p-ISSN: 1907-8056
e-ISSN: 2527-5410
AGROINTEK
Jurnal Teknologi Industri Pertanian
journal homepage: journal.trunojoyo.ac.id/agrointek

PHYSICOCHEMICAL AND FATTY ACID PROFILE OF FISH OIL FROM RED SNAPPER HEADS (*Lutjanus malabaricus*) REFINED FROM VARIOUS NaOH CONCENTRATIONS

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Article history
Received:
8 July 2021
Revised:
10 September 2021
Accepted:
14 September 2021

Keyword
Red snapper; heavy metal; fish oil; neutralization; peroxide.

ABSTRACT

The red snapper fillet industry only uses 40–50% of its fish meat; the rest becomes waste. Red snapper fillet waste can be used as raw material for fish oil rich in Polyunsaturated Fatty Acid through the extraction process. Extracted fish oil quality can be improved through a neutralization process. This research aims to determine the degree of Baume NaOH to produce fish oil with the best physicochemical properties. This study used a simple completely randomized design with three replications. The results showed that the difference in the degree of Baume NaOH significantly affected all characteristics of fish oil except yield and density. The neutralization process with the addition of NaOH 16°Be effectively improves the quality of fish oil and can maintain the unsaturated fatty acid content of fish oil. Neutralized fish oil has the characteristics of an acid value of 2.67 mg KOH/g, a peroxide value of 0.79 meq/kg, a p-anisidin value of 1.34 meq/kg, and a total oxidation value of 2.90 meq/kg. Identification of fatty acids and heavy metal contamination in fish oil before and after neutralization showed that the dominant fatty acid in fish oil was in the form of saturated fatty acids (50.58-67.51%) and heavy metal contamination in the form of arsenic 3.61-4.07 ppm.

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DOI 10.21107/agrointek.v15i4.11098

INTRODUCTION

Fish is a rich food in unsaturated fatty acids, omega-3 fatty acids (Abbas, 2009). One type of fish that is rich in unsaturated fatty acids is red snapper (*Lutjanus malabaricus*). Red snapper is a demersal fish with a high economic value widely caught in Indonesian waters (Prisantoso, 2017). Based on statistical data from the Ministry of Marine Affairs and Fisheries (2020), snapper production in Indonesia reached 351,107 tons/year.

The red snapper industry currently developing is the red snapper fillet industry (Rostini, 2013). In the red snapper fillet processing industry, the edible portion of fish meat is only 40–50%, and the rest is waste in the form of heads, gills, entrails, bones, fins, skin, and scales (Ifa, 2018). One of the efforts to utilize fishery industry waste can be made by processing waste into fish oil (Apituley, 2020). According to Suseno (2013), fish oil produced from waste or processing by-products has a dark color, higher peroxide value, and high free fatty acid content.

Extracted fish oil is a mixture of several compounds such as glycerides, free fatty acids, phospholipids, sterols, tocopherols, pigments, and toxic substances such as heavy metals (Suseno, 2012). Putri (2020) reported that fish oil extracted from the head of red snapper using the wet rendering method for 1 hour at a temperature of 80°C has a yellowish cloudy color characteristic. The color analysis of that fish oil was as follows : brightness level (L*) 43.5, redness level (a*) -0.9, and yellowness level (b*) 2.7. In addition, the extracted red snapper head fish oil also has a peroxide value of 0.98 meq/kg and a free fatty acid content of 1.02%. The relatively high levels of free fatty acids and peroxides can inhibit the utilization of oil. Therefore, it is important to refining fish oil to remove impurities such as non-triglyceride compounds, dyes, odors, and toxic substances to produce high-quality oil that is fit for consumption.

Extracted fish oil is a mixture of several compounds such as glycerides, free fatty acids, phospholipids, sterols, tocopherols, pigments, and toxic substances such as heavy metals (Suseno, 2013). Several studies regarding the purification of fish oil include purification of fish oil by-product of canning with alkali neutralization (Ratih, 2016) and purification of sardine fish oil by-product of fish canning with bentonite adsorbent (Suseno, 2013).

Several studies regarding the purification of fish oil include purification of fish oil by-products of canning by neutralization of alkali (NaOH and KOH) 10°Be, 14°Be, and 18°Be (Ratih, 2016) and purification of mackerel fish oil from fish meal processing by-product with alkali neutralization used NaOH 22°Be, 24°Be, and 26°Be (Feryana, 2014).

