Journal of
Food and Pharmaceutical Sciences

Original Article

Physicochemical Characterization and Fatty Acid Profiles of Fish Oil from Catfish (*Clarias gariepinus)*

Karina Primatyas Ningrum[1], Abdul Rohman[2,3*] and Ronny Martien[4]

[1] Master in Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Gadjah Mada, Jl. Sekip Utara, Sleman, Yogyakarta 55281, Indonesia; karina.primatyas2398@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 Pharmaceutical Technology, Faculty of Pharmacy, Universitas Gadjah Mada, Jl. Sekip Utara, Sleman, Yogyakarta, 55281, Indonesia; ronnymartien@ugm.ac.id
* Corresponding author: Abdul Rohmann | Email: abdulkimfar@gmail.com

Received: 20 March 2023; Revised: 11 June 2023; Accepted: 05 July 2023; Published: 16 July 2023

Abstract: Indonesian aquaculture cultivation production of catfish has been growing faster than other fish. Catfish has a potential source of fish oil and contains polyunsaturated fatty acids that have health benefits. The objective of this study was to perform physicochemical characterization and determined the fatty acid profile of catfish oil. This study used fish oil extracted from the head and the flesh of catfish using dry rendering with a mechanic press. The result showed that catfish flesh oil (CFO) and catfish head oil (CHO) were significantly different parameters ($p<0.05$) in terms of physicochemical characteristics including acid, peroxide, iodine, and saponification value. The best characteristics are CFO than CHO for acid value, peroxide value, iodine value, and saponification value following the number is 2.17 mg KOH/g, 5.15 meqO2/kg, 76.13 I_2/100 g, and 175.86 mg KOH/g. The predominant fatty acids were oleic, palmitic, and linoleic acids. These catfish oils are found suitable for functional food.

Keywords: Catfish oil; physicochemical characterization; fatty acid profile

1. INTRODUCTION

Indonesia is the largest aquaculture production in Southeast Asia and the third largest world production after China and India. This shows that Indonesia has big influence in increasing the competitiveness and acceptability of aquaculture products in regional and international markets [1]. Catfish known as "ikan lele" in Indonesia is one of the freshwater fish cultivation commodities that grows fast compared to other commodities. From 2009 to 2013, catfish experienced the largest increase in production, namely 88.98%, above gourami, patin, and carp [2].

Catfish (*Clarias gariepinus*) have the potential to use its oil as a source of Omega-3 unsaturated fatty acids. Catfish oil is known to contain 40.32% saturated fatty acids, while total unsaturated fatty acids saturation is 59.65% [3]. Unsaturated fatty acids (Polyunsaturated Fatty acids (PUFA)) have several benefits such as helping the process of brain development (intelligence), the development of the sense of sight, and the immune system of infants and toddlers. PUFA also has an important role for the body in disease prevention, such as lowering cholesterol and preventing heart disease, hypertension, arthritis, and other inflammatory and autoimmune diseases [4], [5].

Catfish is a potential candidate which is considered as the cheapest source of animal protein and unsaturated fatty acids. To ensure the quality of catfish oil, it is necessary to carry out characterization to differentiate and characterize the fish oil. The characterization is based on

Indonesian national standards (SNI) [6] and international fish oil standards (IFOS) [7] consisting of chemical and physical properties. The character and quality of fish oil are influenced by many factors which cause different results, including the extraction process, the place of origin of the sample, the treatment of the sample, and the personnel [8].

Catfish can be consumed as nutritious food. The largest part of fish that is commonly consumed is the flesh and the head. The chemical composition of fish such as protein and fat is important from the nutrition perspective of human health [9]. The physicochemical properties of edible fats and oils are important for their characterization [10]. These physicochemical parameters include acid value, peroxide value, iodine value, and saponification value [11]. The fatty acid composition of fish is affected by environmental factors, species, and the production area [12]. This study aimed to perform the physicochemical characteristics and fatty acid composition of flesh and head catfish.

2. MATERIALS AND METHODS

2.1. Materials

Catfish was obtained from freshwater fish cultivation in Sleman, Yogyakarta, Indonesia. The reagents used were ethanol p.a, potassium hydroxide (KOH), hydrochloric acid (HCl), chloroform, Wijs reagent, glacial acetic acid (CH_3COOH), potassium iodide (KI), sodium thiosulfate, methanol, sodium hydroxide (NaOH), n-hexane, boron trifluoride (BF_3), and sodium chloride were obtained from Merck (Germany).

