Identify the position of omega 3 and 6 in sn-2 triacylglycerol goldfish oil with hydrolysis by using lipase immobilized of *Mucor miehei*

Maruba Pandiangan[1,2,*], Jamaran Kaban[3], Basuki Wirjosentono[3] and Jansen Silalahi[4]

[1]Postgraduate Student of Chemistry Science, Department of Chemistry, Universitas Sumatera Utara, Medan, Indonesia
[2]Faculty of Agriculture, Universitas Katolik Santo Thomas, Medan, Indonesia
[3]Department of Chemistry, Universitas Sumatera Utara, Medan, Indonesia
[4]Department of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia

* *email: maruba_pandi@yahoo.com*

Abstract. Omega 3 and 6 fatty acids are very useful to be consumed for improving human health. Omega 3 and 6 are in position (stereospecific numbering) sn-1, 2 and 3 in fat molecules. Moreover, the position will affect the digestion and absorption of fatty acids in the body. In the fat metabolism of the body, fatty acids in the sn-2 position are not hydrolyzed so they can be absorbed properly. Therefore, it is necessary to identify the position of omega 3 and 6 fatty acids in fatty molecules so that the potential of fish oil as a source of omega 3 and 6 can be known. Goldfish oil from cages and ponds was extracted using the soxhletation method. Analysis of fatty acid composition by gas chromatography (GC) was previously esterified using BF_3, while for the position determination of hydrolysis of fatty acids using lipase enzyme. The results showed that the composition of unsaturated fatty acids was more than saturated fatty acids. Omega 3 and 6 fatty acids in the fat molecule are found more in the sn-2 position than in the sn-1 + sn-3 position. Based on the composition and position of fatty acids, the goldfish oil from cages and ponds contains of fatty acids omega 3 and 6, at sn-2 position so that more is absorbed in fat metabolism in the body. Thus, the goldfish oil has the potential as a source of omega 3 and 6 from the trout.

Keywords: goldfish oil, fatty acids, position omega 3 and 6

1. Introduction

Fish oil is a rich source in omega 3 and 6 multi- unsaturated fatty acids (PUFA) that give health benefits with cardiovascular effects that proved beneficial [1,2]. Natural fatty acids including fatty acids omega 3 are linolenic acid (C18:3 *n*-3), eicosapentaenoic acid (EPA;C20:5 *n*-3), docosahexaetanoic acid (DHA;C22:6 *n*-3), while for omega 6 are linoleic acid (C18:2 *n*-6) and arachidonic acid (ARA;C20:4 *n*-6). Both EPA and DHA fatty acids are the main fatty acids for the functional effects of fish oil [3,4]. Linolenic fatty acids (C18: 3 *n*- 3) which is included in the typical omega 3 and linoleic fatty acids (C18: 2 *n*- 6) which is included in typical omega 6 is essential fatty acids that is fatty acids that the body needs and contain double bonds which cannot be synthesized by the human body [5]. The American Heart Association recommends the consumption of 1g/day of omega 3 fatty acids by patients with coronary heart disease [6]. Omega 3 fatty acids is about 30% of

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.
Published under licence by IOP Publishing Ltd

the total fatty acids in natural fish oil. Esthers concentrates from omega 3 fatty acids have been formulated to provide higher amounts of EPA and DHA per dose for each patients [7].

The contents of fatty acid omega 3 in trout are generally lower than marine fish, but nevertheless they can still be used as a source of compound unsaturated fatty acids. The composition of fat and fatty acids of fish are varies. Some factors that influence this one, namely species, season, geographical location, gonad maturity and the size of the fish. Fat contents and fatty acid composition in tilapia are affected by fish species and season when harvested [8].

Long-term consumption of EPA and DHA has been proved that it gives a positive impact on patients with coronary heart disease, which is able to reduce the risk of sudden death by 45% compared to patients who do not consume EPA and DHA, reduce blood cholesterol, especially LDL, anti-platelet aggregation, and anti inflammation [9]. EPA and DHA are also useful for healing keloid symptoms. Consume the food that is rich of Omega 3 has also been shown to be effective in reducing the risk of coronary. Based on research, it is known that the sudden death rate of coronary in Eskimo races is at the lowest level compared to other races in the world. This is closely related to the habits of Eskimo races who often consume the food that is rich of omega 3 [10,11].

