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Refining of tilapia (*Oreochromis niloticus*) viscera oil with different sodium hydroxide concentrations

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Abstract

Crude fish oil cannot be applied to various products or consumed because its characteristics did not meet the food safety standards. Thus, it needs a purification process using NaOH to reduce the value of free fatty acids. This study was performed to determine the effect of NaOH on the characteristics of Tilapia's viscera oil and to determine the best concentration. This study was an experimental laboratory using the Completely Randomized Design with different concentrations of NaOH 1%, 2% and 3% with triplication. Parametric data were analyzed by ANOVA followed by HSD, while non-parametric data were analyzed by Kruskal-Wallis and followed by the Mann-Whitney test. The difference in NaOH concentration indicated that the higher the NaOH concentration, the lower the value of free fatty acids, peroxide, p-Anisidine, and total oxidation. The results showed that the different concentrations of NaOH showed a significantly different effect (P<5%) on free fatty acids, peroxide number, p-Anisidine, total oxidation, and organoleptic. The best concentration of NaOH in this study was 2% with the value of free fatty acids, peroxide value, p-Anisidine, total oxidation, and organoleptic of 1.51%, 2.044 meq/kg, 9.50 meq/kg, 13.20 meq/kg, and 8.06; respectively.

1. Introduction

Tilapia is one of the high fishery commodities in Indonesia. According to the Ministry of Marine Affairs and Fisheries, the average distribution of aquaculture production and tilapia production is second after seaweed. In 2018, the production was around 1,169,144,54 tons and increased to 1,337,831,69 tons in 2019. In 2020, the temporary data was recorded at around 364,747,10 tons and was estimated to increase from the previous year (Directorate General of Aquaculture, 2021). The high production of tilapia encourages the growth of tilapia side products utilization, especially viscera. The viscera amounted to around 7.5-15% of fish weight, which was a by-product of the fish processing industry. Viscera contain polyunsaturated fatty acids, phospholipids, fat-soluble vitamins that have the potential to be used as fish oil (Martins *et al*., 2015; Mota *et al*., 2019). According to Villamil *et al*. (2017), viscera have high protein and lipid, including omega-3, squalene, and fat-soluble vitamins.

Fish oil is beneficial for the body because it is rich in nutrients, especially polyunsaturated fatty acids (PUFA), reducing the risk of coronary heart disease. The other content, omega-3, is perfect for developing brain systems and the sense of sight and growth and development in toddlers. According to Hsu *et al*. (2020), long-term consumption of EPA and DHA has been shown to positively impact patients with coronary heart disease, lowering blood cholesterol, especially LDL.

The oil is extracted by a rendering method. The result is crude fish oil which is then purified. The neutralization process is an oil refining process that removes free fatty acids by using caustic soda. According to Hidayat *et al*. (2019), neutralization separates free fatty acids from crude oil. The separation is done by reacting free fatty acids with bases to form soap. The commonly used base is caustic soda (NaOH) because it is cheap and more efficient.

Aqueous NaOH is proven to have better quality than other bases in the neutralization oil process (Purwasasmita *et al*., 2015). The quality of the neutralized oil is believed to be influenced by the concentration of NaOH. Santoso *et al*. (2018) explained that the neutralization process of red fruit oil with a NaOH of 1.25 N resulted in free fatty acid levels of 0.31% and yields ranging from 40-54%. There is a tendency that the higher the normality of the alkaline

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solution, the lower the level of free fatty acid produced. However, it does not affect the peroxide value of around 0.4 meq/kg. This study was conducted to determine the effect of NaOH concentration on the chemical and organoleptic characteristics of tilapia fish oil.

2. Materials and methods

2.1 Materials

The material used in this study was the viscera of tilapia (*Oreochromis niloticus*) obtained from PT. Aquafarm, Semarang, Indonesia. NaOH (Merck) and distilled water were obtained from PT. Bratachem, Semarang, Indonesia.

