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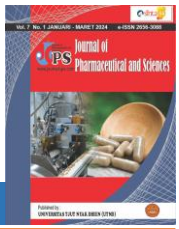

Comparative study of the fatty acid profiles of catfish oil and keting fish oil through purification with bentonite

Studi perbandingan profil asam lemak minyak ikan lele dan minyak ikan keting melalui pemurnian dengan bentonit

Nadia Miftahul Jannah [a]*, Karina Primatyas Ningrum [b]

[a] *Analysis of pharmacy, Faculty of pharmacy, Sultan Agung Islamic University, Central of Java, Indonesia.*
[b] *Clinical and Communities of pharmacy, Pharmacy, Al-Fatah Health College, Bengkulu, Indonesia.*

Corresponding Authors: nadiamj@unissula.ac.id

Abstract

Fish generally have high omega-3 and omega-9 fatty acid content and are beneficial for health. This makes fish oil develop into a food supplement. Increasing the amount of free fatty acids in oil can potentially reduce the quality and damage of fish oil. Adsorbents have bleaching properties that can affect fish and fish oil content colour changes. This study aims to determine the effect of bentonite adsorbent treatment on the fatty acid profile of catfish oil and keting fish oil analysed using the Gas Chromatography Mass Spectrometry (GC-MS) method. Catfish oil (CFO) and Keting fish oil (KFO) samples were extracted without organic solvents and then treated with bentonite as an adsorbent. The extracted oil was derivatised and then injected into the GC-MS instrument system. There were 29 types of fatty acids detected in each sample, namely 12 types of saturated fatty acids (SFA), eight types of monounsaturated fatty acids (MUFA), and nine types of double unsaturated fatty acids (PUFA). The effect of purification with bentonite was analysed using One-way ANOVA from Minitab19 software, showing that there were differences in the content of fatty acid profiles of CFO and KFO that were purified and not purified with bentonite, shown in each category of fatty acids SFA, MUFA and PUFA, which had a p-value <0.05.

***Keywords*: Fish Oil, Fatty Acids, Bentonite, GC-MS.**

Abstrak

Secara umum ikan memiliki kandungan asam lemak omega-3 dan omega-9 yang tinggi dan bermanfaat bagi kesehatan. Hal ini menjadikan minyak ikan dapat dikembangkan menjadi suplemen makanan. Peningkatan jumlah asam lemak bebas pada minyak dapat berpotensi menurunkan mutu dan kerusakan minyak ikan. Adsorben memiliki sifat *bleaching* yang dapat mempengaruhi perubahan warna pada minyak ikan dan kandungan minyak ikan. Penelitian ini bertujuan untuk mengetahui pengaruh perlakuan adsorben bentonit terhadap profil asam lemak pada minyak ikan lele dan keting yang dianalisis menggunakan metode *Gas Chromatography Mass Spectrometry* (GC-MS). Sampel minyak ikan lele (CFO) dan minyak ikan keting (KFO) diekstraksi tanpa menggunakan pelarut organik kemudian diberi perlakuan bentonit sebagai adsorben. Minyak hasil ekstraksi diderivatisasi kemudian diinjeksikan ke sistem instrumen GC-MS. Terdapat 29 jenis asam lemak terdeteksi pada masing-masing sampel yaitu 12 jenis asam lemak jenuh jenuh (SFA), 8 jenis asam lemak tidak jenuh ikatan tunggal (MUFA), dan 9 jenis asam lemak tidak jenuh ikatan rangkap (PUFA). Pengaruh pemurnian dengan bentonit dianalisis menggunakan *One-way* ANOVA dari perangkat lunak Minitab19 menunjukkan bahwa terdapat perbedaan kandungan profil asam lemak CFO dan KFO yang dimurnikan dan tidak dimurnikan dengan bentonit, ditunjukkan pada masing-masing kategori asam lemak SFA, MUFA dan PUFA yang memiliki nilai p<0,05.