The nutritional value of fat is determined by the composition and distribution (position) of the fatty acids in the glycerol molecule. The position of fatty acids in the fat molecule is determined by *stereospecific numbering (sn),* namely sn-1, 2 and 3 positions in the fat molecule (triacylglycerol = TAG) can also affect the nutritional value of fat, because this position will affect the metabolic processes in the body. Lipase enzymes has a role in hydrolyzing fatty acids in the TAG structure when the fat metabolism are in the body. There are three sources of lipase that actively hydrolyze fat in digestion before being absorbed, namely saliva lipase, gastric lipase and pancreatic lipase. Lipase enzyme in humans works specifically at sn-1,3 position and does not hydrolyze acyl in sn-2 position [12,13]. Recently has been found the effect of lipid decline of fish oil which is related to the distribution/position of EPA and DHA triacylglycerol. In addition, it seems that DHA which is bound in position sn-2 triacylglycerol, can reduce the cholesterol level and rat serum triacylglycerol [14-16].

Since there are many benefits of fish oil for health, thus the Indonesia's fishery potential needs to be mapped to find the local fish oil sources, that can be used as sources of omega 3 and 6. As a first step, on this occasion goldfish *(Cyprinus carpio)* was chosen as a sample considering that it is a type of local fish, that can be obtained easily in Indonesia in large enough quantities, reaching more than 1 million tons.

2. Research Method

The material used is goldfish cultivated in cages and ponds obtained from the Medan City market. Reagents for testing the fatty acid composition were n-hexane, NaOH 0.5N, methanol, BF_3, saturated NaCl, and Na_2SO_4 anhydrous, while for the hydrolysis process were CaCl 0.063 M, Tris-HCl buffer solution, ethanol, immobilized lipase enzyme from *Mucor miehei* specifically on sn-1,3 position (Lipozyme®TL IM). Materials that were used for physico-chemical tests such as acetic acid-chloroform solvent, saturated KI, distilled water, 1% starch indicator, 0.01N sodium thiosulfate, 0.5 N KOH, 0.5 N HCl, pp indicator, 0.1 N KOH, 95% ethanol, chloroform, iodine-bromide reagent, 15% KI, and 0.1 N. sodium thiosulfate

The equipment used in the study were vacuum ovens, soxhlet apparatus, separating funnels, centrifuges, ovens, *whatman* 42 filter paper, digital scales, water baths, rotary evaporators, glassware, and gas chromatography Shimadzu QP 2010 ULTRA brand with FID detector. The column used was a DB-23, length 30 meters, column temperature 40^o - 250 ^{o}C, the rate of temperature rise of 20 ^{o}C/min, detector temperature of 260 ^{o}C, nitrogen carrier gas, column rate of 0.72 mL/min, the rate of flow 37.7mL/minute [17].

This study consists of five phases: 1. Preparation of fish oil, 2. Characterization the chemical physical features of fish oil, 3. esterification of fatty acids, 4. Analysis of fatty acids by GC, 5. Evaluation the nutritional value of fish oil, and 6. Determination the comparison of omega 3 and 6. Flowchart of the research method as in Figure 1 below.