2.2 Oil purification

Oil purification was based on Ratih *et al*. (2016) with modification. The first process was degumming; around 50 g of crude fish oil was weighed and heated at 70°C, 15% warm distilled water was added from the volume of oil for 5 mins, then put into a separating funnel to separate the oil from the water and gum. Then boiled at 70°C, added NaOH 0.1N 1% (A), 2% (B), and 3% (C), respectively, and stirred for 10 mins to create the foam. Non-neutralized oil was used as a control (K). The oil was then put into a separatory funnel and distilled water with a 1:1 ratio, and then oil, soap, and water are separated. After that, it was centrifuged at 3000 rpm for 10 mins to separate the remaining soap and water.

2.3 Free fatty acid analysis

Free fatty acid analysis was performed following Association of Official Analytical Chemists (AOAC) (2007) by weighing and dissolving 10 g of the sample into 25 mL of 95% ethanol. Then the mixture was heated at a temperature of 40 for 10 mins and two drops of phenolphthalein indicator were added and shaken. The solution was then titrated with 0.05M NaOH until a pink colour appeared and did not disappear for 30 s. The free fatty acids percentage was calculated by multiplying the number of mL NaOH, the molarity of NaOH and the molecular weight of the oleic fatty acids (282.5) divided by the sample weight and multiplied by 100%.

2.4 Peroxide value analysis

Peroxide value analysis was performed following AOAC (2012) by weighing 5 g of the sample and adding 30 mL of glacial-chloroforms acetic acid solution (3:2). The mixture was then stirred until homogeneous using a magnetic stirrer. Then 0.5 mL of saturated KI solution was added and allowed to stand for 1 min while stirring. Then 30 mL of distilled water was added to the solution and titrated with 0.1 N $Na_2S_2O_3$ until the yellow colour almost disappeared. Then 0.5 mL of 1% starch solution was added and titrated again with 0.1 N $Na_2S_2O_3$ until the blue colour disappeared. The peroxide value is calculated by multiplying the ml and normality of $Na_2S_2O_3$ divided by the sample's weight and multiplied by 1000.

2.5 Anisidine value analysis

Anisidine value analysis was performed following Watson (1994) by preparing test solutions 1 and 2. Test solution one was prepared by dissolving 0.5 g of sample into 25 mL of trimethylpentane, then spectrophotometrically measured 350 nm. Test solution two was prepared by adding 1 mL of 0.25% p-anisidine solution into 5 mL of test solution 1, then shaken and allowed to stand for 10 mins under the dark. Test solution two was then calibrated at a wavelength of 350 nm.

$$Anisidine\ value = \frac{25 \times (1{,}2\ absorbance\ of\ solution\ 1 - absorbance\ of\ solution\ 2)}{Weight\ of\ Sample}$$

2.6 Total oxidation analysis

Total oxidation analysis was performed following Perrin (1996) by adding twice the peroxide value with the obtained anisidine number.

2.7 Sensory analysis

The sensory analysis was tested by thirty panellists semi-trained with a rating scale of 1-9 for oil turbidity, colour, and odour. A score of nine indicated a good oil by its bright and clean appearance, yellow colour, and solid odour (Badan Standardisasi Nasional, 2011).

2.8 Statistical Analysis

This research was performed in triplicate. Parametric data were analyzed using ANOVA. A further HSD test is performed if there is a significant difference, while non-parametric data is analyzed using the Mann-Whitney test.

3. Results and discussion

3.1 Oil quality after degumming

The oil quality after degumming was shown in Table 1. The free fatty acid in the oil after degumming was 2.47%. This value was considered high as the standard value for fish oil, according to the IFOS, was < 1.5%. The free fatty acid is formed due to the hydrolysis of triacylglycerol, which is catalyzed in water. The degumming process in this study was the addition of water, leading to a higher free fatty acid. According to Dominguez *et al*. (2019), free fatty acids are produced by hydrolysis and oxidation reactions combined with neutral fats. This reaction is accelerated in the presence of heat, water, acidity, and catalysts (enzymes).

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Table 1. Quality test after degumming.

