Journal of
Food and Pharmaceutical Sciences

Original Article

Physicochemical Characterization Refined Patin Fish Oil (*Pangasius micronema*) using Bentonite and Activated Carbon

Indah[1,2], Abdul Rohman[2,3*] and Lily Arsanti Lestari[2,4]

[1]Master in Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Gadjah Mada, Jl. Sekip Utara, Sleman, Yogyakarta 55281, Indonesia; indah2020@mail.ugm.ac.id

[2]Center of Excellence Institute for Halal Industry & Systems, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia; abdulkimfar@gmail.com

[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, Jl. Sekip Utara,Sleman, Yogyakarta 55281, Indonesia; abdulkimfar@gmail.com

[4]Department of Nutrition and Health, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Jl. SekipUtara, Sleman, Yogyakarta 55281, Indonesia; lily_al@ugm.ac.id

*Corresponding author: Abdul Rohman | Email: abdulkimfar@gmail.com

Received: 30 May 2022; Revised: 30 June 2022; Accepted: 22 July 2022; Published: 31 August 2022

Abstract: This study aims to determine fish oil's physical and chemical quality from the flesh of *Pangasius microcinema*. The quality of extracted fish oil can be improved through a refining process. Before and after the refining process, the quality of crude fish oil was analyzed, including acid value (AV), saponification value (SV), iodine value (IV), peroxide value (PV), and p-anisidine value (An-V). The results of the characterization of patin fish oil (PFO) in meat were carried out with 3 refinings, namely refining without activated carbon refining treatment with bentonite, the value of susceptibility to acid value, peroxide value, saponification value, iodine value, and p-anisidine value, which ranged from 1.92-2.64 mg KOH/g, peroxide value 3.76-4.87 meq O2/kg, iodine value 80.46-81.39 g I_2/100g, saponification value 189,48-196.75 mg KOH/g, and p-anisidinee value 5.95-8.93 meq/kg. The best refining process is the oil extracted from patin fish oil meat oil and distilled using bentonite from dry rendering because the processed fish oil is fulfill standard in the standards set by the *International Association of Fish Flour Producers, Indonesian Fish Oil Standards,* and as food fish oil grades.

Keywords: patin, characteristics, bentonite, activated carbon

1. INTRODUCTION

Indonesia is an importer of *omega-3 fatty acids*, although actually, it has potential for a local source of *omega-3 acid* which has not been fully optimized. One source of *omega-3 fatty acids* is starting fish oil. The patin fish (*Pangasius micronema),* on the other hand, has received little attention. It is well known that polyunsaturated fatty acids (PUFA) in meat PFO, particularly *docosahexaenoic acid* (DHA) and *eicosapentaenoic acid* (EPA) [1]. PFOs are reported to contain monounsaturated fatty acids of 35.0–44.4% and polyunsaturated fatty acids of 9.3–19.3%, and among these, PFO contains omega-3 fatty acids of approximately 4.7% [2].

The common method to extract fish oil is the dry rendering method. The extracted oil may contain impurities and other compounds such as *free fatty acids, monoglycerides*, *diglycerides*, *phosphatides*, *hydrocarbons, carbohydrates*, *proteins*, and degradation products that are not good for humans [3]. Therefore, this oil must be purified to meet the standard quality of fish oil for human consumption. The method commonly used to improve the quality of the oil is chemical refining. The chemical refining steps are done by adding an adsorbent [4]. The adsorbent can absorb impurities from components, pigments, and free fatty acids in the oil. The adsorbents that can be used in the refining process are activated carbon, bentonite, attapulgite, and chitosan [5]. showed that the addition of bentonite can reduce the free acid number of catfish oil from 1.72 after the addition of 2% bentonite to 1.150.23 and the peroxide number from 5.18 to 0. Other studies conducted by

