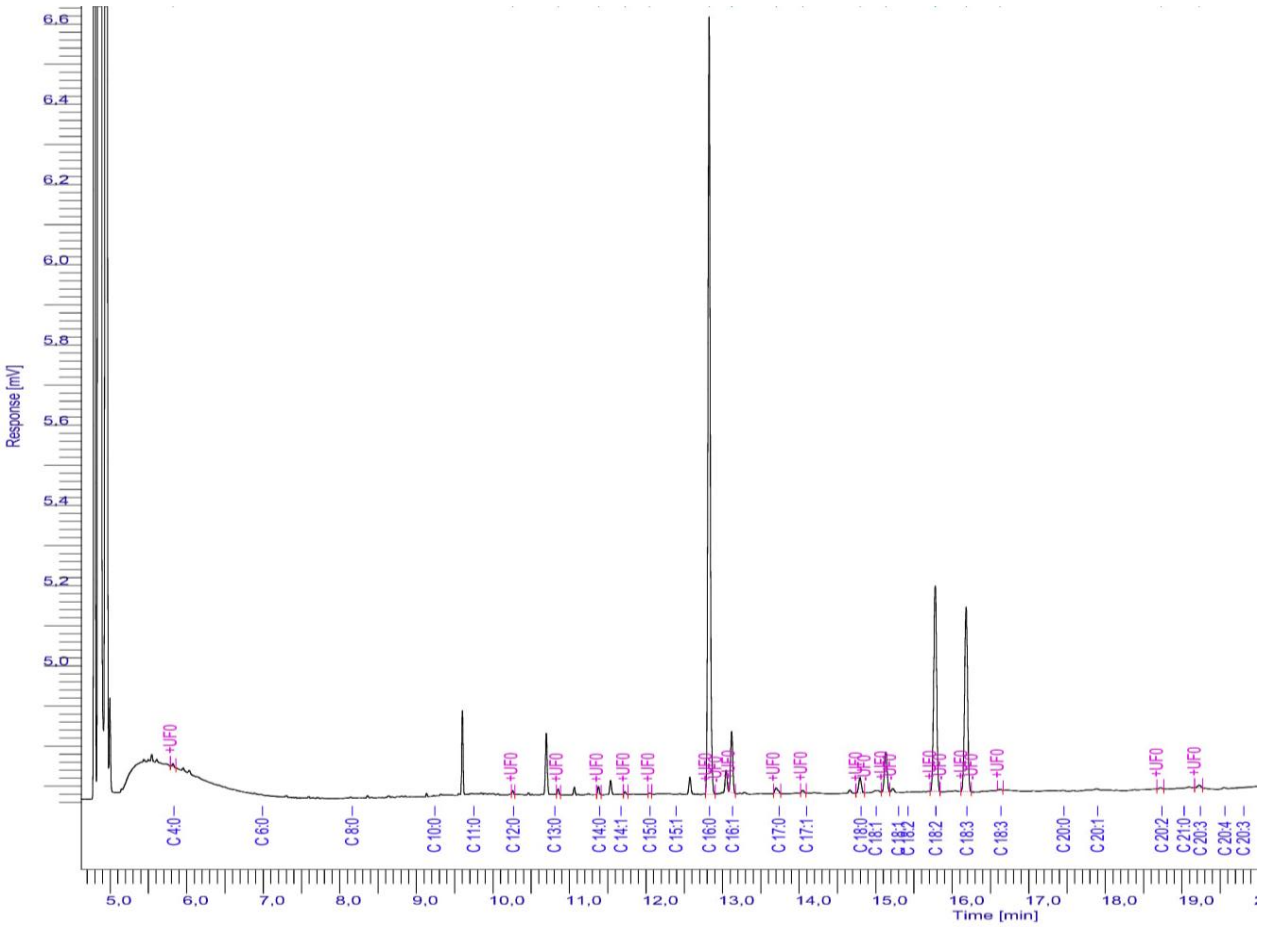

Figure 6. The GC-MS chromatogram of crude oil extract from tuna by-products

Table 2. The fatty acid components of crude oil extract from tuna by-products

Saturated Fatty Acid (SFA)	Result
Butyric acid (C4:0)	0.15%
Lauric Acid (C12:0)	0.16%
Tridecanoic Acid (C13:0)	0.29%
Myristic Acid (C14:0)	0.39%
Palmitic Acid (C16:0)	33.35%
Heptadecanoic Acid (C17:0)	0.54%
Stearic Acid (C18:0)	1.30%
Total of SFA	36.18%
Mono-Unsaturated Fatty Acid (MUFA)	
Palmitoleic Acid (C16:1)	6.36%
Heptadecanoic Acid (C17:1)	13.00%
Oleic Acid/ω-9 (C18:1)	15.83%
Total of MUFA	35.19%
Poly-Unsaturated Fatty Acid (PUFA)	
Linoleic Acid/ω-6 (C18:2)	4.17%
Linolenic Acid/ω-3 (C18:3)	2.19%
Eicosapentaenoic Acid (EPA)/ω-3 (20:5)	7.70%
Docosahexaenoic Acid (DHA)/ω-3 (22:6)	5.88%
Total of PUFA	19.94%

By and large, fatty acid profile analysis is needed to determine the fatty acid content contained in the crude oil extract of tuna by-products, which includes saturated fatty acids (SFA), namely fatty acids without double bonds, mono-unsaturated fatty acids (MUFA), namely fatty acids with a single, double bond, and poly-unsaturated fatty acids (PUFA), namely fatty acids with multiple double bonds. Fatty acid profile of the crude extract of tuna by-product oil resulting from extraction at a temperature of 50°C, where extraction results at 50°C obtained a fatty acid profile of SFA>MUFA>PUFA. This is based on research by Homayooni et al. (2014) in the extraction of sardines, in that the highest SFA levels were obtained at extraction temperatures of 50-60°C. Figure 6 depicts variations in the fatty acid composition of the three tuna by-product samples. The findings of this study showed that the dominant type of SFA was palmitic acid at 33.35%, MUFA was oleic acid at 15.83%, and PUFA was EPA at 7.70%. Several types of marine fish are known to have SFA content ranging from 4.84% to 46.79%, MUFA content ranging from 9.71% to 21.31%, and PUFA content ranging from 4.6% to 31.90% (Kandyliary et al. 2020). Similar results were also reported by Luczynska et al. (2014), who obtained SFA content ranging from 18.53% to 28.72%, MUFA content ranging from 17.95% to 49.89%, n-6 content ranging from 2.40% to 11.51%, and n-3 content ranging from 18.74% to 45.42%. PUFA type is dominated by DHA of 5.88% and EPA of 7.70%. Similar results were also found in previous research, which reported that the n-3 content in marine animals (green tiger prawn, European squid, gilthead seabream, shi drum, and john dory) was around 7.11% to 38.70% (Durmus 2019). The type of PUFA that predominates in seawater fish is n-3 (Devadason et al. 2016). The presence of EPA and DHA in fish oil from tuna by-products: viscera, eyes, and liver shows the potential for processing it into raw materials for producing fish oil with high benefits, such as immunostimulant agents. Omega-3 fatty acids can be converted to the precursors of anti-inflammatory mediators such as resolvins and protectins, EPA and DHA. These mediators stimulate macrophage phagocytosis for removing apoptotic cells, enhance the sorting of inflammatory chemokines, enhance neutrophil infiltration, and lower inflammation (Rehman et al. 2016).

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