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EFFECT OF DIFFERENT BENTONITE CONCENTRATIONS ON THE CHARACTERISTICS OF PURE TILAPIA (*OREOCHROMIS NILOTICUS*) FISH OIL

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ABSTRACT

Tilapia (Oreochromis niloticus) is a freshwater fish widely cultivated in Indonesian waters. Tilapia processing generates substantial byproduct waste, as only the flesh is typically utilized, leaving behind organs such as the head, bones, skin, and viscera. Tilapia viscera can be valorized as a source of fish oil. Fish oil is a fatty acid-rich product containing omega-3. Fish oil intended for human consumption must conform to food-grade standards through a multi-stage refining process. One critical stage in fish oil production is purification via bleaching. The bleaching process using bentonite aims to reduce free fatty acids and improve the color of the fish oil. This study aimed to determine the effect of bentonite concentration on the characteristics of tilapia fish oil and to identify the optimal concentration. An experimental laboratory method with a completely randomized design (CRD) was employed, using a single factor of varying bentonite concentrations (0%, 3%, 5%, and 7%) with three replications. Parameters evaluated included yield, peroxide value (PV), free fatty acid (FFA) content, p-anisidine value (p-AV), clarity, TOTOX value, slip melting point, and organoleptic assessment. Data were analyzed using ANOVA and Kruskal-Wallis tests. Results showed that yield values ranged from 65% to 100%; PV ranged from 3.30 to 7.69 meq/kg; FFA ranged from 1.20% to 2.50%; slip melting point ranged from 31.65°C to 34.96°C; p-AV ranged from 4.47 to 10.35 meq/kg; TOTOX value ranged from 12.43 to 25.74 meq/kg; clarity ranged from 62.33% to 73.00%; and the organoleptic confidence interval was $8.05 < \mu < 8.18$.

KEYWORDS: Tilapia; Fish Oil; Refining; Bentonite.

1. INTRODUCTION

Tilapia (Oreochromis niloticus) is a freshwater aquaculture commodity widely farmed throughout Indonesia. Tilapia is highly favored by consumers owing to its affordable price and relatively high nutritional value, which has contributed to steadily increasing consumption rates each year. According to data from the Ministry of Maritime Affairs and Fisheries (2018), tilapia production has risen annually—from 640,568 thousand tons in 2016 to 890,909 million tons in 2017, representing an increase of 39.08% over that period.

The high production volume of tilapia inevitably leads to a corresponding increase in processing byproducts. Since utilization of tilapia is largely limited to the flesh, substantial waste accumulates in the form of heads, fin bones, skin, and viscera. Viscera that are not properly managed can have adverse environmental impacts. According to Hildawianti et al. (2017), fish processing waste that is frequently left unutilized includes visceral offal, which contains high concentrations of protein and unsaturated fatty acids. One viable application for fish viscera is the extraction of fish oil.

Crude fish oil obtained through extraction typically contains numerous impurities that diminish its quality; therefore, a refining stage is essential. Fish oil refining is conducted to elevate its quality to meet the standards of the International Fish Oil Standard (IFOS). The refining process involves several stages: degumming, neutralization, and bleaching. In this study, the bleaching stage was carried out to remove pigments from the oil, thereby improving its clarity relative to oils produced by earlier refining methods, with the aim of increasing consumer appeal. Additionally, bleaching reduces the free fatty acid content of the oil, thereby preventing lipid oxidation. The bleaching process is carried out with the aid of an adsorbent—a solid substance capable of absorbing specific components from a fluid phase. Common adsorbents include activated carbon, bentonite, and zeolite, each possessing distinct adsorption characteristics. According to Polii (2016), bentonite offers several advantages as an adsorbent, including a sufficiently large surface area and a high capacity for swelling, making it particularly well-suited for this application. Bentonite was selected as the adsorbent in this study due to its superior adsorptive capacity.