The quality of an oil is determined through the determination analyzed including acid value, saponification value, iodine value, peroxide value, and p-Anisidine value. of the acid value and the value of peroxide [6]. The acid value shows the presence of free fatty acids in the oil. While the value of peroxide indicates the degree of damage to fish oil [7]. An oil that can last a long time if the acid content maximum free fat in oil is 0.5% (equivalent to oleic acid) or a maximum acid value of 1 mg KOH per gram sample [8]. Acid content-free fat that is still above the limit maximum can be improved through the process of refining by the refining method until the acid content fat meets the requirements [9], Steps that can be taken to prevent this are oil purification with adsorbents that can absorb impurities from components, pigments, and free fatty acids in the oil can lead to improved quality . This study aims to study the refining of meat a of PFO, to determine the physicochemical characteristics both before and after refining [10]

2. MATERIALS AND METHODS

2.1 Materials

Patin fish oil (Ikan Patin in Indonesia) was purchased from the central fish market in Central Java (in Juana Pati), Indonesia. The reagents used were ethanol p.a (Merck, Germany), potassium hydroxide (KOH), hydrochloric acid (HCl), chloroform, wijs reagent, glacial acetic acid (CH3COOH), potassium iodide (KI), sodium thiosulfate, and distilled water. Adsorbent: bentonite and activated carbon.

2.1.1 Patin Fish oil (PFO) preparation

The Patin fish samples were extracted from body parts of flesh and head of Patin fish to get Patin Fish Oil (PFO) according to [4]. All body parts of fish samples were cut into small pieces and then dried in a cabinet dryer for about 24 hours, except the flesh of Patin which is dried for about 1 day at a temperature of 50°C [11]. PFO was extracted using direct pressing with 100 kN force for 2 min. The samples were subjected to centrifugation at 5000 × g for 10 min to separate the sediment. The obtained PFO was treated with activated charcoal, previously dried using an oven at 105°C, as a bleaching agent [12]. During the bleaching procedure, a-10 grams of PFO were added with activated carbon concentrations of 10%. After that, PFO was heated and centrifuged at 6.500 rpm for 10 min to get clear PFO [13].

2.2 Patin Fish Oil (PFO) characterization

2.2.1 Determination of acid value

Acid value (AV) was determined according to the AOAC official method (2000) with some modifications. Oil samples (for the head, 1 g, and flesh, 1 g) were accurately weighed into Erlenmeyer 250 mL and then added with 50 mL of neutralized ethanol 95% and 2 mL of phenolphthalein indicator solution 1%. The oil samples were then titrated with 0.1 N KOH-ethanolic until the appearance of the first permanent pink color. The titration was titrated in three replicates. The permanent pink colour persisted for at least 30 s during titration. AV was calculated as:

$$\text{Acid value (mg KOH/g)} = \frac{\text{KOH volume (ml)x N KOH X 56,1}}{\text{mass of samples (g)}}$$

2.2.2 Determination of peroxide value

Measurement of peroxide value (PV) can be used as an indication of peroxides contained in the analysed oil. PV was determined according to the AOAC official method (2000). A gram of each sample was accurately weighed into a 250 mL Erlenmeyer flask then 30 mL of acetic acid and chloroform (3:2) were added, and swirled to mix well. The mixture was added with 0.5 mL of saturated potassium iodide solution and allowed to stand for exactly 1 min in a dark room. After that, the mixture was added with 30 mL of distilled water and swirled to mix. A starch indicator (1 mL) was added and then titrated with 0.1 N sodium thiosulfate until the blue colour disappeared. PV was calculated as:

$$\text{Peroxide value } \left(\text{meq}\frac{\text{Q2}}{\text{1000 g}}\right) = \frac{\text{vol. Na tiosulfat x N Na tiosulfat x 1000}}{\text{mass of samples (g)}}$$