2.1.1. Fish oil Preparation

Catfish oil was extracted from body parts of the flesh and the head. Catfish were cleaned and all body parts were cut into small pieces and then dried in a cabinet dryer for about 24 hours at a temperature of 50^{o}C. Catfish oil (CFO) was extracted using direct pressing with 100kN force for 2 min. The oil was separated from debrish by centrifugation at 5000 rpm for 10 minutes to get crude fish oil [8].

2.2. Catfish oil (CFO) characterization

Determination of the physical-chemical properties based on the standard method of the Association of Official Analytical Chemists (AOAC), the aspect of the properties of fish oil consists of determining the physical-chemical number; acid, peroxide, iodine, saponification value and analysis of the fatty acid profile [13].

2.2.1. Determination of acid value

Oil samples (for the head 1 g and flesh 1 g) was 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 titrated with 0.1 N KOH-ethanolic until the appearance of the first permanent pink color. The titration was titrated in three replicates. The acid value was calculated as:

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

2.2.2. Determination of peroxide value

1 gram of each sample fish oil was accurately weighed into a 250 mL Erlenmeyer flask then 30 mL of acetic acid and chloroform (3:2) were added, and 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. Then titrated with 0.1 N sodium thiosulfate until the blue color disappeared. Peroxide value was calculated as:

$$\text{Peroxide value} = \frac{\text{Sodium thiosulfate vol (mL) x N Sodium thiosulfate x 1000}}{\text{mass of samples (g)}}$$

2.2.3. Determination of iodine value

The iodine value was determined according to the AOAC official method (2000) by the Wijs method. 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. 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 color disappeared. 1 mL indicator was added and the titration was continued until the blue color disappeared. Iodine value was calculated as:

$$\text{Iodine value} = \frac{\text{Sodium thiosulfate vol (Blank}-\text{sample) x N Sodium thiosulfat x 12,69}}{\text{mass of sample (g)}}$$

2.2.4. Saponification value

An approximate 1 g of fish oil was accurately dissolved with 50 mL KOH-ethanolic in an Erlenmeyer flask and then mixed until homogeneous. The solution was heated at temperatures 80-85oC for 30 mins. After that, the solution was cooled, and added with 1 mL of phenolphthalein. The mixture was titrated with 0.5 N HCl until the pink color has just disappeared. Saponification value was calculated as:

$$\text{Saponification value} = \frac{\text{HCl volume (Blank}-\text{sample)}x\ N\ Hcl\ x\ 56,1}{\text{massa of sample (g)}}$$

2.2.5. Fatty acid composition

The fatty acid constituents were analysed by Agilent 8890 GC apparatus coupled to Agilent GC/MSD 5977B mass spectrometry detector. Fatty acid methyl ester (FAME) was prepared by taking 200 µl of catfish oil added with 1.0 ml of n-hexane, and 200 ml of NaOH solution in methanol was heated for 10 min while shaken out. The mixture was then added with a 1.5 ml BF_3 solution and heated for 10 min. After that, added with 1.5 ml saturated NaCl and vortexed for 10 min. Supernatants that contained fatty acid methyl ester (FAME) derivatives were taken and injected into the gas chromatograph system [14], [15]. Supelco 37 component FAME mixture (Sigma Aldrich) was used as the reference standards fish oil. Sample was injected using a 7693A autosampler. A split mode injection was used with a split ratio of 1:10. Helium was used as carrier gas, and the injector temperature was set at 250°C. Compounds were separated on a HP-INNOWAX 60 m, 0.25 mm, and 25 µm capillary column. The column oven temperature program started at 150°C for 1 min then ramped from 150 to 200°C during 15 min at a rate of 15°C/min.

2.3. Statistical analysis

The results of CFO characterization were analyzed using One-way ANOVA from Minitab 19 software with a significance level for all analyses of $p<0.05$. Furthermore, the data was carried out using the one-way ANOVA method to see differences in the physicochemical characterization of fish oil between the different body parts of fish oil groups.

3. RESULTS AND DISCUSSION

The flesh and head of catfish were taken and the oils contained were extracted using cold pressing [8]. This method is suitable for use on an industrial scale because the extraction process does not use harmful solvents and the extraction process does not use heating so that the quality of the fish oil is maintained and it is safe for consumption. Cold pressing methods was successfully to obtain crude catfish head oil (CHO) and catfish flesh oil (CFO). Visually, CHO and CFO showed in Figure 1. The CFO had a more light-yellow color compared to CHO having yellow-orange color. This result because of the different characteristic of catfish oil from flesh and head. The different of physical characteristics (such as hardness), stability, the nutritional value of lipids can caused by the composition of fatty acids [16], [17].