The use of bentonite as an adsorbent in fish oil refining has been demonstrated by Hastarini et al. (2012), who reported that the addition of bentonite to fish oil not only improves color but also reduces other components such as undesirable aroma compounds, heavy metals, lipid

oxidation products (including peroxides, aldehydes, and ketones), free fatty acids, and phosphatide content.

Based on prior research by Sari et al. (2016), refining of fish oil using 1% bentonite at a specified heating temperature for 30 minutes yielded a peroxide value of 5.79 meq/kg and a free fatty acid value of 0.76%. It has been established that increasing bentonite concentration enhances the clarity of fish oil. The objective of the present study was to determine the effect of different bentonite concentrations on the yield, free fatty acid content, peroxide value, p-anisidine value, total oxidation value, clarity, slip melting point, and organoleptic characteristics of refined tilapia fish oil.

2. MATERIALS AND METHODS

2.1 Materials and Equipment

The primary raw material used in this study was tilapia viscera obtained from PT. Aquafarm. Equipment used included an oven, centrifuge, spectrophotometer, analytical balance, and water bath.

2.2 Extraction of Tilapia Fish Oil

Fish oil extraction followed the procedure described by Rozi et al. (2019). Tilapia viscera were cleaned and non-essential parts such as intestines were removed. The cleaned viscera were placed in an oven at 90°C for 1 hour. The extracted viscera were then separated into oil and solid fractions. The oil was centrifuged at 3,500 rpm for 10 minutes and stored in dark-colored bottles at low temperature.

2.3 Refining of Tilapia Fish Oil

The refining procedure followed Sari et al. (2016). Degumming was performed by adding 8% NaCl solution at a specified heating temperature for 15 minutes, followed by filtration. The subsequent neutralization step involved the addition of 1N NaOH at a concentration of 2%, with heating for 1 hour. Distilled water was added in a 1:1 ratio and the mixture was separated using a separatory funnel, followed by centrifugation at 3,000 rpm for 10 minutes. The bleaching step was performed by adding activated bentonite at concentrations of 0%, 3%, 5%, and 7%, with heating and stirring for 20 minutes, followed by centrifugation at 3,500 rpm for 10 minutes. The refined oil was stored in dark glass bottles at −18°C prior to analysis.

2.4 Yield Determination (Apituley et al., 2020)

Yield was determined as the ratio of the volume of oil obtained to the weight of the raw material, expressed as a percentage:

Yield (%) = (Volume of oil obtained / Weight of raw material) × 100%

2.5 Peroxide Value (AOAC, 2005)

The peroxide value was determined by iodometric titration based on the amount of iodine liberated from potassium iodide by peroxides, using sodium thiosulfate as the titrant and starch solution as the indicator. A 5 g sample was placed in a 250 mL Erlenmeyer flask and combined with 30 mL of acetic acid–chloroform solution (3:2 v/v), followed by 0.5 mL of potassium iodide (KI) solution. The mixture was gently swirled and 30 mL of distilled water was added. Titration was performed with 0.01 N sodium thiosulfate until the solution turned yellow. Subsequently, 0.5 mL of 1% starch indicator was added, turning the solution blue. Titration was continued with constant swirling until the blue color disappeared. The peroxide value was calculated as follows:

PV (meq/kg) = [(V sample − V blank) × N × 1000] / W

2.6 Free Fatty Acid (FFA) Determination (AOAC, 1995)

A 14 g fish oil sample was placed in a 250 mL Erlenmeyer flask and 25 mL of 95% ethanol was added, followed by heating. Two milliliters of phenolphthalein (PP) indicator was added and the sample was titrated with 0.1 N KOH until a persistent pink color appeared for at least 30 seconds. The FFA content was calculated using the following formula:

FFA (%) = [(V KOH × N KOH × M oleic acid) / (W sample × 1000)] × 100%

2.7 Slip Melting Point (AOCS, 2003)

Three glass capillary tubes (approximately 1 mm diameter) were immersed in pre-melted sample to fill to a height of approximately 1 cm. The filled capillary tubes were allowed to solidify at a specified temperature for 16 hours. The capillary tubes were attached to a thermometer with their tips aligned. The assembly was immersed in a beaker of water maintained below the anticipated slip melting point. The beaker was placed on a hotplate and temperature was raised gradually until the oil in the capillary tube became clear. The temperature at which the liquid in the capillary became clear was recorded as the slip melting point. Measurements were performed in triplicate.