***Kata Kunci*: Minyak Ikan, Asam Lemak, Bentonit, GC-MS**

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Introduction

Indonesia is reported to have more than 900 species of freshwater fish. [1]. The substantial fish production in Indonesia presents an opportunity to utilise local fish as a raw source of fish oil. Fish oil is one of the fishery products with high demand and is often used as a dietary supplement, white butter, and other industrial materials. [2]. Generally, fish oil contains high levels of omega-3, omega-6, and omega-9 fatty acids, which are beneficial for health. The polyunsaturated fatty acids (PUFA) contained in fish oil have several benefits, such as aiding brain development (intelligence), the development of the visual senses, and the immune system of infants and toddlers. For example, omega-3 PUFA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are reported to play an essential role in children's nutritional intake. [3,4]. These fatty acids can also be used to prevent several diseases such as cardiovascular diseases, inflammation, arthritis, and diabetes mellitus [5].

Indonesia is rich in fish with relatively high fish content, such as snakehead fish oil, milkfish oil, and catfish oil. Preliminary studies show that these fish oils contain abundant omega-3 and omega-6 fatty acids. Therefore, exploring fish oil based on local Indonesian fish is necessary.

Catfish *(Clarias gariepinus)* and keting fish *(Mystus gulio)* are two local Indonesian species that can be used. Both fish have whiskers and are freshwater catfish. Fish processing has advanced to the point that it may now be utilised to produce fish oil as a byproduct in addition to fresh flesh. Fatty acid components in the oil are highly susceptible to oxidation, which causes the fatty acids to separate from the glycerol bond and increase the quantity of free fatty acids. The oil may become damaged and lose quality due to the rise in free fatty acids. [6]. Utilising adsorbents for oil purification is one way to try to stop this.

Adsorbents can bind free fatty acids to maintain oil quality. They also absorb impurities from the oil's components, pigments, and free fatty acids. One adsorbent that can be used in the fish oil purification process is bentonite. [7,8]. Several studies related to the use of bentonite in the fish oil purification process have been reported, including research conducted by Nurbayasari et al. (2017) [9], which stated that using 2% bentonite can improve the quality of catfish oil that meets SNI standards. This study is supported by Indah et al. (2022) [7], who stated that purifying catfish oil using a 2% bentonite adsorbent resulted in better oil quality than untreated oil. Oil quality can be determined by characterisation by selecting the acid number, peroxide number, iodine number, saponification number, and fatty acid profile. [10].

This study was conducted to compare the fatty acid profiles of purified and unpurified catfish oil (CFO) and keting fish oil (KFO) with 2% bentonite using Gas Chromatography Mass Spectrophotometry (GC-MS). GC-MS analysis was chosen because of its speed and sensitivity [11].

Experimental Section

This study consists of three stages: sample preparation, extraction of catfish and keting fish oil, and analysis of the effect of purification with bentonite on the fatty acid profile of catfish and keting fish oil using the GC-MS method.

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Materials and Apparatus

The equipment used in this study includes glassware (Pyrex), microtubes (Eppendorf, Biologix), digital analytical balance (Mettler Toledo, Ohaus), 15 mL conical tubes (Iwaki), hotplate magnetic stirrer (Stuart CB162), magnetic bars, micropipettes (Eppendorf), ultrasonicator (J.P. Selecta), vortex mixer (Thermolyne), blue tips, yellow tips, white tips (Biologix and Eppendorf), and GC-MS (Agilent 8890). The materials used are catfish from Yogyakarta, Indonesia, and keting fish from Central Java, Indonesia. The reagents used are methanol, sodium hydroxide (NaOH), n-hexane, boron trifluoride (BF_3), and sodium chloride obtained from Merck (Germany).

Sample Preparation

Catfish oil (CFO) and keting fish oil (KFO) were taken from the fish's entire body, from tail to head. The catfish and keting fish were cleaned, and the whole body was cut into small pieces, then ready for extraction.

Fish Oil Extraction

The extraction of CFO and KFO was carried out using the dry rendering method, which involves a cabinet dryer for approximately 24 hours at a temperature of 50-55°C combined with a hydraulic press machine with a force of 100-150 kN for 2 minutes. [12]To obtain the fish oil extract, the oil was separated from impurities by centrifugation at 5000 rpm for 10 minutes.

