Determination and identification of omega 3 and 6 fatty acids position in nile tilapia oil

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Abstract. The fat nutritional value is determined by the composition and distribution (position) of fatty acids in glycerol molecules. Position is determined by stereospecific numbering (sn) ie sn-1, 2 and 3 positions, where the position will affect the metabolic processes in the body. This study aims to determine the components and position of omega 3 and omega 6 fatty acids in triacylglycerol of nile tilapia oil (*Oreochromis niloticus* L.). Nile tilapia oil is obtained by extracting the soxhletation method. Analysis of fatty acid composition by gas chromatography (GC), for determining the position of fatty acid hydrolysis by using specific immobilized lipase enzymes from *Mucor miehei* at sn-1,3 position. The results showed that the composition of unsaturated fatty acids is more than saturated fatty acids. The percentage of omega fatty acids at sn-2 position is higher than in sn-1+sn-3 position. Omega 3 in sn-2 of 2.81% consisted of linolenic acid of 0.91%, EPA of 0.69%, and DHA of 1.21%, at sn-1+sn-3 of 1.55% consisted of linolenic acid by 0.10%, EPA by 1.29% and DHA by 0.16%. Omega 6 is linoleic acid in sn-2 is 13.52% and at sn-1+sn-3 is 1.04%. Based on the composition of fatty acid nile tilapia oil contained omega 3 and 6, and in the sn-2 position more so it is good to be consumed to improve human health.

Keywords: nile tilapia oil, fatty acids, position, omega 3 and 6

1. Introduction

Fish oil has special features of its fatty acid composition. Fish fat contains non-saturated fatty acids, polyunsaturated fatty acids (PUFA) which are known as omega 3 and omega 6. Natural fatty acids which include omega 3 fatty acids were linolenic acid (C18:3,w-3), eicosapentaenoic acid or EPA (C20:5,w-3), and docosahexaenoic acid or DHA (C22:6,w-3), while for omega 6 were linoleic acid (C18:2,w-6), and arachidonic acid or ARA (C20:4,w-6). The more dominant in fish oil were DHA, ARA, and EPA [1,2]. Linolenic fatty acids and linoleic fatty acids were essential fatty acids that the body needs and contained double bonds that could not be synthesized by the human body [3,4].

Consumption of omega 3 had also been shown to be effective in reducing the risk of coronary. Based on the research, it was known that the sudden death rate was caused by coronary in Eskimo races at the lowest level compared to other races in the world. It is closely related to the habits of Eskimo races who often consume omega 3 [5-7]. The content of omega 3 fatty acids in trout was generally lower than marine fish, but can still be used as a source of plural unsaturated fatty acids. Fat

content and fatty acid composition in fish is varies. Some factors that greatly affect this included species, season, geographical location, gonad maturity, and the size of the fish [8].

Consumption of fish/capita in Indonesia is still relatively low as much as 41 kg every year. It occured, since the fish which are known that contain omega 3 and omega 6 such as whales, tuna, cod, salmon, and mackerel are found in traditional markets rarely and have relatively high prices. Besides of high prices, another obstacle to consume marine fish as a source of omega 3 and omega 6 fatty acids is the continuous and massive exploration of ocean resources that will damage or disrupt biodiversity. The utilization of some types of marine fish needs to be reduced and limited by finding other alternative source, in this case it was hoped the trout that can be cultivated have the potential to replace marine fish [9].

The nutritional value of fats and oils was determined by the composition and distribution (position) of the fatty acids in the glycerol molecule. The position of fatty acids in the fat molecule was determined by stereospecific numbering (sn), namely sn-1, 2 and 3 positions in the fat molecule (triacylglycerol=TAG), that can also affect the nutritional value of fat, because this position will affect the metabolic processes in the body. Lipase enzymes play a crucial role in hydrolyzing fatty acids in the TAG structure when fat metabolism in the body. There were three sources of lipase that actively hydrolyze fat in the digestion before being absorbed, namely saliva lipase, gastric lipase, and pancreatic lipase. Lipase enzyme in humans worked specifically at sn-1, 3 position and it does not hydrolyzed acyl in sn-2 position [10,11]. Considered to that there are benefits of fish oil for health, Indonesia's fishery potential needs to be mapped to find local fish oil sources that have the opportunity to be used as raw materials for the pharmaceutical industry. As an initial stage, nile tilapia is chosen as a sample considering that this fish is the number three aquaculture in the world, with Indonesia as one of the largest nile tilapia fish fillets in the world [12-15].