2.8 p-Anisidine Value (AOCS, 1998)

Test solution 1 was prepared by dissolving 1 g of sample in 25 mL of trimethylpentane. Test solution 2 was prepared by adding 1 mL of p-anisidine solution (2.5 g/L) to 5 mL of test solution 1, mixing, and protecting from light. A reference solution was prepared by adding 1 mL of p-anisidine solution to 5 mL of trimethylpentane. Absorbance of test solution 1 was measured at 350 nm. Test solution 2 was measured at 350 nm exactly 10 minutes after preparation. The p-anisidine value (p-AV) was calculated as:

$$p\text{-}AV = 25 \times (1.2 \times A_2 - A_1) / W$$

2.9 TOTOX Value (AOCS, 1998)

The total oxidation value (TOTOX) was calculated by summing twice the peroxide value and the p-anisidine value:

$$TOTOX = 2PV + p\text{-}AV$$

2.10 Clarity (AOAC, 1995)

A cuvette was cleaned and filled with the reference standard, which was measured at 100% transmittance. The cuvette was then replaced with one containing the oil sample and clarity was measured as percent transmittance. Oil was diluted ten-fold by mixing 1 mL oil with 9 mL n-hexane as solvent. The wavelength used for clarity measurement was 450 nm.

2.11 Organoleptic Assessment (SNI 2346:2011, BSN 2011)

Organoleptic assessment was conducted using a score sheet to guide panelists in evaluating product quality according to defined quality grade specifications. A total of 30 semi-trained panelists participated. Data from the score sheets were tabulated and analyzed using non-parametric statistical methods at a 95% confidence level.

2.12 Statistical Analysis

Both parametric and non-parametric analyses were applied. Parametric data analysis was used for yield, peroxide value, and FFA results; where data indicated normality and homogeneity ($P > 5\%$), one-way Analysis of Variance (ANOVA) was applied. Where significant differences were detected, Honestly Significant Difference (HSD) post hoc tests were performed to determine differences between treatments.

3. RESULTS AND DISCUSSION

Results of the chemical characterization of refined tilapia fish oil with different bentonite concentrations are presented in Table 1. The bentonite concentrations applied were 0% (K), 3% (A), 5% (B), and 7% (C). As bentonite concentration increased, values for yield, PV, FFA, p-AV, slip melting point, and TOTOX all decreased, while clarity improved.

Table 1. Chemical characteristics of pure tilapia fish oil.

Parameter	K (0%)	A (3%)	B (5%)	C (7%)
Yield (%)	100.00 ± 0.00^{d}	84.00 ± 2.64^{c}	76.33 ± 3.21^{b}	65.00 ± 3.60^{a}
Peroxide Value (meq/kg)	7.69 ± 1.18^{c}	5.97 ± 0.65^{bc}	4.35 ± 0.72^{ab}	3.30 ± 0.12^{a}
FFA (%)	2.50 ± 0.14^{b}	2.24 ± 0.12^{b}	1.58 ± 0.11^{a}	1.20 ± 0.24^{a}
Slip Melting Point (°C)	34.96 ± 0.63^{c}	33.90 ± 0.56^{bc}	32.80 ± 0.80^{ab}	31.65 ± 0.15^{a}
p-Anisidine Value (meq/kg)	10.90 ± 0.65^{c}	9.20 ± 0.44^{bc}	8.11 ± 0.92^{b}	4.47 ± 0.73^{a}
TOTOX Value (meq/kg)	25.75 ± 3.49^{c}	21.13 ± 0.49^{bc}	17.39 ± 2.51^{b}	11.07 ± 0.55^{a}
Clarity (%)	62.33 ± 3.78^{a}	67.00 ± 2.64^{ab}	69.33 ± 4.04^{ab}	73.00 ± 2.64^{b}

Note: Data represent means of three replications ± standard deviation. Values followed by different superscript letters within the same row indicate significant differences ($p < 5\%$).