Fish Oil Purification

The extracted CFO and KFO were purified with bentonite, which was previously activated using an oven at 105°C. The purification procedure was done by taking 10 grams of CFO and KFO each and adding 2% bentonite. After that, CFO and KFO were heated and centrifuged at 6,500 rpm for 10 minutes to obtain clear CFO and KFO. The obtained oil was then collected and stored in dark containers. [7,10,12].

Fatty Acid Profile Analysis

The fatty acid profiles of each sample were analysed using an Agilent 8890 GC with an Agilent GC/MSD 5977B mass spectrometry detector. The fatty acids from CFO and KFO were converted into fatty acid methyl esters to be analysed by GC-MS. The methyl esters were formed by taking 200 µl of each sample, dissolving it with 1.0 ml of n-hexane, and adding 200 µl of NaOH solution in methanol while shaking. The mixture was then added with 1.5 ml of BF_3 solution and 1.5 ml of saturated NaCl solution and stirred for 10 minutes. [13]. The supernatant containing the fatty acid methyl ester derivatives was taken and injected into the GC-MS system. A 37-component fatty acid methyl ester mix from Supelco (Sigma Aldrich) was used as a reference standard for fish oil.

Data Analysis

The fatty acid profiles of CFO and KFO before and after purification with bentonite were analysed using One-way ANOVA from Minitab19 software with a significance level for all analyses set at $p<0.05$. This data was used to observe the differences in the fatty acid profiles of CFO and KFO before and after purification with bentonite.

Results and Discussion

Samples were prepared by cleaning the fish intestines, removing the fins, cutting them into fist-sized pieces, and thoroughly washing all the fish samples. Cutting the fish body aims to enlarge the sample surface area, thereby maximising the contact area of the fish sample with heat during the extraction process and ensuring proper extraction. [14].

The extraction of CFO and KFO was carried out without using organic solvents. The chosen method was dry rendering, which involves drying with a cabinet dryer assisted by a hydraulic press. This method is straightforward and does not require chemicals. [15,16]. The prepared samples were then extracted at 50°C for 24 hours. The temperature in fish oil extraction aims to damage the tissue by coagulating the proteins in the cell walls, allowing the oil to be extracted. The selected temperature minimises the oxidation process

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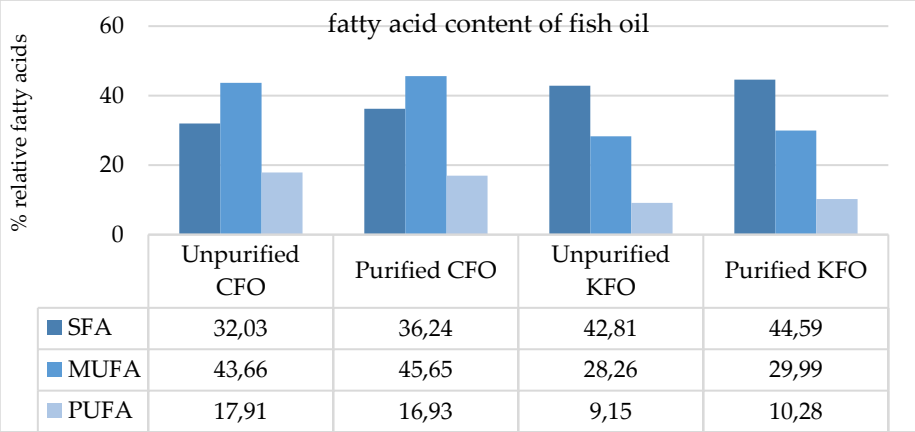
during extraction due to excessive heating. The drying time is set to maximise heat contact with the sample during the drying process. [12,17].

The dried samples were then wrapped in clean, porous filter cloth to avoid contamination during the pressing process and allow the oil to escape through the filter cloth's pores. The extraction process is marked by the hydraulic press pressing the sample down and forcing the oil out from the sides of the cylinder. The obtained oil was then collected in dark containers to prevent oxidation. [10].