2.2.3 Determination of iodine value

The iodine value was determined according to the AOAC official method (2000). A 300 mg of oil samples were accurately weighed and placed in 250 mL Erlenmeyer, added with 25 mL chloroform followed by 20 mL of wijs solution. The solution was allowed to react in a dark room for 30 mins. A 10 mL of 10% potassium iodide along with 50 mL of deionized water were added to each sample. The mixture was titrated using 0.1 N sodium thiosulfate until the yellow colour disappeared. Starch indicator (1 mL) was added and the titration was continued until the blue colour disappeared. IV was calculated as:

$$\text{Iodine value (mg } I_2\text{/g)} = \frac{\text{vol.Na2S2O3 blanko} - \text{vol.Na2S2O3 sampel x N Na2S2O3 x 12,69}}{\text{massa sampel (g)}}$$

2.2.4 Saponification value

The saponification value (SV) was expressed as the number of milligrams of potassium hydroxide (KOH) required to saponify 1 g of oil. Determination of SV was carried out according to the AOAC official method (2000). An approximate 1 g of oil was accurately dissolved with 50 mL KOH-ethanolic in an Erlenmeyer flask then mixed until homogeneous. The solution was heated at temperatures 80-85^{o}C for 30 mins. After that, the solution was cooled and added with 1 mL phenolphthalein. The mixture was titrated with 0.5 N HCl until the pink colour has just disappeared. SV was calculated as:

$$\text{Saponification value} = \frac{(\text{HCl volume of blanko} - \text{HCl volume of sampel}) x\ N\ Hcl\ x\ 56,1}{\text{mass of sampel (g)}}$$

2.2.5 Determination of p- anisidine value

An-V was determined by dissolving 0.5 gram of PFO samples in 25 mL of n-hexane [14]. This solution was measured by UV-vis spectrophotometry against n-hexane as a reference and recorded as A1 (test solution 1). Solution 2 was prepared by adding 1 mL of p-anisidine solution (2.5 g/L) into 5 mL of test solution 1, and then shaken and kept away from light. The reference solution was prepared by adding 1 mL of p-anisidine solution (2.5 g/L) into 5 mL of n-hexane solution, shaken, and kept away from light [15]. The absorbance value of the test solution 1 was measured at 350 nm. Test solution 2 was measured at 350 nm exactly 10 min after the solution was prepared and was calculated as:

$$\text{p} - \text{anisidine value} = \frac{25\ x\ (\ 1.2\ A2 - A1)}{\text{Mass of Samples (g)}}$$

2.3 Statistical analysis

The results of PFO characterization were analyzed using One-way Annova from Minitab 19 software with a significance level for all analyzes of $p<0.05$ Furthermore, the data was carried out using the one-way ANOVA method to see differences in the effect of fish oil administration between treatment groups. If the results of the analysis there are significant differences, then the analysis will be continued with the tukey test to find significant differences.

3. RESULTS AND DISCUSSION

Patin fish oil extracted from the flesh and head of Patin fish to obtain crude PFO was subjected to a refining process using activated carbon and bentonite. Visually, PFO purified with activated carbon and bentonite revealed clearer color than crude PFO as shown in Figure 1.

(A) (B) (C)

Figure 1. The visual appearance of crude Patin Fish Oil (PFO) (A), PFO treated with activated carbon (B) and PFO treated with bentonite (C).

The adsorbents used are activated carbon and bentonite. This treatment is expected to remove the unattractive and unfavorable taste, aroma, and color of the oil, thereby extending its shelf life. Research conducted by [16], showed that the addition of bentonite can reduce the free acid number of catfish oil from 1.72 after the addition of 2% bentonite to 1.150.23 and the peroxide number from 5.18 to 0. Other studies conducted by [17], show that the addition of activated carbon to tilapia fish oil has a relatively small peroxide value of 1.220.005% and an acid value of 3.120.23%. This shows

that bentonite and activated carbon can reduce fat oxidation products, namely peroxides, aldehydes, and ketones.

All PFO s are subjected to characterization by determining their acid (AV), peroxide (PV), iodine (IV), and anisidine values (An-V), and the characterization results were compiled in Table 1. AV and PV represented the hydrolytic and oxidation degrees of PFO; therefore, the higher AV and PV indicated the low quality of edible oils, while IV and An-V were characteristics of certain oils. Among three PFO samples, PFO treated with bentonite revealed the lowest AV and PV.