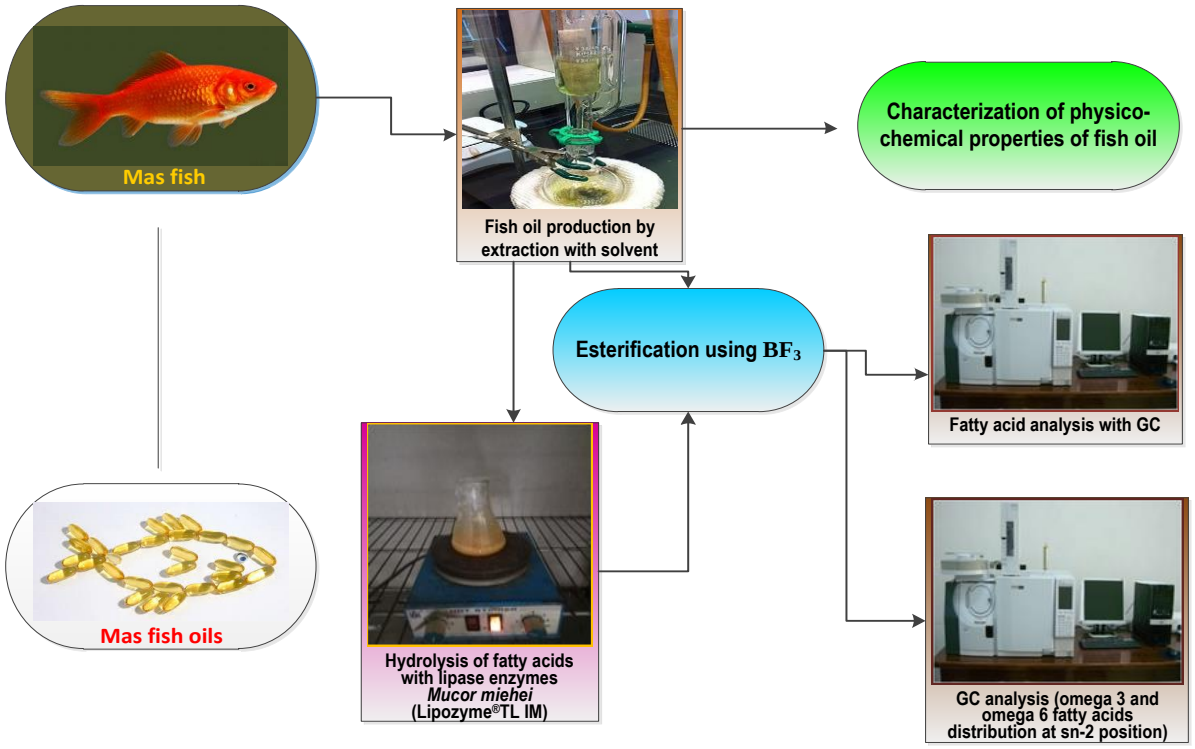


Figure 1. Flowchart of research method

Fish oil was obtained by using soxhletation method based on SNI. 01-2354.3-2006. As much as 500 g fillet of fish were washed, and then cut into small parts, then dried in a vacuum oven for 3 hours at a temperature of 70 °C. Then extracted for ± 50 minutes at a temperature of ± 80 °C with n-hexane solvent. After that the extract obtained was distilled at ± 70 °C for ± 60 minutes. Then the extract was distilled in oven at a temperature of ± 50 °C for ± 25 minutes. Furthermore, the obtained fish oil was characterized by chemical physical properties, and fatty acid composition [18].