Several methods purification of fish oil can be done, including adding alkali and adsorbents to fish oil (Feryana, 2014). The common alkaline compound added in the neutralization step is NaOH (Bija, 2017). This study aims to determine the degree of Baume NaOH that can produce fish oil with the best physicochemical properties nature of 80°C has a yellowish cloudy color characteristic, with the results of the color analysis as follows: brightness level (L*) 43.5, level redness (a*) -0.9, and yellowness level (b*) 2.7. In addition, the extracted red snapper head fish oil also has a peroxide value of 0.98 meq/kg and a free fatty acid content of 1.02%. The relatively high levels of free fatty acids and peroxides can inhibit the utilization of oil. Therefore, it is crucial to refining fish oil to remove impurities such as non-triglyceride compounds, dyes, odors, and toxic substances to produce high-quality oil that is fit for consumption.

METHODS

Materials

The raw material used in this research is the head of red snapper from PT. Inti Luhur Fuja Abadi, Pasuruan, East Java. The head of the red snapper used has a size ranging from 200-300 g/head. The chemicals used included aquadest, sodium hydroxide (NaOH), potassium hydroxide (KOH), hydrochloric acid (HCl), 96% ethanol, phenolphthalein indicator, glacial acetic acid, chloroform, saturated potassium iodide (KI), starch indicator, sodium thiosulfate (Na2S2O3) 0.05 N, p-anisidin, isooctane, 20% boron trifluoride (BF3), potassium hydroxide (KOH) 0.5 M in methanol, saturated sodium chloride (NaCl), anhydrous sodium sulfate (Na2SO4), hexane, nitrogen (N2) gas, saturated nitric acid (HNO3), and standard Ge, In, Bi, Rh 10 mg/L.

Equipments

The equipments used in this study were freezer, gas stove, steam pan, stainless basin,

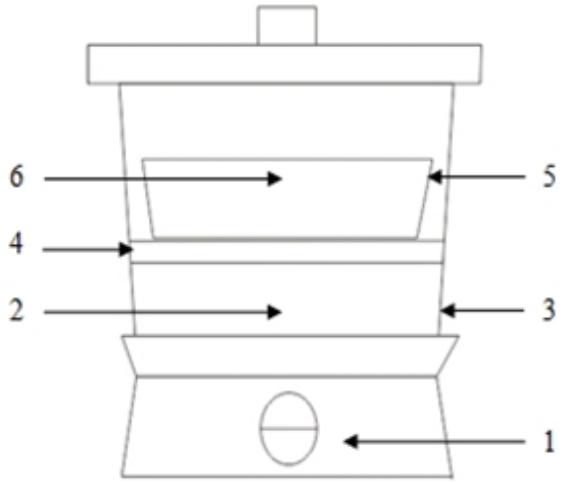
cutting board, knife, stainless spoon, filter cloth, aluminum foil, plastic, spatula, measuring cup, Erlenmeyer, dropper pipette, measuring pipette, filler, beaker glass, measuring flask, glass bottle, thermometer, set of back cooler, stir bar, centrifuge, analytical balance, hot plate stirrer, titration kit, water bath shaker, vortex, colorimeter, UV-Vis spectrophotometer, a set of GC-FID and ICP-MS analyzers.

Pretreatment

The red snapper head waste was cleaned, cut to 5x5x5 cm to optimize oil production during extraction, and weighed 500 g. After that, the snapper head waste was stored in a frozen condition until the time of extraction.

Fish Oil Extraction (Wet Rendering Method)

Fish oil extraction was carried out based on the Putri (2020) method. The red snapper head that has been prepared is placed in a stainless steamer, 500 mL of distilled water has been added as a heat-conducting medium. The extraction process was carried out using hot steam at 80°C for 1 hour. The extracted oil will later be accommodated in a stainless basin in the frying pan. Then the fish oil was centrifuged at 6000 rpm for 5 minutes.