Figure 1. The visual of Catfish Head Oil (left) and Catfish Flesh Oil (right).

All catfish oil is subjected to characterization by determining their acid (AV), peroxide (PV), iodine (IV), and saponification (SV), the characterization results were compiled in Table 1. The acidity of oil is an important quality parameter related to the presence of free fatty acid (FFA) and other non-lipid acid compounds [11]. The acid value (AV) was determined to express the acidity of studied fats and oils. Acid value can be used to determine the degree of hydrolysis in an oil sample during storage. The quality of oil will decrease if the value of the acid number is higher [10]. The study free fatty acid of catfish oil from fresh and frozen shown that catfish oil from fresh is better than frozen [18]. Based on SNI dan IFOS, the standard of free fatty acids in oil is 3.0 mg KOH/g and catfish oil head and flesh have met the quality requirements. The acid value of catfish head oil and catfish flesh oil were significantly different ($p<0.05$).

Table 1. Physicochemical properties of Catfish Oil

Parameters	CHO	CFO
Acid value (mg KOH/g)	2.17±0.1200	1.58±0.0528
Peroxide value (meqO_2/1000g)	5.15±0.1466	4.15±0.1145
Iodine value (g I_2/100 g)	76.13±2.0083	85.42±2.2198
Saponification value (mg KOH/g)	175.86±5.3900	191.71±5.5150

CHO= Catfish Head Oil, CFO= Catfish Flesh Oil

Peroxide value is the most value to determine the degree of oil damage during oxidation. The oil damage can occur due to the oxidation process by oxygen from the air binding unsaturated fatty acid in the oils during heat processing [19]. The smaller PV means better quality. Based on statistical analysis CHO dan CFO were significantly different ($p<0.05$). In this study peroxide value of CFO are meet the requirement based on SNI dan IFFO that is < 5.0 meqO_2/1000g.

Iodine value and saponification value to determine of composition fatty acid from oil [10]. Iodine value is a measure of overall unsaturation degree, defined as the number of grams of iodine absorbed by 100 g of fats or oils. High iodine value shows that the oils contain a higher degree of unsaturation and have good quality. Saponification value is an index of the average molecular mass of fatty acid in the oil sampels. SV is the number of miligrams of potassium hydroxide required to neutralize the fatty acid resulting from complete hydrolysis of 1 g of oil samples. The high SV indicates that the oil sampels had a lower molecular weight of fatty acid. The Iodine value and saponification value of catfish head oil and catfish flesh oil were significantly different ($p<0.05$).

The fatty acid compositions of CHO and CFO were shown in Table 2. Palmitic acid, oleic acid, and linoleic acid are the three fatty acids that dominate in both oil samples. The results obtained were similar as reported by Srimiati (2015) and Pandiangan (2020). The different of percentage of fatty acid profile is caused the other research using Soxhlet method. Other factors contributing to these differences include harvesting seasons, fish food and body part which may influence the fat and lipid profile [21].