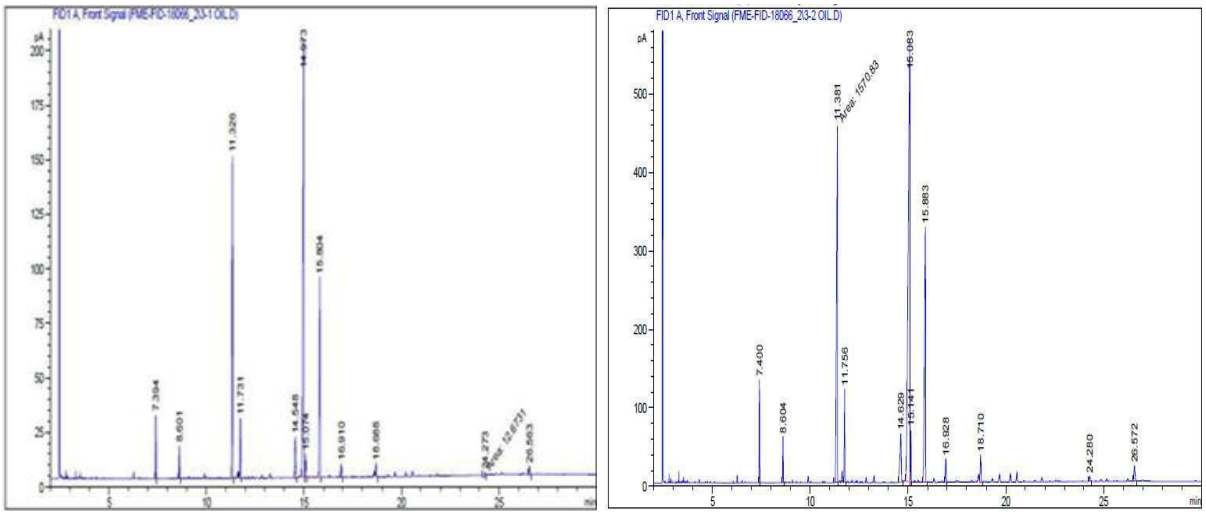
Subsequent, the oil was weighed as much as 25 mg oil in a closed test tube, and it was added with 1 mL of 0.5 N NaOH (in methanol), then shaken for 1 minute. The tube was tightly closed and heated in a 100 °C water bath for 5 minutes, and then cooled to a temperature ranging from 30-40 °C. Added 1 mL of BF_3 and closed tightly back to the tube, then heated in a 100 °C water bath for 5 minute. Next, it is cooled to a temperature of 30-40 °C and then added with 1 mL of n-hexane and shaken vigorously for 30 seconds. Added 2 mL of saturated NaCl, so that two layers are formed, namely water and n-hexane layer. The formed n-hexane layer is separated so that only the water layer remains. The water layer is extracted again with 1 mL of n-hexane. The formed n-hexane layer is taken and combined with the first n-hexane layer. N-hexane extract was added 50 mg Na_2SO_4 anhydrous and left for 15 minutes, then evaporated. Water-free liquid phase is injected as much as 1 µL for analysis using a gas chromatography tool [19,20].

The formula 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%). Δ = [33.33% -% SFA] + [33.33% -% MUFA] + [33.33% -% PUFA]. If Δ is 0 then the fish oil has good nutritional value, the greater the deviation, the worse the nutritional value. The physical features testing: cloudy point, where as the chemical features testing : peroxide number, saponification number, fatty acids levels, iodine number [21].

3. Results and discussion

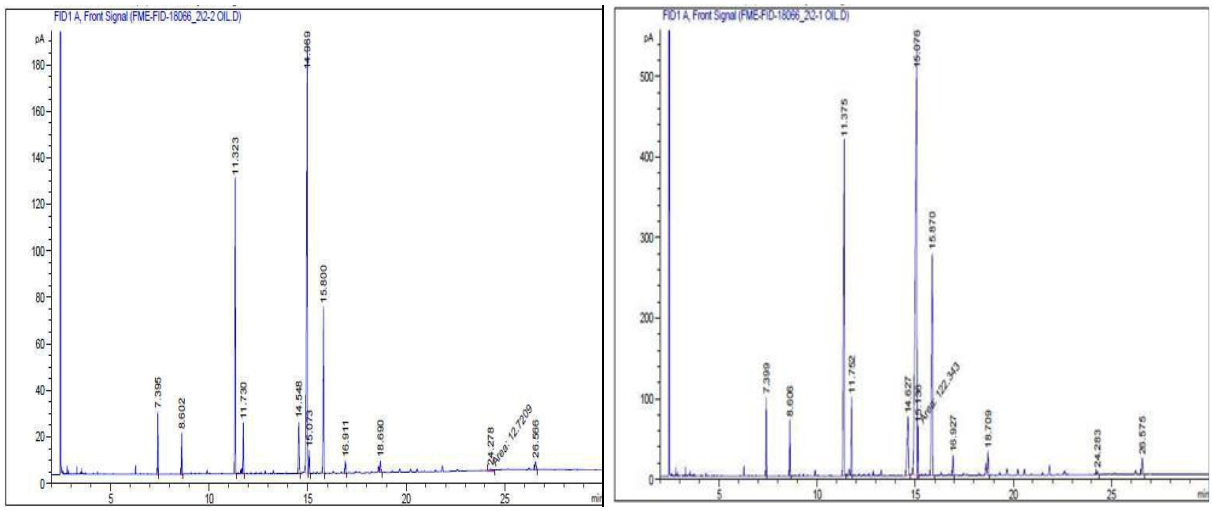
Analysis of the type and quantity of fatty acids that contained in fish oil extracts was done by GC. The analysis by GC consists of two stages. Fatty acids must be estered first into fatty acid methyl