Analysis	Results
Free fatty acid (%)	2.47±0.28
Peroxide value (meq/kg)	3.59±0.34
p-Anisidine (meq/kg)	9.82±0.77
Total oxidation (meq/kg)	14.57±0.64

Values are presented as mean±SD of triplicates.

After degumming, the peroxide value was 3.59 meq/kg and still below the IFOS standard of <5 meq/kg. The low peroxide value did not always indicate the oil was not appropriately oxidized because the slow oxidation rate indicated proper oil storage at low temperatures. According to Ayu *et al*. (2019), the low peroxide value can be caused by the rate of formation of new peroxide, and it is lower than the rate of degradation of peroxides into other compounds because peroxides rapidly degrade and react with other compounds.

The result of the p-Anisidine test on oil after degumming was 9.82 meq/kg. The anisidine value of the degummed oil met the IFOS standard, which was less than 20 meq/kg. Hydroperoxide compounds from the oxidation will immediately degrade into secondary products such as aldehydes and ketones, and the p-Anisidine test could detect these compounds (Ambindei *et al.,* 2020). The high peroxide value was in line with high p-Anisidine. The degumming process used heat could increase the peroxide value so did the p-Anisidine value. According to Kamini *et al*. (2016), p-Anisidine is one of the methods used to measure the level of secondary oxidation products (carbonyl compounds). A high peroxide value is followed by p-Anisidine thus the oil will undergo further degradation.

The total oxidation in the oil test after degumming was 14.57 meq/kg. The oil from the degumming process had low total oxidation, which is still below the IFOS standard of <26 meq/kg. Total oxidation was influenced by the results of the peroxide and p-Anisidine numbers. The total oxidation was calculated from the peroxide and p-Anisidine numbers. According to Mohammadi *et al*. (2013), oxidation increases linearly with the peroxide and p-Anisidine. The lower the total oxidation value, the better the quality of the oil. Total oxidation is an overall parameter of the oxidation state of the oil.

3.2 Viscera oil after neutralization

3.2.1 Free fatty acid

The results obtained in the free fatty acid test were 2.46% in sample K, and A had the highest free fatty acid of 1.86%. In contrast, samples B and C were 1.51% and 1.37%, respectively. The result showed that the concentration of NaOH in the neutralization process considerably affected the value of free fatty acids. According to Purwasasmita *et al*. (2015), alkali significantly decreases the free fatty acids levels and reacts to form soap. Rahayu *et al.* (2021) explained that in the neutralization process, a saponification reaction occurs between free fatty acids with NaOH or KOH to form salts of fatty acids. This salt formed is called soap.

The results of free fatty acids produced in this study after treatment were 1.37% to 1.86%. The results obtained were lower with the analysis of free fatty acids from mackerel fish oil of 1.93% to 2.68% with a concentration of NaOH 22^{O}Be (Feryana *et al*., 2014). The difference in the concentration of NaOH used in the neutralization process affected the free fatty acid levels. A high concentration of NaOH could increase soap precipitation. The precipitate was compact and bound water, thus reducing the water content in the oil. The reduced water content in the oil could reduce hydrolysis, which caused the formation of free fatty acids. According to Musyaroh and Hidayat (2018), the neutralization process with a high concentration of NaOH resulted in more deposits being formed. Soap that settles compactly will be easily separated, and the water content of the neutralizing oil is low.

Free fatty acids were related to the peroxide number, which was obtained from lipid oxidation. High free fatty acids led to the rancidity of the oil and were characterized by oil oxidation. According to Ayu *et al*. (2019), free fatty acids are produced from the hydrolysis process of triglycerides. Increasing the hydrolysis reaction rate would accelerate rancidity characterized by free fatty acid levels and high peroxide numbers.

The high value of free fatty acids would cause an unfavourable flavour and result in a less favourable smell and taste of the oil. It showed that the value of free fatty acids was related to the sensory parameters of fish oil. According to Moghanjoghi *et al*. (2015), free fatty acids are associated with an unpleasant flavour in the oil. Evaporation of free fatty acids will produce volatile compounds that have an unpleasant taste.