Description :
1. Gas Stove
2. Aquadest
3. Pan
4. Pan divider
5. Bowl
6. Fish Head

Figure 1 Red Snapper Oil Extraction Scheme

Fish Oil Neutralization

Fish oil neutralization was carried out by referring to the Feryana (2014) method with modifications. First, the extracted fish oil was weighed as much as 25 g and then added with NaOH 10°Be, 12°Be, 14°Be, and 16°Be following the predetermined amount. Then it was heated for 30 minutes at 50°C. Then the fish oil was centrifuged at 6000 rpm for 25 minutes.

Analysis of Physicochemical Properties of Fish Oil

Crude oil and neutralized oil were analyzed for physicochemical properties, including yield analysis (Haris, 1983), density (Chew, 2016), peroxide value (AOCS, 1998), free fatty acids (AOCS, 1998), acid value (AOCS, 1998), saponification value (AOAC, 2000), ester value (Hutami, 2015), p-anisidin value (AOCS, 1998), and total oxidation (AOCS, 1998).

Fish Oil Color Analysis

Fish oil color analysis was carried out based on the Suseno (2012) method using a color reader. The results are expressed as L*, a*, b*, C*, and h* values. The value of C* or chroma results from the calculation of the equation C* = [(b*2 + a*2)1/2]. While the value of h* or hue results from the calculation of the equation h = [tan−1 (b*/a*)].

Determination of Best Treatment

The best treatment in this study was determined by selecting the treatment that required the lowest degree of baume NaOH to produce quality characteristics of fish oil following the WHO-Codex Alimentarius Commission standards.

Fish Oil Fatty Acid Profile Analysis

Analysis of the fatty acid profile of fish oil was carried out based on the AOCS (1993), AOAC (2000), and Ratnayake (2006) methods. Fish oil of 2-3 g was prepared and then methylated by adding 1.5 mL of 0.5M KOH solution in methanol into a 10 mL screw tube. The solution was heated to a temperature of ± 100°C and cooled to room temperature, then added 20% BF3 in methanol, then heated again at a temperature of ± 100°C. Then the solution was cooled and shaken until the solution temperature was around 30°C, and saturated NaCl and hexane were added, then vortexed for ± 2 minutes. After forming two layers, transfer the organic phase layer to a 2 mL tube containing anhydrous Na2SO4 and let stand for 15 minutes. Finally, the solution was put into a 2 mL vial. Detection of the fatty acid content of the oil was carried out using Perkin Elmer's GC-FID. The inlet temperature is set at 225°C. A sample of 0.1µL was injected into the GC-FID

device operated using a Supelco SP2560 column with a column length specification of 100 m, a diameter of 0.25 mm, and a thickness of 0.2 m. The carrier gas used is N2, with a flow rate of 18 cm/second. The oven is set at 240°C with a temperature rise rate of 2.5°C/min. FID's detection results can show the types of fatty acids in fish oil, including saturated fatty acids, unsaturated fatty acids, MUFA (Monounsaturated Fatty Acid), PUFA (Polyunsaturated Fatty Acid), and total fatty acids in the sample. In addition, chromatogram detection results can be used to measure the levels of each fatty acid.

Heavy Metal Pollution Analysis

Analysis of fish oil heavy metal contamination was carried out based on the AOAC (2014), AOAC (2015), and Creed (1994) methods. First, fish oil as much as 0.5-1.5 mL is put into the vessel. Then add 10 mL of concentrated HNO_3, let stand for 15 minutes. After that, close the vessel and digest it in a microwave digester (150˚C for 10 minutes), then hold at a temperature of 150˚C for 15 minutes. The results of digestion are cooled, then put into a 50 mL volumetric flask. Rinse the vessel with aquadest quantitatively, combine the rinse results with the destruction results in a 50 mL volumetric flask. Next, add 0.4 mL of the standard internal mixture of Ge, In, Bi, Rh 10 mg/L and dilute with distilled water to the mark, homogenize. The solution was then filtered through a 0.20 m RC/GHP filter. Measure the intensity of the sample solution in the ICP MS system. As analytes with Ge standard internal, Hg and Pb analytes with Bi standard internal, Cd and Sn analytes with In standard internal.

Statistical Analysis

All experiments were performed in three replications with analysis of variance (ANOVA) (SPSS Inc, Version 22) performed with a completely randomized design. Duncan's test ($p < 0.05$) was used to detect differences among mean values offish oil properties in all test intervals.