esters to make it easier to become a gas because of the low ester vapor point. After it was separated, in the GC would be obtained chromatogram which indicates the number of compounds in the oil and the abundance of the compounds were in the percentage area. The chromatogram fish oil can be seen in Figure 2, 3 and the composition of fatty acids that contained in gold fish oil in Table 1.



(a) (b)

Figure 2. Chromatogram of goldfish oil from cages (a) before hydrolysis, (b) after hydrolysis



(a) (b)

Figure 3. Chromatogram of goldfish oil from ponds (a) before hydrolysis, (b) after hydrolysis

Based on Figures 2 and 3 and Table 1, it is known that saturated fatty acids and unsaturated fatty acids goldfish oil from cages have considerable differences, total saturated fatty acids are 29.845%, while total unsaturated fatty acids are 74.530%. After hydrolysis the total saturated fatty acids was 30.269%, while the total unsaturated fatty acids was 70.452%. In goldfish oil from ponds before hydrolysis obtained total saturated fatty acids 29.057% and total unsaturated fatty acids 74.187%. After hydrolysis total saturated fatty acids was 31.355%, while total unsaturated fatty acids was 70.767%. The biggest component of saturated fatty acids in goldfish from cages and ponds is palmitic

acid (C:16-0) which is 23.190% and 22.040%. Unsaturated fatty acids C:18-3 (linolenic acid), C:20-3 (ericosatrienoic acid), C:20-5 (EPA), C:22-6 (DHA) are omega 3, unsaturated fatty acids C:18-2 (linoleic acid), C:18-3 (γ-linoleic acid) are omega 6, and unsaturated fatty acids C:18-1 (oleic acid), C:20-1 (eicosenoic acid) are omega 9. Based on GC data, it can be concluded that the total omega 9 fatty acids in goldfish oil from cages and ponds was higher by 36.443% and 36.773%, compared to the total omega 3 by 3.735% and 2.293% as well as omega 6 as big 17.014% and 16.718%.

Table 1. The fatty acid composition that contained in goldfish oil before hydrolysis and after hydrolysis with chromatography gas

Type of fatty acids	Carbon	Name of fatty acids	Amount (%)			
			Goldfish oil cages		Goldfish oil ponds	
			Before	After	Before	After
Saturated fatty acids	C:14-0	Myrictic acid	1.690	1.800	1.465	2.165
	C:16-0	Palmitic acid	23.190	21.640	22.040	22.620
	C:18-0	Stearic acid	4.022	5.686	4.543	5.415
	C:21-0	Heneicosanoic acid	-	0.396	0.419	-
	C:24-0	Lignoceric acid	0.943	1.143	1.009	1.155
Total Saturated fatty acids (SFA)			29.845	30.269	29.057	31.355
Unsaturated fatty acids	C:16-1	Palmitoleic acid	3.940	3.074	3.574	3.282
	C:17-1	Cis-10-Heptadecanoic acid	0.523	0.425	0.474	0.425
	C:18-1	Oleic acid $^{W-9}$	46.030	45.890	46,890	46.780
	C:18-2	Linoleic acid^{W-6}	17.850	16.120	17.960	15.870
	C:18-3	γ-Linoleic acidt $^{W-6}$	0.801	0.446	0.503	0.388
	C:18-3	Linolenic acid^{W-3}	1.188	1.057	1.155	1.120
	C:20-1	Ericosenoic acid^{W-9}	1.472	1.279	1.310	1.232
	C:20-2	Ericosadienoic acid	0.786	0.780	0.724	0.518
	C:20-3	Ericosatrienoic acid^{W-3}	0.617	0.482	0.549	0.396
	C:20-5	Ericosapentaenoic acid^{W-3}	0.556	0.434	0.495	0.380
	C:22-6	Docosahexanoic acid^{W-3}	0.767	0.465	0.553	0.376
Total Monounsaturated fatty acids			51.965	50.668	52.248	51.719
Total Polyunsaturated fatty acids			22.565	19.784	21.939	19.048
Total Unsaturated fatty acids			74.530	70.452	74.187	70.767