2. Research Methods

The material used was nile tilapia fish that was bought from the Medan City market. The reagents for testing the fatty acid composition were NaOH 0.5N, methanol, BF_3, saturated NaCl, n hexane, and anhydrous Na_2SO_4, while for the hydrolysis process was 0.063 M CaCl, Tris-HCl buffer solution, ethanol, lipase enzyme from Mucor miehei specific to sn-1,3 position (Lipozyme ® TL IM).

Fish oil is obtained by means of a dry rendering method. A total of 500 g of fish meat fillets were washed thoroughly and then cut into small cubes and then dried in a vacuum oven for 3 hours at a temperature of 70ºC, then pressed for fish oil. Fish oil obtained from oven drying and pressing is then mixed. Fish oil that has been mixed is then added 2.5% NaCl, then heated at 50ºC. Next separated by a funnel and taken the oil. Then centrifuged at 7000 rpm for 20 minutes. Furthermore, the obtained fish oil was characterized by chemical physical features and fatty acid composition [15].

The test of physical features was cloudy point, while for chemical properties were peroxide number, saponification number, free fatty acid content, and iodine number [16]. Total of 6 g of oil was weighed in 125 mL erlenmeyer. Then distilled water was added as much as 10 mL, 2.5 mL of $CaCl_2$ 0.063 M, 5 mL of Tris-HCl buffer solution, 100 mg of lipase, then it was incubated at a temperature of 37±0.5ºC with a variation of incubation time of 10 hours and shaking was done every 1 hour, for 10 minutes. Then it was activated with 50 mL of ethanol. Then the mixture was moved to a separating funnel, shaken and let it be until two layers were formed. The top layer (fatty acid) was taken and added with 50 mL of ethanol. Then it was evaporated on top of a water bath in a evaporating dish that was known to be heavy. The fatty acid layer obtained from the evaporation results is weighed to a constant weight [17].

The oil was weighed as much as 25 mg in a closed test tube, next it was added with 1 mL of 0.5 N NaOH solution (in methanol), then shaken for 1 minute. The tube is tightly closed and heated in a 100ºC water bath for 5 minutes, then cooled to a temperature ranging from 30-40ºC. After that, added 1 ml BF_3 and closed the tube again, then heated in a 100 ºC water bath for 5 minutes. Then cooled to a temperature of 30-40ºC, then added 1 mL of n-hexane and shaken for 30 seconds. Added 2 mL of saturated NaCl solution so that two layers are formed, namely water and n-hexane layer. The formed n-hexane layer was separated so that there were only the water layer remains. The water layer was extracted again with 1 mL of n-hexane. The formed n-hexane layer was taken and combined with the

first n-hexane layer. N-hexane extract was added with 50 mg of anhydrous Na_2SO_4 and left for 15 minutes, then evaporated. Water-free liquid phase was injected as much as 1 µL, anf for analysis was using a gas chromatography tool [18]. Flowchart of the research method as in Figure 1.