RESULTS AND DISCUSSIONS

Yield

The addition of NaOH solution with different Baume degrees gave no significant effect ($p>0.05$) on the yield of neutralized fish oil. However, the yield of fish oil after neutralization in this study was higher than that of Suseno (2015) study, where sardine fish oil from industrial by-products after going through the neutralization process used a 16-22°Be NaOH solution at a temperature of 40-90°C for 10 minutes has a yield of 59.60-89.03%.

Table 1 Yield Neutralized Oil

Treatments		Yield (%)
Neutralization (NaOH)	10°Be	94.70[a]
	12°Be	94.06[a]
	14°Be	91.37[a]
	16°Be	90.56[a]

Description: Different letters in the same column showed a significant difference ($p<0.05$)

The amount of non-oil fraction content influences neutralized fish oil yield. According to Hastarini (2012), purification processes such as neutralization cause the loss of impurity compounds in fish oil, resulting in neutralization resulting in decreased oil weight. In the process of neutralization or alkali refining, selecting the type of alkali, concentration, amount of alkaline solution, and the separation technique between the saponified fraction and the unsaponified fraction are essential factors affecting the yield of fish oil purification (Estiasih, 2012). The slight difference in Baume degrees of NaOH solution and high process efficiency may cause the yield of the neutralization process to be not significantly different.

Oil Density

The density of extracted fish oil and after neutralization in this study is similar to the research conducted by Lamas (2019), where the density of extracted *Zearaja flavirostris* fish liver oil was 1.08 g/cm^3, and the density of *Zearaja flavirostris* fish liver oil after going through the purification process decreased to 0.96 g/cm^3.

The addition of NaOH solution with different degrees of Baume gave no significant effect ($p<0.05$) on the density of neutralized fish oil. The density value of fish oil is influenced by the components contained in the sample (Bija, 2017). The high-density value of red snapper head fish oil extracted and after neutralization was caused by the dominant palmitic fatty acid content of 29.87% and 34.97% (Table 4). The palmitic fatty acid has a molecular weight of 256 g/mol and is classified as a long-chain fatty acid composed of sixteen carbon chains (Tuminah, 2009). According to Istiqlaal (2018), fish oil with a dominant extended

carbon chain fatty acid component will have a relatively more significant molecular weight.

Free Fatty Acid

Testing free fatty acids in fish oil aim to determine how much damage the oil has due to the hydrolysis process (Putri, 2020). The addition of NaOH solution with different Baume degrees gave significant results ($p<0.05$) on the free fatty acids of neutralized fish oil.

The free fatty acid content of fish oil before neutralization was 3.53% (Table 2). After going through the neutralization process, it decreased to 1.22% (Table 2). The free fatty acids of fish oil extracted and after neutralization in this study in line with research conducted by Ratih (2016). In that research, the lemuru fish oil before the neutralization process had free fatty acids of 4.66%. After the neutralization process used a temperature of 70°C by adding 10-18°Be NaOH solution, the free fatty acids of lemuru oil decreased to 0.89-1.40%.

Red snapper head fish oil decreased the value of free fatty acids. The higher the Baume degree or the concentration of NaOH solution added to the oil due to the NaOH solution will remove the free fatty acid content in fish saponifying the impurity compounds in the oil. So that the higher the Baume degree of the NaOH solution added, the more free fatty acids will be saponified, and the levels of free fatty acids in fish oil will be lower than before neutralization.

Acid Value

The acid value describes the amount of free fatty acid content in the oil formed due to the hydrolysis reaction of triglycerides (Panagan, 2011). Statistical analysis showed that the addition of NaOH solution with different degrees of Baume gave results that had a significant effect ($p<0.05$) on the acid value of fish oil. The acid value of red snapper head fish oil extracted initially was 7.74 mg KOH/g. After going through the neutralization process, it decreased to 2.67 mg KOH/g (Table 2). The changed in acid value after neutralization was caused by the acid value of fish oil which positively correlates with the value of free fatty acids (Feryana, 2014).

The results of the research on red snapper head fish oil after neutralization with the addition of NaOH 16°Be in this study were following the fish oil standard by the WHO-Codex Alimentarius Commission, which stated that the maximum limit for the acid number in fish oil was no more than 3 mg KOH/g to be declared suitable for consumption, which is 2.67 mg KOH/g.