In goldfish oil from cages of omega 3 fatty acids before hydrolysis as much 3.128% which consisted of linolenic acid 1.188%, ericosatrienoic acid 0.617%, EPA 0.556%, DHA 0.767%, and after hydrolysis as much 2.438% consisting of linolenic acid 1.057%, ericosatrienoic acid 0.482%, EPA 0.434%, DHA 0.465%. Omega 6 fatty acids before hydrolysis as much 18.651% consisting of linoleic acid 18.651%, γ-linoleic acid 0.801% and after hydrolysis as much 16.566% consisting of linoleic acid 16.120%, γ-linoleic acid 0.446%. Omega 9 fatty acids before hydrolysis as 47.502% which consisted of oleic acid 46.030% and ericosenoic acid 1.279%, and after hydrolysis as 47.169% consisting of oleic acid 45.890% and ericosenoic acid 1.311%. Likewise, goldfish oil from ponds omega 3 fatty acids before hydrolysis as 2.752% which consisted of linolenic acid 1.155%, ericosatrienoic acid 0.549%, EPA 0.495%, DHA 0.553%, and after hydrolysis as 2.272% consisting of linolenic acid 1.120%, ericosatrienoat acid 0.396%, EPA 0.380%, DHA 0.376%. Omega 6 fatty acids before hydrolysis as 18.463% consisting of linolenic acid 17.960%, γ-linoleic acid 0.503% and after hydrolysis as 16.258% consisting of linoleic acid 15.870%, γ-linoleic acid 0.388%. Omega 9 fatty acids before hydrolysis as 48.200% consisting of oleic acid 46,890% and ericosenoic acid 1.310%, and after hydrolysis it was 48.012% which consisted of 46.780% oleic acid and 1.232% ericosenoic acid.

The position of unsaturated fatty acids in goldfish oil triacylglycerols was presented in Table 2.

Table 2. The position of unsaturated fatty acids in goldfish oil triacylglycerols

Carbon	Name of fatty acids	Amount (%)			
		Goldfish oil cages		Goldfish oil ponds	
		sn-2	sn-1+sn-3	sn-2	sn-1+sn-3
C:16-1	Palmitoleic acid	3.074	0.866	3.282	0.292
C:17-1	Cis-10-Heptadecanoic	0.425	0.098	0.425	0.049
C:18-1	Oleic acid $^{W-9}$	45.890	0.140	46.780	0.110
C:18-2	Linoleic acid^{W-6}	16.120	1.730	15.870	2.090
C:18-3	γ-Linoleic acidt $^{W-6}$	0.446	0.355	0.388	0.115
C:18-3	Linolenic acid^{W-3}	1.057	0.131	1.120	0.035
C:20-1	Ericosenoic acid^{W-9}	1.279	0.193	1.232	0.078
C:20-2	Ericosadienoic acid	0.780	0.006	0.518	0.206
C:20-3	Ericosatrienoic acid^{W-3}	0.482	0.135	0.396	0.153
C:20-5	Ericosapentaenoic acid^{W-3}	0.434	0.122	0.380	0.115
C:22-6	Docosahexanoic acid^{W-3}	0. 465	0.302	0.376	0.177
	Total Omega 3	2.438	0.690	2.272	0.480
	Total Omega 6	16.566	2.085	16.258	2.205
	Total Omega 9	47.169	0.333	48.012	0.188