Table 1. The acid value (AV), Peroxide value (PV), iodine value (IV) and Anisidine Value (An-V) of Crude Patin fish oil (PFO) and PFO purified with activated carbon and bentonite by statistical analysis of anova one- way.

Parameters	Crude PFO	PFO treated with activated carbon	PFO treated with bentonite	Indonesian Standard	IFOS
Peroxide value (meq/1000 g)	4.87±0,145	4.290 ±0.082	3.76±0.137	< 5.0	< 5.0
Acid Value (mg KOH/g)	2.64±0,099	1.92±0.0704	2.42±0.105	< 3.0	< 3.0
Iodine value (g I_2/ 100 g)	81.39±1.19	81.99±1.281	80.46±1.061	< 140 g	95-118
Saponification value (mg KOH/g)	196.75±1,45	194.87±2,05	189.48±2.77	-	175-201
Anisidine Value (meq/kg)	8.93±0.164	7.07±0,0634	5.95±0.115	≤ 20	≤ 20

Based on SNI, the standard of free fatty acids in oil is 3.0 mg KOH/g and all treatments are considered to have met the quality requirements of SNI. When compared with Ayu and Diharmi (2019) the acid value obtained in this study was higher, whereas [17], study showed an acid value of 0.37 0.01 mg KOH/g showed lower free fatty acids than in this study. This is possible due to two factors, including the hydrolysis and storage prosesses. The hydrolysis process can occur during the extraction process or storage. Oils and fats consist of triacylglycerol (TAG) which can be hydrolyzed into free fatty acid molecules catalyzed by lipase enzymes and the presence of water. the presence of water can support the hydrolysis reaction which causes a lot of free fatty acids to be formed [18]. Without treatment, the PFO peroxide value of meat was 4.87 PFO treated with activated carbon was 4.29, and PFO treated with bentonite was 3.76 meq/1000 g. These results indicate that PFO treated with bentonite has better quality than other treatments. In this study, peroxide values were high because the oil was not refined. Peroxide or hydroperoxide is an intermediate species, which is an unstable species that can react with KI quickly [19]. Iodine value (IV) is a measure of overall unsaturation degree, defined as the number of grams of iodine absorbed by 100 g of fats or oils [20]. IV determines the stability of oils to oxidation [21]. High IV shows that the oils contain a higher degree of unsaturation and have good qualities [22]. In this study, IV for PFO crude oil 81.39±1.19, PFO treated with activated carbon 81.99±1.281, and PFO treated with bentonite 80.46±1.061. Based on SNI, the allowable IV was 82-88 g I_2/100 g [23]. Reported that the acceptable fish oils were oils with typical IVs of 95-118 g I_2/100 g [24]

Saponification value (SV) is an index of the average molecular mass of fatty acid in the oil samples. SV is the number of milligrams of potassium hydroxide required to neutralize the fatty acid

resuresultinglted from complete hydrolysis of 1 g of oil samples [25]. The high SV indicates that the oil samples had a lower molecular weight of fatty acid [25]. The SVs obtained were PFO crude oil 196.75±1.45, PFO treated with activated carbon 189.48±2.77, and PFO treated with bentonite 189.48±2.77.

Based on SNI p-anisidine value < 20 meq/kg, the value of anisidine number in this study met the requirements. The results of the p- anisidine value PFO number are presented in table 1. The p-anisidine value PFO value in meat has a much smaller value, this is in accordance with the peroxide value obtained The p-anisidine value has a lower value than the peroxide value. This is presumably because the formation of peroxides as intermediates in fat oxidation will increase in the presence of high temperatures and the presence of metals which causes the hydroperoxide compound to be unstable and will undergo problem solving into a complex mixture of aldehydes, ketones, and other products called secondary oxidation products [25].