3.1 Yield

Yield results indicated a significant effect of bentonite concentration on oil yield. Yield values decreased progressively with increasing bentonite concentration. This trend is attributable to the capacity of bentonite to adsorb components present in the oil. According to Hastarini et al. (2012), the heating stages of the refining process can cause weight loss in the oil. The refining process removes impurities from the crude oil, thereby reducing the total oil mass following purification.

Yield is an important parameter in fisheries product processing, as it quantifies the efficiency of raw material utilization. Fish oil yield is influenced by the fat content of the fish. Higher fat content correlates with higher oil yield. According to Andhikawati (2020), fish oil yield is influenced by several factors including fat content, moisture content, and protein content. The fat content of fish is in turn influenced by feed composition.

3.2 Peroxide Value

Peroxide value results differed significantly across treatments. Based on normality and homogeneity tests, treatments K, A, and C were significantly different. The use of adsorbents in oil refining substantially affects PV because the adsorbent can absorb peroxide compounds within the oil. According to Rahayu and Purnavita (2014), the capacity of acid-activated bentonite to adsorb peroxide compounds is attributed to silanol groups (Si-OH) formed from SiO_2 in bentonite under acidic activation conditions.

Deterioration of fish oil quality is primarily caused by oxidation. Oxidation is driven by unsaturated fatty acids reacting with oxygen, and increased oxidation results in elevated peroxide values. Oxidation produces hydroperoxides, which cause rancidity in the oil. According to Suadi et al. (2017), oxidation occurs when oil is contaminated by air, leading to the formation of peroxides and hydroperoxides that produce rancid off-flavors.

3.3 Free Fatty Acid (FFA) Content

Results demonstrated that different bentonite concentrations significantly influenced FFA content. Higher bentonite concentrations reduced FFA values progressively. According to Anwar et al. (2016), the reduction in FFA is attributable to the adsorptive activity of activated bentonite through silanol groups (Si-OH) formed from SiO_2 during acid activation. The oxygen atom of the silanol group forms a hydrogen bond with the hydrogen atom of the carboxyl group of the free fatty acid, enabling adsorption onto the adsorbent surface and consequently reducing FFA levels.

3.4 Slip Melting Point

ANOVA results indicated significant differences ($P < 5\%$), leading to rejection of the null hypothesis. Treatment K differed significantly from treatments A, B, and C. Slip melting point is influenced by processing temperatures during the refining stages of degumming, neutralization, and bleaching. According to Efendi et al. (2020), the melting point of an oil is influenced by the degree of unsaturation and chain length of its fatty acids. Shorter chain lengths and higher degrees of unsaturation correspond to lower melting points.

3.5 p-Anisidine Value (p-AV)

Results showed that all treatments (K, A, B, and C) were significantly different from one another. Decreasing p-AV with increasing bentonite concentration reflects the reduction of hydroperoxide compounds. According to Sembiring et al. (2018), activated bentonite can

adsorb or reduce secondary lipid oxidation products including aldehydes, ketones, and alcohols, thereby lowering the p-anisidine value.

The p-anisidine test measures secondary oxidation in oil arising from primary oxidation reactions that generate non-volatile carbonyl by-products. Primary oxidation produces hydroperoxides, whereas secondary oxidation of oil produces aldehydes. According to Huli et al. (2014), the p-anisidine assay quantifies secondary oxidation products resulting from the decomposition of hydroperoxides into aldehydes and ketones.

3.6 TOTOX Value

TOTOX values differed among treatments and were derived from the combined peroxide value and p-anisidine value. According to Suseno et al. (2019), the total oxidation value is the sum of primary and secondary oxidation, calculated as two times the peroxide value plus the p-anisidine value. The TOTOX value represents both hydroperoxide content and its degradation products, providing a comprehensive measure of oil oxidation status.