From Table 2 it can be seen in goldfish oil from cages total fatty acids omega 3 at sn-2 position was 2.438% consisting of linolenic acid 1.057%, ericosatrienoic acid 0.482%, EPA 0.434%, DHA 0.465%, while in position sn-1+sn-3 was 0.690% ie linolenic acid 0.131%, ericosatrienoic acid 0.135%, EPA 0.122% and DHA 0.302%. Omega 6 fatty acids at sn-2 position were 16.566% consisting of linoleic acid 16.120%, γ-linolic acid 0.446%, and in sn-1+sn-3 was 2.085% ie linoleic acid 1.730%, γ-linoleic acid 0.355%, while omega 9 at sn-2 position was 47.169%, namely oleic acid 45.890%, ericosenoict acid 1.279%, and at sn-1 + sn-3 position were 0.333% ie oleic acid 0.140%, ericosenoic acid 0.193%. In goldfish oil from ponds total omega 3 fatty acid at sn-2 position was 2.271% consisting of linolenic acid 1.120%, ericosatrienoic acid 0.396%, EPA 0.380%, DHA 0.376%, while in sn-1+sn-3 position was 0.480% ie linolenic acid 0.035%, ericosatrienoic acid 0.153%, EPA 0.115% and DHA 0.177%. Omega 6 fatty acids at sn-2 position was 16.258% consisting of linoleic acid 15.870%, γ-linoleic acid 0.388%, and sn-1+sn-3 was 2.205% ie linoleic acid 2.090%, γ-linoleic acid 0.115%, while omega 9 at sn-2 position was 48.012% namely oleic acid 46.780%, ericosenoic acid 1.232%, and sn-1+sn-3 was 0.188% ie oleic acid 0.110%, ericosenoic acid 0.078%. Judging from the position of omega fatty acids in triacylglycerol of goldfish oil from cages and ponds, it can be concluded that the dominant omega fatty acids in sn-2 position are omega 9 as much 47.169% and 48.012%, then omega 6 as much 16.566% and 16.258%, next omega 3 as much 2.438% and 2.272%.

One method that used to determine the nutritional value of an oil or fat is based on its fatty acid composition, namely by calculating the ratio of the ideal fatty acid group with the percentage of SFA: MUFA:PUFA that were 33.33%:33.33%:33.33%, and calculating the percentage deviation of absolute value or the difference from the percentage of the class of fatty acids in vegetable oil or animal fat with an ideal composition value of 33.33% for each ideal group of fatty acids. The nutritional value of goldfish oil based on deviations from the ideal composition can be seen in Table 3.

Table 3. Nutritional value of goldfish oil

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
Goldfish oil from cages	31.819 (1.51)	51.965 (18.63)	22.565 (10.76)	30.90
Goldfish oil from cages is hydrolyzed	27.295 (6.03)	50.668 (17.33)	19.784 (13.54)	36.90
Goldfish oil from ponds	31.355 (1.97)	52.248 (18.91)	21.939 (11.39)	32.27
Goldfish oil from ponds is hydrolyzed	29.057 (4.27)	51.719 (18.38)	19.048 (14.28)	36.93

Based on Table 3 the composition of fatty acids in goldfish oil from cages, goldfish oil from cages is hydrolyzed, goldfish oil from ponds and goldfish oil from ponds is hydrolyzed consisted of consecutive SFA of 31.819%, 27.819%, 31.355%, and 29.057%, MUFA respectively of 51.965%, 50.668%, 52.248% and 51.719%, then PUFA respectively 22.565%, 19.784%, 21.939% and 19.048%. Compared to the ideal composition of fish oil, the total deviations respectively were 30.90%, 36.90%, 32.27% and 36.93%. This data states that the nutritional value of goldfish oil has not met the ideal composition, where the ratio of the three types of fatty acids has not met the ratio of 33.33% and the total deviation is very high.The ratio of omega 3 fatty acids and omega 6 from goldfish oil that was obtained from analysis by gas chromatography, can be seen in Table 4.