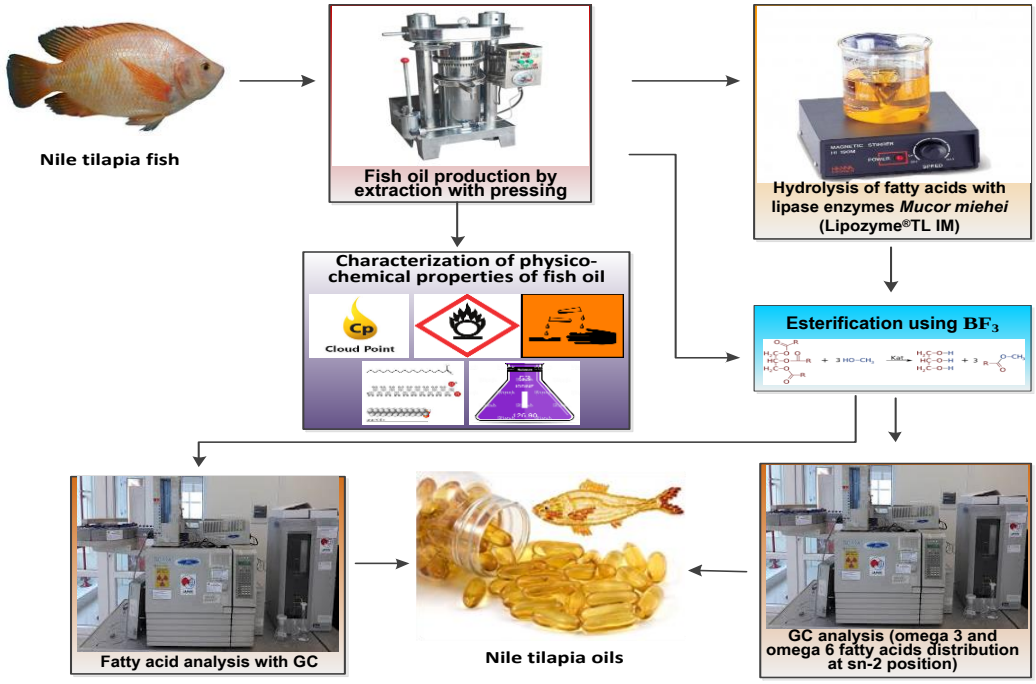

Figure 1. Flowchart of research method

The instrument used was Shimadzu QP 2010 ULTRA gas chromatography (GC) with FID detector. The column used is DB-23, 30 meters long, column temperature 40°-250°C, temperature rise 20°C/minute, detector temperature 260°C, nitrogen carrier gas, column rate 0.72 mL/minute, flow rate 37.7mL/minute [19]. The pattern for finding the deviation value is the absolute value (Δ) of the difference between the percentage of each class of fatty acids with an ideal value (33.33%). Where Δ obtained from [33.33%-%SFA]+[33.33%-%MUFA]+[33.33%-%PUFA]. If Δ is 0 then the fish oil is of good nutritional value, the greater the deviation, the worse the nutritional value [16].

3. Results and Discussion

The fish oil yield was the percentage of oil content that was obtained from the extraction of nile tilapia meat used. The yield was obtained from the comparison of the oil mass and the mass of the sample used. The sample of nile tilapia were 347.1 g and from the extraction was obtained fish oil 24.6 g and the calculation of the yield was 7.1%. Physical and chemical features of nile tilapia oil were analyzed by determination of turbid point, peroxide number, saponification number, free fatty acid content, and iodine number that is presented in Table 1.

Table 1. Physical and chemical properties of nile tilapia oils

Characteristics	Unit	Amount
Physical properties		
Cloudy point	°C	70.2
Chemical properties		
Peroxide number	meq/kg	2.66
Saponification	mg KOH/g	29.45
Free fatty acid	%	3.19
Iodine number	mg/100g	15.45

The cloudy point test was conducted to determine the presence of contamination by strange materials or mixing oil. The cloudy point was determined by heating the oil that had been added to the solvent and then left to form turbidity. The temperature at the start of turbidity was called the turbidity point. The rate of peroxide showed the level of damage from a fish oil, where the greater of the number of peroxide, thus the quality of fish oil became low. Table 1 showed that the rate of peroxide from the average yield of nile tilapia samples tested was 2.66 meq/kg. It showed that the rate of peroxide from nile tilapia oil had reached the standard peroxide requirements in fish oil up to 5.0 meq/kg [20]. Unsaturated fatty acids could bind oxygen to the double bond to form peroxide. Unsaturated fatty acids were increasingly to oxygen by increasing the number of double bonds in the molecular chain [21].