4. CONCLUSION

Based on the results of the physicochemical constants, the PFO of the meat was carried out 3 treatments, namely without treatment, purification of activated carbon with bentonite had an acid number range between 1.92-2.64 mg KOH/g, peroxide number between 3.76-4.87 meq O2/kg these two numbers. as a parameter of oil quality. The higher these values, the more likely the formation of free fatty acids that reduce the oil quality. Iodine number is between 80.46-81.99 g I2/100g, saponification number is between 189.48-196.75 mg KOH/g, and anisidine number is 5.95-8.93 meq/kg as characteristics of the oil. The results showed that the PFO of meat with bentonite purification obtained the lowest acid and peroxide values compared to other samples.

References

[1] E. Hastarini, E. H. Fardiaz, D., Iranto, dan S. Budijanto, "Characteristics of Fish Oil Produced from Fillet Processing Waste of Siam (Pangasius hypopthalmus) and Jambal (Pangasius djambal) Catfi sh," *Jurnal Agritech*, vol. 32, no. 04, hlm. 403–410, 2013.

[2] R. B. Hashim, E. F. Jamil, F. H. Zulkipli, dan J. M. Daud, "Fatty acid compositions of silver catfish, Pangasius sp. farmed in several rivers of Pahang, Malaysia.," *Journal of oleo science*, vol. 64, no. 2, hlm. 205–209, 2015, doi: 10.5650/jos.ess14191.

[3] N. Nazir, A. Diana, dan K. Sayuti, "Physicochemical and Fatty Acid Profile of Fish Oil from Head of Tuna (Thunnus albacares) Extracted from Various Extraction Method," *International Journal on Advanced Science, Engineering and Information Technology*, vol. 7, hlm. 709, Apr 2017, doi: 10.18517/ijaseit.7.2.2339.

[4] U. Nadhiro, S. Subekti, W. Tjahjaningsih, dan Patmawati, "Quality characteristics of Bali sardinella (Sardinella lemuru) oil purified with bentonite as an adsorbent," *IOP Conference Series: Earth and Environmental Science*, vol. 137, no. 1, 2018, doi: 10.1088/1755-1315/137/1/012012.

[5] R. T. Rosmalina, W. Kosasih, dan S. Priatni, "the Effects of Adsorbent Materials on the Lipid Quality of Lemuru Fish Oil and the Enrichment of Omega-3 Using Lipase," *Jurnal Teknologi dan Industri Pangan*, vol. 32, no. 1, hlm. 16–26, 2021, doi: 10.6066/jtip.2021.32.1.16.

[6] AOAC, *Official Methods of Analysis of AOAC International AOAC Internation*. 2000.

[7] M. J. J. Martins, L. Purnamayati, dan R. Romadhon, "Pengaruh Suhu Wet Rendering yang Berbeda terhadap Karakteristik Ekstrak Kasar Minyak Ikan Lele (Clarias sp.)," *agriTECH*, vol. 41, no. 4, hlm. 335, 2021, doi: 10.22146/agritech.49875.

[8] I. Maulana, S. Sukraso, dan S. Damayanti, "THE CONTENT OF FATTY ACIDS IN INDONESIA'S FISH OIL," *Jurnal Ilmu dan Teknologi Kelautan Tropis*, vol. 6, Nov 2014, doi: 10.29244/jitkt.v6i1.8635.

[9] A. Rasyid, "Asam Lemak Omega-3 Dari Minyak Ikan," *Oseana*, vol. XXVIII, no. 3, hlm. 11–16, 2003.

[10] S. Bija, S. H. Suseno, dan U. Uju, "Purification of Sardine Fish Oil Through Degumming and Neutralization," *Jurnal Pengolahan Hasil Perikanan Indonesia*, vol. 20, no. 1, hlm. 143, 2017, doi: 10.17844/jphpi.v20i1.16501.

[11] Kamini, P. Suptijah, J. Santoso, dan S. H. Suseno, "Extraction by Dry Rendering Methode and Characterization Fish Oil of Catfish (Pangasius hypopthalmus) Viscera Fat by Product of Smooked Fish Processing," *Jurnal Pengolahan Hasil Perikanan Indonesia*, vol. 19, no. 3, hlm. 196, 2016, doi: 10.17844/jphpi.v19i3.14477.