TOTOX values were directly related to PV and p-AV results. Treatment K, which had the highest PV and p-AV, correspondingly exhibited the highest TOTOX value. Conversely, lower PV and p-AV values resulted in lower TOTOX values. According to Suseno et al. (2020), a high peroxide value combined with a high p-anisidine value indicates poor oil quality.

3.7 Clarity

Clarity results indicated that treatments K and C were significantly different, while treatments A and B were not significantly different. Clarity values increased with increasing bentonite concentration, as the adsorbent was effective in absorbing impurities and improving oil color. According to Marwati et al. (2015), increased clarity is attributable to the hygroscopic nature of bentonite, which absorbs water present in the oil. Dissolved water causes turbidity; upon absorption by bentonite, the oil becomes clearer. The greater surface area provided by higher bentonite concentrations allows more impurity adsorption and enhanced clarity.

3.8 Organoleptic Assessment

Organoleptic assessment of the refined tilapia fish oil with different bentonite concentrations was conducted for three parameters—turbidity, color, and odor—using a 1–9 scale with 30 panelists. The results are presented in Table 2.

Table 2. Organoleptic assessment results of pure tilapia fish oil.

No.	Treatment	Turbidity	Color	Odor	Confidence Interval
1	K (0%)	7.33 ± 1.18^{a}	7.20 ± 1.10^{a}	7.60 ± 1.19^{a}	$7.25 < \mu < 7.50$
2	A (3%)	7.40 ± 1.10^{a}	7.27 ± 1.46^{a}	8.00 ± 1.26^{a}	$7.54 < \mu < 7.77$
3	B (5%)	8.27 ± 0.98^{b}	8.13 ± 1.14^{b}	8.07 ± 1.01^{a}	$8.05 < \mu < 8.26$
4	C (7%)	8.20 ± 1.80^{b}	8.00 ± 1.02^{b}	8.07 ± 1.01^{a}	$7.99 < \mu < 8.18$

Note: Data represent means of three replications ± standard deviation. Values followed by different superscript letters within the same column indicate significant differences ($p < 5\%$).

For the turbidity parameter, treatment K was not significantly different from treatment A, while treatment A differed significantly from treatments B and C. Treatments B and C were not significantly different. The highest mean turbidity score was recorded for treatment C, while the lowest was observed for treatment K (0% bentonite). Increasing bentonite concentration progressively improved turbidity scores, reflecting enhanced clarity of the oil.

For the color parameter, treatment K was not significantly different from treatment A, while treatments B and C were significantly different. The highest mean score was recorded for treatment B, and the lowest for treatment A. The addition of adsorbent during bleaching improves oil color. According to Sumartini et al. (2019), the refining process effectively removes pigments from oil, which are adsorbed onto the adsorbent surface during heating.

For the odor parameter, statistical analysis indicated no significant differences between treatments K, A, B, and C. The addition of bentonite did not significantly alter odor characteristics. According to Sabar et al. (2015), oil that has undergone refining exhibits a less pungent odor, attributable to polar components in the adsorbent that bind odor-causing (off-flavor) compounds.

4. CONCLUSIONS

The addition of bentonite during the bleaching stage at concentrations of 0%, 3%, 5%, and 7% significantly influenced the characteristics of refined tilapia fish oil. Increasing bentonite concentration progressively reduced yield, free fatty acid content, peroxide value, p-anisidine value, and total oxidation value, while improving oil clarity and organoleptic characteristics.