The free fatty acid of purified tilapia fish oil followed the Indonesian National Standards (SNI), in which samples A and B had less than 2%. Based on the Badan Standardisasi Nasional (2013), the maximum free fatty acid level in fish oil was 1 to 2%. It indicated that neutralized tilapia viscera oil was under the standard. International Fish Oil Standard (IFOS) explained that the free fatty acids are less than 1.5%. Thus, the neutralized tilapia viscera oil in the sample was lower than the IFOS standard.

3.2.2 Peroxide value

Table 2 shows that the highest peroxide value was

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obtained in sample K for 3.87%, while samples A, B, and C were 3.45 meq/kg, 2.04 meq/kg, and 1.64 meq/kg, respectively. The decrease in peroxide value after neutralization was due to the precipitation of the peroxide compound. The higher the concentration of NaOH used, the lower the peroxide value. As peroxide also precipitates with free fatty acids that form soap and separate from the oil. Peroxide compounds that have short carbon chains will immediately dissolve in water. During the neutralization process, some of the peroxides will dissolve in water and separate from the oil. Sharma *et al*. (2012) explained that the decrease in peroxides in the neutralization process is also due to peroxide compounds with short carbon chains that are soluble in water and caused by free fatty acids. A small amount of peroxide and free fatty acids is deposited, and some of the peroxides also precipitate.

The peroxide value in this study was 1.64 meq/kg to 3.45 meq/kg after treatment. The results were lower than the sardine fish oil, purified at 65°C for 20 mins for 3.23 meq/kg (Balance *et al*., 2019). The difference in interval and temperature used during purification will affect the number of peroxides produced. According to Bija *et al*. (2017), the increased peroxide level is due to the high temperature during purification, thus accelerating oxidation. Peroxide values were related to the number of hydroperoxides that significantly affect the quality of the oil.

Fish oil oxidation was affected by the presence of free fatty acids. The higher the free fatty acids, the higher the oxidation rate, thus the peroxide number increases. The high free fatty acids resulted from the increase in the oil's hydrolysis process, leading to oxidation thus the oil smells rancid. According to Kamini *et al*. (2016), increased hydrolysis can increase the potential for rancidity. Free fatty acids are produced due to the hydrolysis of triglycerides and oxidation of fatty acid double bonds.

The peroxide value was closely related to the p-Anisidine value as the hydroperoxides resulting from primary oxidation will undergo secondary oxidation. Hydroperoxide compounds will be degraded into simpler compounds such as aldehydes and ketones. The p-Anisidine test was carried out to determine the product of the secondary oxidation of oil in the form of aldehydes, ketones, and alcohol. According to Haq *et al*. (2016), peroxide measures the total hydroperoxide produced in primary oxidation. The primary oxidation products are then broken down to produce compounds in aldehydes and alcohols, secondary oxidation products. To determine the amount of secondary oxidation can be done with p-Anisidine.

The peroxide value of purified tilapia oil was less than 5 meq/kg. It indicated that the standards followed the peroxide value of neutralized tilapia viscera oil. IFOS explained that the peroxide level is less than 5.0 meq/kg, and tilapia viscera oil can be categorized as having a good peroxide value.

3.2.3 p-Anisidine

Based on Table 2, the highest p-Anisidine was found in samples A and K at 11.22 meq/kg and 11.04 meq/kg, respectively, while sample B was 9.50 meq/kg, and the lowest value was found in sample C at 8.92 meq/kg. The value of p-Anisidine was non-linear with the concentration of NaOH. It was because the hydroperoxide in the oil had decreased, also did the aldehydes and ketones. Feryana *et al*. (2014) explained that the analysis of p-Anisidine is a parameter to measure the secondary oxidation in oil. The p-Anisidine is a derivative of hydroperoxide compounds in primary oxidation that take shape in aldehydes and ketones. It causes changes in the odour of the oil and becomes a parameter of the rancidity of the oil.