[12] H. Mulyani dan A. Sujarwanta, *LEMAK DAN MINYAK*. Metro: Lembaga Penelitian UM Metro, 2018.

[13] Y. Huang, F. Li, G. Bao, M. Li, dan H. Wang, "Qualitative and quantitative analysis of the influence of biodiesel fatty acid methyl esters on iodine value," *Environmental Science and Pollution Research*, vol. 29, no. 2, hlm. 2432–2447, 2022, doi: 10.1007/s11356-021-15762-w.

[14] A. Rohman, S. Riyanto, A. Windarsih, K. Irnawati, dan Rosiana Putri, A., *Karakterisasi Minyak Dan Lemak*. Yogyakarta.: Indonesian Journal of Pharmacy Pustaka Pelajar, 2019.

[15] S. H. Suseno dan T. Faradiba, "MINYAK IKAN DAN HABBATUSSAUDA Characterization and Stabilization of Product Formulation of Fish and Habbatussauda Oil," vol. 16, hlm. 142–149, 2013.

[16] L. Sembiring, M. Ilza, dan A. Diharmi, "Characteristics of Pure Oils from Belly Fat (Pangasius hypophthalmus) with Bentonite Purification," *Jurnal Pengolahan Hasil Perikanan Indonesia*, vol. 21, no. 3, hlm. 549, 2018, doi: 10.17844/jphpi.v21i3.24742.

[17] Prasetyowati, A. Kurniawan, dan D. Saputra, "Pemurnian Minyak Jelantah dengan Adsorben Bentonite and Arang Aktif," *Jurnal Teknik Kimia*, vol. 17, no. 5, hlm. 59–65, 2011.

[18] D. F. Ayu, A. Diharmi, dan A. Ali, "Characterization of the oil from the abdomen part of smoked catfish (Pangasius hypophthalmus) processing by-product," *Jurnal Pengolahan Hasil Perikanan Indonesia*, vol. 22, no. 1, hlm. 187–197, 2019, doi: 10.17844/jphpi.v22i1.26473.

[19] A. Putri, A. Rohman, W. Setyaningsih, dan S. Riyanto, "Determination of acid, peroxide, and saponification value in patin fish oil by FTIR spectroscopy combined with chemometrics," *Food Research*, vol. 4, hlm. 1758–1766, 2020, doi: 10.26656/fr.2017.4(5).030.

[20] N. Hani, J. Nuraini, dan K. Y. Lee, "Studies on the extraction and characterization of fish oil from wastes of seafood processing industry," *Asian J Food Agro Ind*, vol. 2, hlm. 959–973, Jan 2009.

[21] M. Maliki dan I. H. Ifijen, "Extraction and Characterization of Rubber Seed Oil," vol. 4, no. 6, hlm. 24–27, 2020.

[22] T. Babalola dan A. D.F, "Chemical and quality evaluation of some alternative lipid sources for aqua feed production," *Agriculture and Biology Journal of North America*, Jun 2011, doi: 10.5251/abjna.2011.2.6.935.943.

[23] T. Bako, V. I. Umogbai, dan J. O. Awulu, "Criteria for the extraction of fish oil," *Agricultural Engineering International: CIGR Journal*, vol. 19, no. 3, hlm. 120–132, 2017.

[24] IFOS, *Fish oil purity standards, [online], (http://www.omegavia.com/best-fish_oilsupplement-3/*. diakses online tanggal 28 Agustus 2021, 2011. doi: 10.17844/jphpi.v20i3.19766.

[25] A. K. Rai, H. C. Swapna, N. Bhaskar, P. M. Halami, dan N. M. Sachindra, "Effect of fermentation ensilaging on recovery of oil from fresh water fish viscera," *Enzyme and Microbial Technology*, vol. 46, no. 1, hlm. 9–13, 2010, doi: 10.1016/j.enzmictec.2009.09.007.