The saponification number showed the size of the molecule of fatty acids that contained in nile tilapia oil. The oil which was composed by short carbon chain fatty acids had a small molecular weight, so that it had a large saponification rate. [21]. The number of nile tilapia oil in Table 1 was 29.45 mg KOH/g, it was indicating lower than the standard. It can be concluded that the saponification number of nile tilapia oil still does not reached the standards value of SNI at 196-200 mg KOH/g [20].

Determination of acid numbers described the amount of free fatty acids that contained in the oil. Since the process of hydrolysis of triacylglycerol that occured, thus the free fatty acid was arised [21]. The greater the acid number, the oil quality became lower. The acid number according to SNI is expressed as the number of mg KOH that was needed to neutralize 1 g of free fatty acid. The acid figure can also be converted in% which was equivalent to oleic acid. Based on Table 1, it was obtained levels of free fatty acids of 3.19 mg KOH/g. Thus, it can be concluded that low-quality nile tilapia oil where the acid value obtained was greater than the standard of acid number according to BPOM which is 0.6 - 1.0 mg KOH/g [20].

Iodine numbers showed the total of unsaturated fatty acids was contained in nile tilapia oil. The double bonds that were found in unsaturated fatty acids would react with iodine. High iodine numbers indicated that the oil contained unsaturated fatty acids. The oil which contained a lot of unsaturated fatty acids, would bind iodine in large quantities (1). Iodine number of nile tilapia oil in Table 1 was 15.45 mg/100g which showed the value was lower than iodine number standard according to SNI 04-7182-2006 that was equal to 45-46 mg/100g [20].

Analysis of the type and quantity of fatty acids that contained in fish oil extracts was carried out by GC. The analyses used was GC through two stages. Fatty acids must be estered first into fatty acid methyl esters to make it easier to become gas because of the low ester vapor point. After that, it was separated in GC, so that chromatogram would be obtained which showed the number of compounds contained in the oil. The chromatogram of nile tilapia oil can be seen in Figure 2 and 3, the composition of fatty acids contained in nile tilapia oil in Table 2.

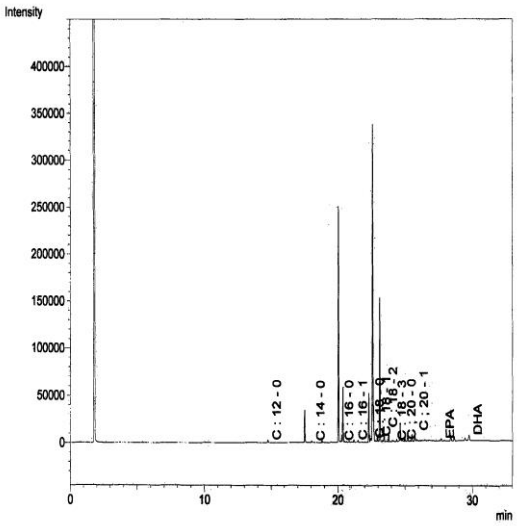

Figure 2. Chromatogram of nile tilapia oils before hydrolysis

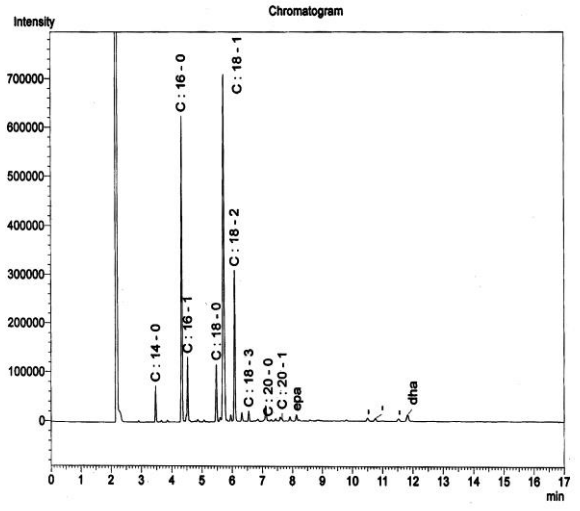

Figure 3. Chromatogram of nile tilapia oils after hydrolysis

Table 2. Composition of fatty acids contained in nile tilapia oils before and after hydrolysis by gas chromatography