The red snapper head fish oil in this study had a lower value than that one in the study of Ratih (2016) research, whose the mackerel fish oil by-product of flouring before the neutralization process had an acid value of 31.52 mg KOH/g. However, after the neutralization process used a temperature of 60°C for 30 minutes by adding 22°Be, 24°Be, and 26°Be NaOH solutions, the acid value of fish oil decreased to 3.84-5.32 mg KOH/g.

Table 2 Physicochemical Characteristics Neutralized Oil

Parameter	Crude Oil	Neutralization (NaOH)				(WHO, 2017)
		10°Be	12°Be	14°Be	16°Be	
Free Fatty Acid (%)	3.53^{c}	2.14^{b}	1,62^{ab}	1,47^{ab}	1,22^{a}	
Peroxide Value (meq/kg)	3.52^{b}	1.24^{a}	1,18^{a}	0,99^{a}	0,79^{a}	≤ 5,00
p-Anisidine Value (meq/kg)	0.46^{a}	1.13^{b}	0,41^{a}	0,64^{a}	1,34^{b}	≤ 20,00
Total Oxidation (meq/kg)	7.65^{b}	3.68^{a}	2,77^{a}	2,64^{a}	2,90^{a}	≤ 26,00
Acid Value (mg KOH/g)	7.74^{c}	4.69^{b}	3,54^{ab}	3,22^{ab}	2,67^{a}	≤ 3,00
Saponification Value (mg KOH/g)	107.79^{c}	93.75^{b}	88,73^{ab}	83,24^{a}	85,63^{a}	
Ester Value (mg KOH/g)	100.06^{d}	89.06^{c}	85,19^{bc}	80,02^{a}	82,96^{a}	
Density (g/cm^{3})	1.10^{a}	1.09 ^{a}	1,08 ^{a}	1,01 ^{a}	1,04 ^{a}	

Description: Different letters on the same line showed a significant difference ($p<0.05$)

Table 3 Color properties Neutralized Oil

Treatment		L*	a*	b*	C*	h*
Crude Oil		48,0[a]	4,0 [a]	10,2[a]	11,0[a]	69,8[a]
Neutralization	10°Be	59,9[b]	5,9 [a]	25,3[b]	26,0[b]	76,9[b]
(NaOH)	12°Be	62,6[bc]	5,5 [a]	26,5[b]	27,1[b]	78,4[bc]
	14°Be	64,5[c]	4,2 [a]	27,7[b]	28,1[b]	81,5[bc]
	16°Be	62,6[bc]	3,3 [a]	25,7[b]	26,0[b]	82,6[c]

Description: Different letters in the same column showed a significant difference (p>0.05)

Peroxide Value

The neutralization process reduced the peroxide value of fish oil from 3.52 meq/kg to 0.79 meq/kg (Table 2). This is because peroxide compounds are more polar than triglycerides, so that they are easily absorbed by the soap formed in the neutralization process. This condition makes the higher the Baume degree of the NaOH solution added, the more soap is formed in the fish oil, the more peroxide compounds are absorbed (Estiasih, 2012).

The addition of NaOH solution with different degrees of Baume gave significant results ($p<0.05$) on the peroxide value of the neutralized fish oil. Therefore, is study's results follow the fish oil standard by the WHO-Codex Alimentarius Commission, which states that the maximum peroxide value in fish oil is not more than 5 meq/kg to be declared fit for consumption which is 0.79-3.52 meq/kg.

These results align with the research conducted by (Suseno, 2017), where Siamese catfish oil from the by-product of processing before purification has a peroxide value of 4.94 meq/kg. After going through the neutralization process using a 12-16°Be NaOH solution at a temperature of 60 °C for 10 minutes, the peroxide value decreased to 2.32-4.27 meq/kg.

Saponification Value

The addition of NaOH solution with different degrees of Baume gave a significant effect ($p<0.05$) on the saponification value of fish oil resulting from neutralization. The saponification value of fish oil before neutralization was 107.79 mg KOH/g (Table 2). After going through the neutralization process, it decreased to 83.24 mg KOH/g (Table 2).

The higher the baume degree of the NaOH solution used, smaller the saponification value of fish oil. This is because the higher the concentration of the alkaline solution used, the double bond fatty acids will be saponified (Ratih, 2016). In addition, the low value of the saponification value is caused by the dominant fatty acid that composes fish oil is a palmitic fatty acid. Palmitic fatty acids have a molecular weight of 256 g/mol and are classified as long-chain fatty acids (Tuminah, 2009). The longer the carbon chain that makes up the dominant fatty acid in fish oil, the smaller saponification value (Panagan, 2011).