Table 2. Fatty acid profile of Catfish oil

Fatty acid	CFO	CHO	A[20]	B[3]
Capric Acid, C10:0	0.02	nd	nd	nd
Lauric Acid, C12:0	1.13	0.09	0.37	nd
Myristic Acid, C14:0	1.28	0.82	1.04	1.41
Pentadecanoic Acid, C15:0	0.09	0.13	0.18	nd
Palmitic Acid, C16:0	25.02	25.11	21.27	27.94
Heptadecanoic Acid, C17:0	0.15	0.2	0.2	nd
Stearic Acid, C18:0	6.79	7.27	5.32	9.07
Arachidic Acid, C20:0	0.2	0.19	0.1	1.9
Heneicosanoic Acid, C21:0	0.02	nd	0.04	nd
Behenic Acid, C22:0	0.09	0.11	0.1	nd
Tricosanoic Acid, C23:0	0.04	0.04	0.03	nd
Lignoceric Acid, C24:0	0.08	0.1	0.06	nd
SFA	**34.91**	**34.06**	**28.71**	**38.42**
Myristoleic Acid, C14:1	0.01	0.02	0.06	nd
Palmitoleic Acid, C16:1	1.21	1.82	3.65	1.88
Cis-10-Heptadecanoic Acid, C17:1	0.08	0.1	0.14	nd
Oleic Acid, C18:1 n9c	41.95	40.55	30.92	37.32
Cis-11-Eicosenoic Acid, C20:1	0.91	0.92	0.53	0.64
Erucic Acid, C22:1n9	0.03	0.03	0.04	0.26
Nervonic Acid, C24:1	0.02	0.05	0.06	nd
MUFA	**44.21**	**43.49**	**35.4**	**40.1**
Linoleic Acid, C18:2n6c	15.49	13.82	12.37	17.07
g-Linolenic Acid, C18:3n6	0.8	0.74	1.31	0.99
Linolenic Acid, C18:3n3	0.47	0.58	0.68	nd
Cis-11,14-Eicosedienoic Acid, C20:2	0.23	0.25	0.3	nd
Cis-8,11,14-Eicosedienoic Acid, C20:3n6	0.83	0.69	0.71	nd
Cis-11,14-Eicosedienoic Acid, C20:2	0.23	0.25	0.03	nd
Arachidonic Acid, C20:4n6	0.37	0.47	0.56	nd
Cis-5,8,11,14,17-Eicosapentaenoic Acid, C20:5n3	0.05	0.11	0.28	0.48
Cis-4,7,19,13,16,19-Docosahexaenoic Acid, C22:6n3	0.28	0.77	1.01	1.01
PUFA	**18.75**	**17.68**	**17.25**	**19.55**

CHO= Catfish Head Oil, CFO= Catfish Flesh Oil, A= Srimiati (2015), B= Pandiangan (2020)

Skin from catfish can also produce Poly Unsaturated Fatty Acid (PUFA) such as 0.80% EPA and 2.48% DHA [22].The unsaturated fatty acid of catfish oil was bigger than saturated oil, there are 44.21% Mono Unsaturated Fatty Acid (MUFA), 18.75% PUFA and 34.91% Saturated Fatty Acid (SFA) for catfish flesh oil and 44.21% MUFA, 17.68% PUFA and 34.06% SFA for catfish head oil. Bigger PUFA composition is good for the health. Based on the fatty acid profiles, shown that catfish oil contained more Omega 9 Oleic Acid (C18:1 n9c), than Omega 6 Linoleic Acid (C18:2n6c).

4. CONCLUSION

Catfish oil has potential application in functional food oils. Physicochemical properties (acid value, peroxide, iodine, and saponification values) of catfish head oil (CHO) and catfish flesh oil (CFO) were significantly different based on an ANOVA with a p-value <0.05. The acid value, peroxide, iodine value, and saponification values were acceptable with standards for fish oil. Fatty acid profiles showed that catfish oil contained more unsaturated fatty acid than saturated fatty acid.

References

[1] Direktorat Produksi dan Usaha Budidaya, *Budidaya Ikan Lele Sistem Bioflok*. Direktorat Produksi dan Usaha Budidaya, 2017.

[2] BPS, "Nilai Produksi Perikanan Budidaya Menurut Komoditas Utama (Ribu Rupiah), 2018," Badan Pusat Statistik, Jakarta, 2018. [Online]. Available: https://www.bps.go.id/indicator/56/1514/1/nilai-produksi-perikanan-budidaya-menurut-komoditas-utama.html

[3] M. Pandiangan, J. Kaban, B. Wirjosentono, and J. Silalahi, "Analisis Asam Lemak Omega 3 dan 6 pada Minyak Ikan Lele secara GC-FID," *J. Ris. Teknol. Pangan Dan Has. Pertan. RETIPA*, pp. 22–29, Sep. 2020, doi: 10.54367/retipa.v1i1.908.

[4] G. P. Zaloga, "Narrative Review of n-3 Polyunsaturated Fatty Acid Supplementation upon Immune Functions, Resolution Molecules and Lipid Peroxidation," *Nutrients*, vol. 13, no. 2, p. 662, Feb. 2021, doi: 10.3390/nu13020662.

[5] M. Srimiati, C. M. Kusharto, I. Tanziha, and S. H. Suseno, "KONSUMSI MINYAK IKAN LELE (Clarias gariepinus) YANG DIPERKAYA OMEGA 3 MEMPERBAIKI LOW DENSITY LIPOPROTEIN (LDL) DAN KOLESTEROL TOTAL PADA LANSIA," *J. Gizi Dan Pangan*, vol. 12, no. 2, pp. 117–122, Jul. 2017, doi: 10.25182/jgp.2017.12.2.117-122.