The purification process aims to remove impurities and water from the extracted oil. The presence of impurities can accelerate oxidation and degradation reactions, while the presence of water can allow hydrolysis reactions to occur. The purification process begins with adding anhydrous sodium sulfate (Na_2SO_4) at 10% of the oil weight. Anhydrous Na_2SO_4 is hygroscopic and can remove water content in the extract, causing separation between oil and water [13,18]. The purification process continues physically by adding an adsorbent, a process often referred to as bleaching. Bleaching aims to remove taste and aroma and lighten the color, thereby extending the shelf life of the produced oil. Bentonite is a commonly used adsorbent because of its swelling ability, high cation exchange capacity, large surface area, and water-absorbing properties. The purification was done using a magnetic stirrer with a temperature controller. Temperature control at 40-50°C avoids oil damage and provides a better purification process [2].

Oil is a triglyceride that is part of the lipid component. The triglyceride group in an oil will have different compositions and levels of fatty acids. These compositional differences can be used to identify an oil's fingerprint pattern. Rohman and Windarsih (2019) The two samples are identical if two identical fatty acid profiles are found [19] .

The analysis of the differences in the physicochemical properties between different molecules in a mixture, separated by passing through a column, is called gas chromatography analysis. Gas chromatography separates fatty acid content in the form of fatty acid methyl esters. The analysis of fatty acid content in CFO and KFO using GC-MS is based on the reading of base peaks and SI, which are comparison values with spectra in the GC-MS software database, namely the 37-component fatty acid methyl ester mix from Supelco (Sigma Aldrich) used as a reference standard for fish oil. The results of the fatty acid profile analysis of CFO and KFO detected by GC-MS from 3 replicates of the same treatment are shown in Table 1. 29 types of fatty acid profiles were identified from the CFO and KFO tests. They are divided into three categories of fatty acids: 12 types of saturated fatty acids (SFA), eight types of monounsaturated fatty acids (MUFA), and nine types of polyunsaturated fatty acids (PUFA). There are three types of fatty acids identified in CFO but not in KFO, and conversely, there are two types of fatty acids identified in KFO but not in CFO. The highest fatty acid content in CFO is oleic acid, with a percentage of 41.92% (unpurified CFO) and 43.65% (purified CFO). In comparison, the highest fatty acid content in KFO is palmitic acid, with a percentage of 26.53% (unpurified KFO) and 27.36% (purified KFO).



	Unpurified CFO	Purified CFO	Unpurified KFO	Purified KFO
SFA	32,03	36,24	42,81	44,59
MUFA	43,66	45,65	28,26	29,99
PUFA	17,91	16,93	9,15	10,28

SFA = *saturated fatty acid;* MUFA = *monounsaturated fatty acid;* PUFA = *polyunsaturated fatty acid*

Picture 1. Histogram of Fatty Acid Profiles in the Categories of SFA, MUFA, and PUFA in CFO and KFO

A higher PUFA composition is beneficial for health and can be utilised as a functional food to prevent coronary heart disease and as a food ingredient. Based on the fatty acid profile, it is evident that CFO contains more omega-9 oleic acid (C18:1 n9c) followed by omega-6 linoleic acid (C18:2n6c) and omega-3 linolenic acid

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(C18:3n3). Meanwhile, KFO contains more omega-9, followed by omega-3 and omega-6. The omega-3 content in KFO is higher than in CFO. On the other hand, catfish, which belongs to the catfish family, has a similar content to catfish, namely omega-9 at 34%, omega-6 at 17%, and omega-3 at 0.72% [20]. However, catfish has the highest omega-6 content compared to catfish and keting fish.