The results of the saponification value were much lower than the research conducted by Ratih (2016), where crude oil from lemuru fish had a saponification value of 112 mg KOH/g. After the neutralization process used a temperature of 70°C by adding 10-18°Be NaOH solution, the amount of saponification of lemuru oil decreased to 102-105 mg KOH/g.

Ester Value

The addition of NaOH solution with different degrees of Baume gave a significant effect ($p<0.05$) on the ester value of fish oil resulting from neutralization. The ester value in fish oil tends to decrease as the Baume degree of the NaOH solution increases (Table 2). In the neutralization process, the added alkaline compound can saponify the fatty acids of fish oil more than it should (Ratih, 2016). Excessive saponification can cause the number of fatty acids to decrease, thereby reducing the ester value.

p-Anisidin Value

The addition of NaOH solution with different Baume degrees gave significant results ($p<0.05$) on the p-anisidin value of neutralized fish oil. It was under fish oil standards by the WHO-Codex Alimentarius Commission (Table 2).

Testing the p-anisidin value in the red snapper head oil research is lower than the Feryana (2014) study, where the mackerel fish oil by-product of flouring before the neutralization process has a p-anisidin value of 19.19 meq/kg and after the neutralization process. Using a temperature of 60°C for 30 minutes by adding

22°Be, 24°Be, and 26°Be NaOH solutions, the p-anisidin value of mackerel fish oil decreased to 7.82 meq/kg, 14.31 meq/kg, and 19.71 meq/kg, respectively.

The p-anisidin value in red snapper head fish oil after neutralization showed higher yields than fish oil extracted. This condition is due to the relatively high content of unsaturated fatty acids in fish oil, namely 39.12% and 49.43% (Table 4). The extracted fish oil and, after neutralization, undergo an oxidation process that triggers the formation of secondary oxidation compounds. In addition, the neutralization process using heating also triggers the formation of secondary oxidation compounds (aldehydes and ketones) from primary oxidation compounds (hydrogen peroxide). According to Suseno (2013), the content of polyunsaturated fatty acids or polyunsaturated fatty acids (PUFA) in fish oil can react with oxygen, light, and heat during the purification and testing process to form secondary oxidation compounds in the form of aldehydes, ketones, and their derivatives from hydrogen peroxide.

Total Oxidation

The total oxidation value shows that the neutralization process can effectively reduce the total oxidation value in fish oil. The addition of NaOH solution with different Baume degrees gave significant results ($p<0.05$) on the total oxidation of neutralized fish oil. This is because neutralization can reduce fat oxidation products such as peroxide (Estiasih, 2012). Although the red snapper head fish oil increased the p-anisidin value, it did not give a more significant portion of the value compared to the peroxide value.

The results of this study are lower than those of Feryana (2014), where mackerel fish oil by-product of flouring before the neutralization process has total oxidation of 75.32 meq/kg and after the neutralization process uses a temperature of 60°C for 30 minutes by adding a solution of NaOH 22 °Be, 24°Be, and 26°Be total oxidation of mackerel fish oil decreased to 24.14 meq/kg, 25.53 meq/kg, and 26.93 meq/kg, respectively.

The test results of red snapper head fish oil before and after neutralization with the addition of four different degrees of Baume using NaOH solution are under the fish oil standard by the WHO-Codex Alimentarius Commission, which states that the maximum limit for total oxidation in fish oil is no more than 26 meq/kg to be able to declare fit for consumption, which is equal to 2.64-7.65 meq/kg.

Color Properties

The addition of NaOH solution with different degrees of Baume gave results that had a significant effect ($p<0.05$) on the values of L*, b*, C*, h* and did not significantly affect ($p>0.05$) the a* value of red snapper head fish oil (Table 2). The results of the analysis of the color value of red snapper head fish oil in this study are in line with the results of Huang (2010) research, where salmon oil before purification has a value of L*, a*, b*, C*, and h* respectively of 27. ,0; 4.2; 8.7; 9.6; and 63.9. Then after neutralization using a 12°Be NaOH solution at a temperature of 65°C for 30 minutes, the color values of L*, a*, b*, C*, and h* respectively became 38.5; 4.2; 31.5; 31.8; and 82.4.