[6] Direktorat Pengolahan dan Bina Mutu Kementerian Kelautan dan Perikanan RI, *SNI Produk Perikanan Nonpangan*. Jakarta, 2019.

[7] IFOS, "Standar For Fish Oil," Food and Agriculture Organization of United Nation, Italy, 2017.

[8] A. Nur Ikhsan, A. Rohman, A. Rosiana Putri, F. Syifa, M. Mustafidah, and R. Martien, "Application of FTIR Spectroscopy and Chemometrics for the Prediction of Radical Scavenging Activities of Fish oils," *Indones. J. Pharm.*, pp. 166–174, Jun. 2021, doi: 10.22146/ijp.1004.

[9] M. Chalamaiah, B. Dinesh kumar, R. Hemalatha, and T. Jyothirmayi, "Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review," *Food Chem.*, vol. 135, no. 4, pp. 3020–3038, Dec. 2012, doi: 10.1016/j.foodchem.2012.06.100.

[10] A. Rohman, *Karakterisasi Minyak dan Lemak*. Yogyakarta: Pustaka Pelajar, 2020.

[11] A. R. Putri, A. Rohman, W. Setyaningsih, and S. Riyanto, "Determination of acid, peroxide, and saponification value in patin fish oil by FTIR spectroscopy combined with chemometrics," *Food Res.*, vol. 4, no. 5, pp. 1758–1766, Jun. 2020, doi: 10.26656/fr.2017.4(5).030.

[12] J. R. Bonilla and J. L. Hoyos Concha, "Métodos de extracción, refinación y concentración de aceite de pescado como fuente de ácidos grasos omega-3," *Cienc. Tecnol. Agropecu.*, vol. 19, no. 3, Aug. 2018, doi: 10.21930/rcta.vol19_num2_art:684.

[13] AOAC, *Official Methods of Analysis*, 17th ed. USA: The Association of Official Analytical Chemists, 2000.

[14] A. Guntarti, K. P. Ningrum, I. G. Gandjar, and N. Salamah, "AUTHENTICATION OF SPRAGUE DAWLEY RATS (RATTUS NORVEGICUS) FAT WITH GC-MS (GAS CHROMATOGRAPHY-MASS SPECTROMETRY) COMBINED WITH CHEMOMETRICS," *Int. J. Appl. Pharm.*, pp. 134–139, Mar. 2021, doi: 10.22159/jap.2021v13i2.40130.

[15] T. Yi *et al.*, "Comparative analysis of EPA and DHA in fish oil nutritional capsules by GC-MS," *Lipids Health Dis.*, vol. 13, no. 1, p. 190, Dec. 2014, doi: 10.1186/1476-511X-13-190.

[16] S. J. Smith, S. J. Iverson, and W. D. Bowen, "Reply: Fatty acid signatures and classification trees: new tools for investigating the foraging ecology of seals," *Can. J. Fish. Aquat. Sci.*, vol. 56, no. 11, pp. 2224–2226, Nov. 1999, doi: 10.1139/f99-192.

[17] S. Pădureţ, "The Effect of Fat Content and Fatty Acids Composition on Color and Textural Properties of Butter," *Molecules*, vol. 26, no. 15, p. 4565, Jul. 2021, doi: 10.3390/molecules26154565.

[18] N. C. Ndidiamaka and O. E. Ifeanyi, "Proximate and physicochemical analysis of oil obtained fromtwo fish species (fresh and frozen)," *Int. J. Adv. Res. Biol. Sci.*, vol. 5, no. 4, pp. 167–177, 2018, doi: http://dx.doi.org/10.22192/ijarbs.2018.05.04.017.

[19] indah, L. A. Lestari, and A. Rohman, "Physicochemical Characterization Refined Patin Fish Oil (Pangasius micronema) using Bentonite and Activated Carbon," *J. Food Pharm. Sci.*, vol. 10, no. 2, pp. 626–633, 2022.

[20] M. Srimiati, C. M. Kusharto, I. Tanziha, and S. H. Suseno, "Effect of Different Bleaching Temperatures on the Quality of Refined Catfish (Clarias Gariepinus) Oil," *Procedia Food Sci.*, vol. 3, pp. 223–230, 2015, doi: 10.1016/j.profoo.2015.01.025.

[21] A. Kumar, M. R. Lindley, and S. S. Mastana, "A time efficient adaptation of GC-FID method for the analysis of PBMC lipid composition," 2014.

[22] A. S. A. Habib and D. P. Sarkar, "Extraction and identification of PUFA from African Catfish (Clarias gariepinus) Skin".