The p-Anisidine test in this study was 8.92 meq/kg to 11.22 meq/kg. The results in this study were higher than the results of the neutralization of the by-product yellowfin tuna oil, which was 6.51 meq/kg. This difference is due to the NaOH concentration used for the oil neutralization process in yellowfin tuna, which is higher than that used in this study at 14.36% (Budiadnyani *et al.,* 2015). It indicates that the concentration of NaOH used affects the value of p-Anisidine.

The p-Anisidine value is related to the peroxide value. The p-Anisidine test is carried out to determine

Table 2. Quality of viscera oil.

Treatment	Free Fatty Acid (%)	Peroxide Value (meq/kg)	p-Anisidine (meq/kg)	Total Oxidation (meq/kg)
K	2.46±0.18^{a}	3.87±0.28^{a}	11.04±0.81^{a}	18.79±1.37^{a}
A	1.86±0.13^{b}	3.45±0.31^{a}	11.22±0.69^{a}	18.12±0.07^{a}
B	1.51±0.10^{c}	2.04±0.21^{b}	9.50±0.40^{ab}	13.20±0.50^{b}
C	1.37±0.06^{c}	1.64±0.13^{b}	8.92±0.84^{b}	11.97±0.22^{b}

Values are presented as mean±SD of triplicates. Values with different superscripts within the same column are statistically significantly different (P<0.05). K: Unneutralized tilapia oil, A: Tilapia oil with 1% NaOH, B: Tilapia oil with 2% NaOH, and C: Tilapia oil with 3% NaOH.

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the secondary oxidation, which continues the primary oxidation. As the oil oxidation process continues, hydroperoxides resulting from primary oxidation will decompose to produce secondary oxidation products such as aldehydes and ketones. This secondary oxidation product gives the effect of further damage to the oil, which will form a non-volatile compound. According to Rozi *et al*. (2016), p-Anisidine analysis is conducted to determine secondary oxidation, characterized by fat degradation initiated by hydroperoxides, resulting in a non-volatile carbonyl by-product. The average p-Anisidine value of purified tilapia oil is less than 20 meq/kg, which indicates that it was meet the standard.

3.2.4 Total oxidation

The average result of the total oxidation value test from highest to lowest was found on samples K, A, B, and then C of 18.79 meq/kg, 18.12 meq/kg, 13.20 meq/kg, and 11.97 meq/kg, respectively. The difference in the results of the three samples is because the total oxidation value was the sum of the test results of the oil oxidation parameters, such as peroxide number and p-Anisidine. According to Dari *et al*. (2017), total oxidation is the sum of the primary and secondary oxidation values (AnV and PV). Total oxidation is a parameter to analyze the primary and secondary oxidation of fish oil.

The total value of the lowest oxidation on research was 11.97 meq/kg. The results were higher than silky shark oil refining results using NaOH at a temperature of 50°C for 13.57 meq/kg (Ukthy and Rozi, 2016). The difference in the total oxidation value was due to differences in the sample and the temperature during refining. Different oil samples have different fatty acid content. High temperatures during refining can affect the quality of the oil because it will accelerate oil oxidation. According to Balance *et al*. (2019), total oxidation is a parameter used to determine the presence of compounds produced by fatty acid degradation. The compounds resulting from oxidation are caused by high temperature, oxygen, metal compounds, and light.

The total oxidation value was related to PV and p-Anisidine as an indicator of oil oxidation damage. The PV and p-Anisidine tests were used to determine the total fish oil oxidation value. The PV or peroxide number was linear with the total oxidation and the p-Anisidine value. Sample A has high PV, p-anisidine values, and total oxidation. In contrast, the PV and p-Anisidine values on sample C were lower than samples A and B, also the total oxidation value was low. According to Kusharto *et al*. (2015), the total oxidation value combines primary and secondary oxidation. Peroxide and p-Anisidine values indicate the oxidation state in the early and late stages. The total oxidation measures the hydroperoxide and its derivative products to be used as a parameter for the level of oil oxidation. The total oxidation value of purified oil was less than 26 meq/kg. It indicated that the total oxidation of purified tilapia viscera oil was met the IFOS standard.