Tabel 1. Result of Fatty Acid Profile Analysis of CFO and KFO

tR	%AUC	Molecular Formula	Compound Name	%Relatif Fatty Acid			
				Unpurified CFO	Purified CFO	Unpurified KFO	Purified KFO
7.402	3.86	C10:0	Capric Acid	nd	nd	0.02	0.02
9.722	3.79	C12:0	Lauric Acid	0.02	1.15	0.16	0.21
12.898	3.85	C14:0	Myristic Acid	0.82	1.3	2.2	2.22
14.805	1.98	C15:0	Pentadecanoic Acid	0.13	0.09	1.3	1.33
16.908	6.33	C16:0	Palmitic Acid	25.11	26.42	26.53	27.36
19.138	2.21	C17:0	Heptadecanoic Acid	0.19	0.15	2.15	2.4
21.48	4.47	C18:0	Stearic Acid	5.42	6.79	9.31	9.74
26.919	5.14	C20:0	Arachidic Acid	0.21	0.2	0.33	0.35
30.474	2.75	C21:0	Heneicosanoic Acid	0.02	0.02	0.11	0.16
34.834	5.46	C22:0	Behenic Acid	nd	nd	0.33	0.37
40.229	3.08	C23:0	Tricosanoic Acid	0.04	0.04	0.17	0.2
46.998	5.74	C24:0	Lignoceric Acid	0.07	0.08	0.2	0.23
13.726	1.69	C14:1	Myristoleic Acid	0.01	0.03	0.02	0.02
15.729	1.82	C15:1	Cis-10-Pentadecenoic Acid	0.01	0.02	0.09	0.11
17.642	1.91	C16:1	Palmitoleic Acid	0.73	0.91	4.37	4.75
19.922	2	C17:1	Cis-10-Heptadecenoic Acid	0.09	0.08	0.85	0.93
22.136	5.92	C18:1n9c	Oleic Acid	41.92	43.65	20.7	21.69
27.848	2.42	C20:1	Cis-11-Eicosenoic Acid	0.82	0.91	2.06	2.32
36.241	2.55	C22:1n9	Erucic Acid	0.04	0.03	0.17	0.17
49.223	2.44	C24:1	Nervonic Acid	0.04	0.02	nd	nd
23.354	1.94	C18:2n6c	Linoleic Acid	14.20	13.69	2.95	3.36
24.372	1.67	C18:3n6	γ-Linolenic Acid	0.68	0.81	0.14	0.15
25.323	1.65	C18:3n3	α-Linolenic Acid	0.50	0.49	1.25	1.29
29.706	1.85	C20:2	Cis-11,14-Eicosadienoic Acid	0.18	0.28	nd	nd
29.706	1.85	C20:2n6	Cis-11,14- Eicosadienoic Acid	0.28	0.23	0.52	0.53
30.974	2.23	C20:3n6	Cis-8,11,14-Eicosatrienoic Acid	0.67	0.78	0.33	0.37
32.12	1.73	C20:4n6	Arachidonic Acid	0.89	0.37	2.09	2.27
35.352	1.52	C20:5n3	Cis-5,8,11,14,17-Eicosapen Taenoic Acid	nd	nd	1.11	1.24
50.63	1.6	C22:6n3	Cis-4,7,19,13,16,19-Docosa Hexaenoic Acid	0.51	0.28	0.76	1.07
Total				**93.60**	**98.82**	**80.22**	**84.86**

tR = *time retention;* %AUC = *area under curve; MF = molecular formula*

The results of the tests using GC-MS show an increase in the % relative fatty acids detected with the purification using bentonite. Based on the fatty acid profile data analysis of CFO and KFO, both purified and unpurified with bentonite, significant differences were observed in SFA, MUFA, and PUFA, indicated by a p-value < 0.05 (Table 2). This is consistent with the research conducted by Indah et al. (2022) [7], which stated that using bentonite in fish oil purification can produce better quality than untreated oil. The differences in % relative values obtained are due to differences in the calculation of total fatty acids due to the varying number of detected fatty acids [21]. Limited research exists on the causes of changes in fatty acid composition.

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However, the adsorption process using bentonite affects the fatty acid composition. Bentonite can reduce free fatty acid values, color components, and other impurities [22].

Table 2. Results of the Analysis of the Effect of Bentonite on the Fatty Acid Profiles of CFO and KFO

Sample	*p Value		
	SFA	MUFA	PFA
CFO	0,002	0,026	0,015
KFO	0,014	0,033	0,041

*p Value: One-way ANOVA test result data.

Conclusions

Twenty-nine types of fatty acid profiles were identified from the CFO and KFO tests. These are divided into three categories of fatty acids: 12 types of saturated fatty acids (SFA), eight types of monounsaturated fatty acids (MUFA), and nine types of polyunsaturated fatty acids (PUFA). The highest fatty acid content in CFO is oleic acid, while in KFO, it is palmitic acid. The effect of purification using bentonite shows differences in the SFA, MUFA, and PUFA profiles in CFO and KFO, indicated by a p-value < 0.05.

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