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6. REFERENCES

1. Apituley, D. A. N., Sormin, R. B. D., and Nanlohy, E. E. E. M. (2020). Characteristics and Fatty Acid Profile of Fish Oil from the Head and Bones of Tuna (Thunnus albacares). Jurnal Teknologi Pertanian, 9(1), 10–19.
2. Association of Official Analytical Chemists. (2005). Official Methods of Analysis (18th ed.). AOAC International, Washington, DC.
3. Association of Official Analytical Chemists. (1995). Official Methods of Analysis (16th ed.). AOAC International, Arlington, VA.
4. American Oil Chemists' Society. (1998). Official Methods and Recommended Practices of the AOCS (5th ed.). AOCS Press, Champaign.
5. American Oil Chemists' Society. (2003). Official Methods and Recommended Practices of the AOCS (5th ed.). AOCS Press, Champaign.
6. Badan Standardisasi Nasional. (2013). Standar Nasional Indonesia (SNI) 2730.1:2013. Minyak Hati Ikan Cucut Botol (Crude Shark Liver Oil) – Bagian 1: Spesifikasi. Jakarta: BSN.
7. Badan Standardisasi Nasional. (2011). Petunjuk Pengujian Organoleptik dan atau Sensori pada Produk Perikanan SNI 2346:2011. Jakarta: BSN.
8. Efendi, S. C., Anggo, A. D., and Wijayanti, I. (2020). Effect of Extraction Temperature in the Dry Rendering Method on the Quality of Crude Liver Oil of Manyung Fish (Arius thalassinus). Jurnal Ilmu dan Teknologi Perikanan, 2(1), 64–69.
9. Hastarini, E., Fardiaz, D., Irianto, H. E., and Budijanto, S. (2012). Characteristics of Fish Oil from Processing Waste of Pangasius Catfish (Pangasius hypopthalmus) and Jambal Catfish (Pangasius djambal) Fillets. Agritech, 32(4), 403–410.
10. Hildawianti, Tiwow, V. M. A., and Abram, P. H. (2017). Analysis of Nitrogen (N) and Phosphorus (P) Content in Tilapia (Oreochromis mosambicus) Visceral Waste from Lake Lindu. Jurnal Akademik Kimia, 6(3), 148–153.
11. Polii, F. F. (2016). Purification of Coconut Oil from Smoked Copra Using Activated Carbon and Bentonite Adsorbents. Jurnal Riset Industri, 10(3), 115–124.
12. Rahayu, L. H., and Purnavita, S. (2014). Effect of Temperature and Adsorption Time on the Physicochemical Properties of Used Cooking Oil Refined Using Aren Starch Residue and Bentonite Adsorbents. Jurnal Ilmiah Momentum, 10(2), 35–41.
13. Rozi, A., Suseno, S. H., and Jacoeb, A. M. (2016). Extraction and Characterization of Pisang Shark Liver Oil. JPHPI, 22(3), 414–422.

14. Sabar, J., Fatimah, F., and Rorong, J. A. (2015). Characterization of Fish Oil from Refined Tuna Processing Waste Using Zeolite by Column Chromatography. Jurnal MIPA, 4(2), 161–164.
15. Sari, R. N., Utomo, B. S. B., Basmal, J., and Hastarini, E. (2016). Purification of Catfish Oil from Smoked Fish Byproducts. Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan, 11(2), 171–182.
16. Sembiring, L., Ilza, M., and Diharmi, A. (2018). Characteristics of Refined Oil from Catfish (Pangasius hypophthalmus) Belly Fat Purified with Bentonite. JPHPI, 21(3), 549–555.
17. Sumartini, Supriyanto, and Hastuti, P. (2019). Characteristics of Tilapia (O. niloticus) Fish Oil Refined Using Activated Carbon and Bentonite from Fillet Processing Waste at PT. Aquafarm Nusantara Semarang. Jurnal Aihara, VIII(2), 96–104.
18. Suseno, S. H., Jacoeb, A. M., and Abdulatip, D. (2019). Stability of Commercial Imported Fish Oil (Soft Gel) in Several Regions of East Java. JPHPI, 22(3), 589–600.
19. Yatuu, S. U., Jusuf, H., and Lalu, N. A. S. (2020). Effect of Kitchen Lemongrass (Cymbopogon citratus) Juice on the Mortality of Aedes aegypti Larvae. Journal of Health Sciences, 2(1), 32–42.