3.3 Sensory

The sensory analysis of purified tilapia viscera oil determines whether the oil was under established standards. The results showed that each treatment had met the National Standard. The test included a sensory sheet for turbidity, colour, and odour with a rating scale of 1 to 9 with 30 panellists. The data were presented in Table 3.

3.3.1 Turbidity

Based on the result, the turbidity in sample A was significantly different from that of samples K, B, and C. The highest turbidity value was on sample A with 1% NaOH and the lowest on sample C with 3% NaOH. The higher the NaOH concentration, the lower the turbidity value, indicating a cloudier and less clear appearance. According to Bija *et al*. (2017), the low turbidity is due to soap stocks that contribute to oil turbidity. The high concentration of NaOH causes an overreaction in the formation of soap. Marrakchi *et al.* (2015) explained that a slight excess of caustic soda causes the continuation of the saponification reaction; thus, further treatment is needed to avoid caustic soda reaction with neutral fat, such as the bleaching process.

There are also various other ingredients in fish oil, such as unsaturated fatty acids that are easy to undergo primary and secondary oxidation. After all, oxidation products also affect the turbidity of the oil. According to Insani *et al*. (2017), primary and secondary oxidation

Table 3. Organoleptic test of viscera oil

Treatment	Turbidity	Colour	Odour	X
K	7.60±1.4^{b}	7.40±1.4^{b}	8.00 ±1.0^{a}	7.66±0.8^{b}
A	8.40±0.9^{a}	7.80±0.9^{ab}	8.33±0.9^{a}	8.17±0.5^{a}
B	7.40 ±0.9^{b}	8.46±0.8^{a}	8.33±0.9^{a}	8.06±0.6^{ab}
C	7.20±0.9^{b}	8.40±0.9^{a}	8.20± 0.9^{a}	7.93±0.5^{ab}

Values are presented as mean±SD of triplicates. Values with different superscripts within the same column are statistically significantly different (P<0.05). K: Unneutralized tilapia oil, A: Tilapia oil with 1% NaOH, B: Tilapia oil with 2% NaOH, and C: Tilapia oil with 3% NaOH

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products affect the colour and turbidity of fish oil. The content of primary and secondary oxidation products is high, and the appearance of the fish oil observed is getting darker.

3.3.2 Colour

Another organoleptic parameter was colour. The colour parameter shows that sample K with 0% NaOH was significantly different from samples B and C with 2% and 3% NaOH, respectively, but not significantly different from sample A with 1% NaOH. The colour of each sample met the quality requirements according to SNI, with the highest value in sample C with 3% NaOH and the lowest average value in the K sample with 0% NaOH.

The neutralization process influences the different colour parameters. NaOH reacting with oil makes the oil's colour yellower than before due to the alkaline reaction with peroxide compounds. According to Sari *et al*. (2016), this colour change is due to the action of alkaline compounds on the peroxide group or the combination of nitrogen compounds and oxidized lipids. Heating without an oxidation process on rancid oil could produce a yellow colour.

3.3.3 Odour

The odour parameter showed that K, A, B, and C samples were not significantly different. The odour of tilapia viscera oil was not significantly different from each sample, indicating that the oil's aroma was the same, which had a specific smell of fish oil and did not smell rancid. According to Widiyanto *et al*. (2015), fish oil has a distinctive odour specific to oil, and a slightly fishy smell. Good quality fish oil has a characteristic fishy odour specific for fish species but does not have a rancid smell.

4. Conclusion

The NaOH concentration affects the purified tilapia viscera oil. The higher the NaOH concentration, the lower the free fatty acid, peroxide value, p-Anisidine, and total oxidation value. 2% NaOH is the best concentration which produces free fatty acids, peroxide, p-Anisidine, total oxidation, and sensory value of 1.51%, 2.044 meq/kg, 9.50 meq/kg, 13.20 meq/kg, and 8.06, respectively.

Conflict of interest

The authors declare no conflict of interest.

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