Type of fatty acids	Fatty acids	Name of fatty acids	Amount (%)	
			Before hydrolysis	After hydrolysis
Saturated fatty acids	C:12-0	Lauric acid	0.34	-
	C:14-0	Myristic acid	3.43	2.70
	C:16-0	Palmitic acid	23.02	15.66
	C:18-0	Stearic acid	5.53	5.50
	C:20-0	Arachidic acid	2.56	1.84
	C:22-0	Behenic acid	-	0.15
	C:23-0	Tricosanoic acid	-	0.16
Total Saturated fatty acids (SFA)			34.88	26.01
Unsaturated fatty acids	C:16-1	Palmitoleic acid	6.39	4.40
	C:18-1	Oleic acid $^{W-9}$	41.12	36.27
	C:20-1	Eicosenoic acid $^{W-9}$	3.53	0.65
	C:18-2	Linoleic acid $^{W-6}$	14.56	13.52
	C:18-3	Linolenic acid $^{W-3}$	1.01	0.91
	C:20-5	Eicosapentaenoic acid $^{W-3}$	1.98	0.69
	C:22-6	Docosahexaenoic acid $^{W-3}$	1.37	1.21
Total Monounsaturated fatty acids (MUFA)			46.24	51.04
Total Polyunsaturated fatty acids (PUFA)			25.41	18.92
Total Unsaturated fatty acids (MUFA+PUFA)			71.65	69.96

Based on the Figures 2 and 3, and Table 2, it was known that saturated fatty acids and unsaturated fatty acids have considerable difference. The total of saturated fatty acids were 34.88%, while the total of unsaturated fatty acids were 69.96% which consisted of MUFA 51.04% and PUFA 18.92%. After hydrolysis, the total of saturated fatty acids was 26.01%, while the total unsaturated fatty acids were 57.65% which consisted of 41.32% MUFA and 16.33% PUFA. The biggest component of saturated fatty acids was palmitic acid (C: 16-0) as much as 23.02%. Unsaturated fatty acids C: 18-3 (linolenic acid), C: 20-5 (EPA), C: 22-6 (DHA) are omega 3, unsaturated fatty acids C: 18-2 (linoleic acid) are omega 6, and unsaturated fatty acids C: 18-1 (oleic acid), C: 20-1 (eicosenoic acid) were omega 9. Based on GC data, it can be concluded that the total omega 9 fatty acids in nile tilapia oil was 44 65%, while total omega 3 fatty acids were 4.36% and omega 6 was 14.56%.

The total of omega 3 fatty acids before hydrolysis were 4.36% which consisted of 1.01% linolenic acid, 1.98% EPA, 1.37% DHA, and 2.81% after hydrolysis which consisted of 0.91% linolenic acid %, EPA 0.69%, DHA 1.21%. Omega 6 fatty acids (linoleic acid) before hydrolysis amounted to 14.56% and after hydrolysis by 13.52%. Omega 9 fatty acids before hydrolysis were 44.65% which consisted of 41.12% oleic acid and 3.53% eicosenoic acid, and 36.92% after hydrolysis which consisted of 36.27% oleic acid and eicosenoic acid 0.65%. The position of unsaturated fatty acids in triacylglycerol of nile tilapia oil is presented in Table 3.