The color in fish oil comes from natural pigments and compounds resulting from the degradation of natural dyes (Sabar, 2015). The color characteristics (L*, a*, b*, C*, and h*) in red snapper head fish oil tend to increase along with the higher the Baume degree of the NaOH solution. It was caused by the absorption of pigments such as chlorophyll and carotenoids by the results of saponification compounds during the neutralization process (Chew, 2020). This condition makes the higher the Baume degree of the NaOH solution added, and the more soap is formed in the fish oil, the more pigment is absorbed so that the value of color brightness increases.

According to Huang (2010), fish oil after neutralization, has a brighter and yellowish color than fish oil before neutralization. Sari (2016) states that refining fish oil will make the resulting oil free from impurity compounds to produce fish oil with a pleasant taste and smell, attractive color, and prolong the oil's shelf life before it is consumed used as a raw material in the industry.

Fatty Acids Profile

Extracted red snapper head fish oil contains 31 different types of fatty acids. While in fish oil, after neutralization, contained 7 different types of fatty acids (Table 7). Testing the fatty acid profile of fish oil extracted and after neutralization showed that the dominant fatty acid content was saturated fatty acid, namely 60.78% and 50.58% (Table 4). They were then followed by monounsaturated fatty acids (MUFA) of 20.56%

and 28.21% and polyunsaturated fatty acids (PUFA), of 18.65% and 21.22% (Table 4).

Red snapper head oil contains three types of omega fatty acids in the extracted red snapper head fish oil, namely omega-3 fatty acids, omega-6 fatty acids, and omega-9 fatty acids with an amount of 14.02%, respectively 0.06% and 10.40% (Table 4). While in red snapper head fish oil after neutralization, only two types of omega fatty acids were detected, namely omega-6 fatty acids (6.12%) and omega-9 fatty acids (22.57%) (Table 4). When the fish oil refining process takes place, it is suspected that oxidation occurs due to exposure to light and oxygen so that the unsaturated double bonds contained in it turn into saturated (Dari, 2017). In addition, the fish oil refining process can remove impurities and reduce water content and components other than oil so that the fatty acid composition increases.

Table 4 Fatty Acids Profile Neutralized Oil

Fatty Acids	Structure	Content (%)	
		Crude Oil	Neutralized Oil
Butyric acid	C 4:0	0.12	0.00
Caproic acid	C 6:0	0.10	0.00
Caprylic acid	C 8:0	0.45	0.00
Capric acid	C 10:0	0.37	0.00
Lauric acid	C 12:0	2.16	0.00
Tridecanoic acid	C 13:0	0.12	0.00
Myristic acid	C 14:0	7.53	6.52
Pentadecanoic acid	C 15:0	1.27	0.00
Palmitic acid	C 16:0	29.87	34.97
Heptadecanoic acid	C 17:0	2.10	0.00
Stearic acid	C 18:0	13.55	9.09
Archidic acid	C 20:0	0.92	0.00
Heneicosanoic acid	C 21:0	0.31	0.00
Behenic acid	C 22:0	0.91	0.00
Tricosanoic acid	C 23:0	0.46	0.00
Lignoceric acid	C 24:0	0.55	0.00
TOTAL SFA		60.78	50.58
Myristolic acid	C 14:1	0.35	0.00
Pentadecenoic acid	C 15:1	0.19	0.00
Palmitoleic acid	C 16:1	7.19	5.64
Heptadekenoic acid	C 17:1	0.66	0.00
Oleic acid	C 18:1	10.40	22.57
Eicocenoic acid	C 20:1	0.80	0.00
Nervonic acid	C 24:1	0.98	0.00
TOTAL MUFA		20.56	28.21
Linoleic acid	C 18:2	0.68	6.12
Eicosadienoic acid	C 20:2	0.46	0.00
Docosadienoic acid	C 22:2	0.12	15.11
Linolenic acid	C 18:3	0.12	0.00
Eicosatrienoic acid	C 20:3	0.37	0.00
Arachidonic Acid	C 20:4	3.14	0.00
Eicosapentaenoic Acid	C 20:5	3.04	0.00
Docosahexaenoic Acid	C 22:6	10.74	0.00
TOTAL PUFA		18.65	21.22
Total Omega 3 Fatty Acids		14.02	0.00
Total Omega 6 Fatty Acids		4.06	6.12
Total Omega 9 Fatty Acids		10.40	22.57