Table 3. Position of unsaturated fatty acids in triacylglycerol of nile tilapia oils

Fatty acids	Name of fatty acids	Amount (%)		
		Before hydrolysis	sn-2	sn-1 + sn-3
C:16-1	Palmitoleic acid	6.39	4.40	1.99
C:18-1	Oleic acid $^{W-9}$	41.12	36.27	4.85
C:20-1	Eicosenoic acid $^{W-9}$	3.53	0.65	2.88
C:18-2	Linoleic acid $^{W-6}$	14.56	13.52	1.04
C:18-3	Linolenic acid $^{W-3}$	1.01	0.91	0.10
C:20-5	Eicosapentaenoic acid $^{W-3}$	1.98	0.69	1.29
C:22-6	Docosahexaenoic acid $^{W-3}$	1.37	1.21	0.16
	Total Omega 3	4.36	2.81	1.55
	Total Omega 6	14.56	13.52	1.04
	Total Omega 9	44.65	36.92	7.73

From the Table 3, it can be seen the total unsaturated fatty acids in sn-2 position of 57.65%, which consisted of MUFA of 41.32% and PUFA of 16.33%, while at sn-1+sn-3 position of 12.31% consisted of MUFA of 9.72% and PUFA of 2.59%. From the position of omega fatty acids on triacylglycerol of nile tilapia oil, it can be concluded that the dominant omega fatty acids at sn-2 position were omega 9 at 36.92%, consisted of 36.27% oleic acid and 0.65% eicosenoic acid. Then omega 6 was linoleic acid by 13.52%, and omega 3 by 2.81% consists of linolenic acid by 0.91%, EPA by 0.69% and DHA by 1.21%. At sn-1+sn-3 omega 9 position was 7.73% which consisted of 4.85% oleic acid and 2.88% for eicosenoic acid, then 1.04% of omega 6, linoleic acid, and omega 3 was 1.55% consisted of linolenic acid of 0.10%, EPA of 1.29% and DHA of 0.16%.

Fish oil is one of the nutrients that contain fatty acids, because it contains about 25% saturated fatty acids and 75% unsaturated fatty acids. One method that was done to determine the nutritional value of an oil or fat is based on its fatty acid composition by counting percentage of deviation from the comparison of the ideal fatty acid group with the percentage of SFA:MUFA:PUFA that was 33.33%:33.33%:33.33%. The nutritional value of nile tilapia oil based on deviations from the ideal composition can be seen in Table 4.

Table 4. Nutritional value of nile tilapia oils

Sample	Fatty acid composition (deviation)			Total deviation (%)
	SFA (%)	MUFA (%)	PUFA (%)	
Ideal composition	33.33 (0.00)	33.33 (0.00)	33.33 (0.00)	0.00
Nile tilapia oil	34.88 (1.55)	51.04 (17.71)	18.92 (14.41)	33.67
Nile tilapia oil is hydrolyzed	26.01 (7.32)	41.32(7.99)	16.33 (17.00)	32.31

Based on the Table 4, the composition of fatty acids in nile tilapia oil and hydrolyzed nile tilapia oil consisted of SFA of 34.88% and 26.01%, MUFA 51.04% and 41.32%, and PUFA 18.92% and 16.33 %. Compared to the ideal composition of fish oil, the total deviation was 33.67% and 32.31%. This data stated that the nutritional value of nile tilapia oil had not reached the ideal composition, where the ratio of the three types of fatty acids had not reached the ratio of 33.33% and the total deviation was very high.

4. Conclusion

The percentage of omega fatty acids at sn-2 position was higher than in sn-1+sn-3 position. Omega 3 at sn-2 position of 2.81% consisted of linolenic acid by 0.91%, EPA 0.69% and DHA 1.21%, at sn-1+sn-3 position at 1.55% consisted of linolenic acid by 0.10%, EPA acid 1.29% and DHA 0.16%. Omega 6 (linoleic acid) at sn-2 position was 13.52% and at sn-1+sn-3 position was 1.04 %. From the fatty acid content, nile tilapia oil contained of omega 3 and omega 6 fatty acids, and the percentage of omega 3 and omega 6 fatty acids at sn-2 position was bigger, so it was very good to be consumed.

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