Table 5. Heavy Metal Contaminant Neutralized Oil

Treatment	Hg (ppm)	Cd (ppm)	Pb (ppm)	As (ppm)
(BSN, 2018)	0.10	0.10	0.10	0.10
Crude Oil	0.00	0.00	0.00	3.92
Neutralized Oil	0.00	0.00	0.00	4.07

The results of this study are in line with the results of Ayu (2019) research, which stated that there was a change in the composition of catfish oil after the neutralization process, namely fish oil before neutralization contained 24 types of fatty acids. After the purification process, it decreased to 7 fatty acids.

Heavy Metal Contaminant

In this study, red snapper head fish oil was detected to contain heavy metal contamination in the form of arsenic. Meanwhile, mercury, cadmium, and lead were not detected in fish oil (Table 5). Meanwhile, heavy metal contamination in the form of arsenic in fish oil after neutralization was detected at 4.07 ppm (Table 5). The results of the arsenic contamination test in extracted red snapper head fish oil were much lower than Huang (2010) study, where salmon oil extracted from processing by-products was detected to contain arsenic contamination of 6.78 ppm. While the arsenic contamination test in red snapper head fish oil after neutralization was much higher than Huang (2010) study, salmon oil after neutralization using NaOH 12°Be at a temperature of 65°C for 30 minutes was detected to contain arsenic contamination of less than 0.2 ppm.

Arsenic contamination in red snapper head fish oil after neutralization in this study was higher than research conducted by Huang (2010). This condition is because, in neutralizing red snapper head fish oil, the temperature used is 50°C, lower than research conducted by Huang (2010), which is 65°C. According to Radja (2021), the magnitude of the temperature affects the amount of kinetic energy in the reacting molecules. The greater the kinetic energy, the faster the molecules move to increase the probability of a practical collision. So the reduction rate of heavy metal contamination in this study is not better than the research results by Huang (2010).

Fish oil after neutralization also had a higher metal contamination than before neutralization due to a decrease in the weight of the oil during the neutralization process. This condition was supported by study of Hastarini (2012), which showed that the purification process such as neutralization causes the loss of impurity compounds in the neutralized fish oil, resulting in a decrease in the final weight of the oil compared to the initial weight of the oil. So that with the reduced weight of fish oil due to loss of impurities and with a fixed amount of arsenic metal contamination, the concentration of arsenic metal in the oil will be detected to increase.

The presence of heavy metal contamination in fish oil is most likely because the habitat or environment of red snapper, which is the raw material in the oil extraction process, has been polluted. According to Darmono (2008), heavy metals can accumulate in the body of fish through several ways, including respiration, food channels (biomagnification), and through the skin (diffusion). Metals are absorbed in fish flesh by the blood, which binds to blood proteins and then distributed throughout the body tissues. This is supported by Tumisem (2011) statement, which states that red snapper is a demersal fish that lives mainly at the bottom of the water and takes materials from the bottom of the water as a food source. Heavy metals have a density of 5 g/cm^3 or more (Supriyantini, 2015). According to Sarjono (2009), heavy metals have properties that are difficult to degrade, easily dissolved in water, deposited in sediments, and can accumulate in aquatic biota bodies.

Fish oil in this study is not in line with SNI 8467: 2018, which states that the maximum limit of heavy metal contamination in the form of mercury, cadmium, lead, and fish oil arsenic is not more than 0.1 ppm to be declared fit for consumption. The red snapper head fish oil extracted and the best neutralization results in this study were 3.92 and 4.07 ppm, respectively (Table 5).

CONCLUSIONS

These results indicate and verify that NaOH 16 Baume can help improve fish oil's physical and chemical quality. Polyunsaturated fatty acids

(PUFA) are highly valued today for their beneficial effects on health. Fish oil is a significant source of these fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The neutralization method affects the fatty acid profile of fish oil. From improving physicochemical properties, adding NaOH 16 Baume is the most effective because it is inexpensive and straightforward.

ACKNOWLEDGMENT

The author would like to thank the University of Muhammadiyah Malang, which financially supported this research through the PPI grant.

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