Table 4 . The ratio of omega 3 and omega 6 fatty acids

Sample	Goldfish oil cages			Goldfish oil ponds		
	n-3	n-6	n-3/n-6	n-3	n-6	n-3/n-6
Before hydrolysis	3.128%	18.651%	(1:6)	2.752%	18.463%	(1:6.7)
Position sn-2	2.438%	16.566%	(1:6.8)	2.272%	16.258%	(1:7.1)
Position sn-1+sn-3	0.690%	2.085%	(1:3)	0.480%	2.205%	(1:4.6)

Based on Table 4 obtained a significant ratio/comparison of goldfish oil. For goldfish oil from cages obtained ratio/comparison of omega 3 and omega 6 before hydrolysis, namely (1:6), and in sn-2 position, namely (1:6.8). Whereas in the sn-1+sn-3 position the ratio/comparison is very small, namely (1:3). On goldfish oil from ponds obtained ratio/comparison of omega 3 and omega 6 before hydrolysis, namely (1:6.7), in sn-2 position namely (1:7.1 and in sn-1+sn-3 position namely (1:4.6). To maintain a healthy, long chain unsaturated fatty acid status ratio/comparison between omega 3 and omega 6 is recommended (1:1) or at least (2:1) which is the optimal ratio [22]. An excessive supply of omega 3 can have an adverse effect on enzymatic activity and the effect on membrane permeability. Excessive intake of omega 6 if it exceeds the ratio (n-6:n-3) that was (20:1) can trigger the pathogenesis of inflammation, increase the risk of cancer, vision damage, autoimmune neurodegenerative diseases. The ratio of omega 3 and omega 6 to fish oil before hydrolysis and in fish oil position sn-2 and fish oil position sn-1+sn-3 were still in the range of ratio requirements, so that it still met the requirements [23].

The chemical and physycal characteristics of goldfish oil were analyzed by the determination of cloudy point, peroxide number, saponification number, fatty acids levels, iodine number presented in Table 5.

Table 5 . The chemical and physycal characteristics of goldfish oil

Characteristics	Unit	Amount	
		Goldfish oil cages	Goldfish oil ponds
Physical properties			
Cloudy point	^{o}C	51.50	54.50
Total solid	^{o}Brix	36.00	34.00
Chemical properties			
Peroxide number	meq/kg	4.80	2.60
Saponification	mg KOH/g	41.45	69.00
Free fatty acid	%	2.69	2.89
Iodine number	mg/100g	13.38	15.07

This cloudy point test was conducted to determine the presence of contamination by foreign materials or mixing oil. This cloudy point was determined by heating the oil that has been added to the solvent until it is clear and then left to form turbidity. The temperature at the start of turbidity is called

the cloudy point. From Table 5 can be seen the cloudy point of goldfish oil from cages and ponds are 51.50 and 54.50ºC.

Table 5 shows that the rate of peroxide from the average yield of goldfish oil from cages and ponds was 4.80 and 2.60 meq/kg. This shows that the rate of peroxide from goldfish oil has met the standard peroxide requirements in fish oil up to 5.0 meq/kg [24]. The rate of peroxide shows the level of damage from a fish oil, where the greater the number of peroxide, the lower the quality of fish oil. The number of saponification of goldfish cages and ponds was 41.45 and 69.00 mg KOH/g, indicating a lower than standard SNI is 196-200 mg KOH/g. The low value of saponification indicated that the formation of fatty acids was longer in the oil chain, so that it has a large molecular weight and a small saponification rate.

Based on the Table 5 obtained levels of oil free fatty acids goldfish cages and ponds respectively 2.69 and 2.89%, greater than the standard number of acids according to BPOM which is 0.6 - 1.0% [24]. The greater the acid number, the lower the oil quality. Iodine number of goldfish oil from cages and ponds was 13.38 and 15.07 mg/100g which showed lower values than standard iodine number according to SNI 04-7182-2006 that is equal to 45 - 46 mg/100g. It can be concluded that the low iodine number indicated that the oil contains unsaturated fatty acids was low.

4. Conclusion

The results showed that the composition of unsaturated fatty acids is bigger than saturated fatty acids. Omega 3 and 6 fatty acids in goldfish oil fat molecules were found to be more in the sn-2 position than in sn-1+sn-3 positions. Omega 3 in sn-2 position in goldfish oil from cages and ponds respectively 2.438% and 2.272% consist of linolenic acid, ericosatrienoic acid, EPA and DHA. Omega 6 at sn-2 position is 16.566% and 16.258% consists of linoleic acid and γ-linoleic acid. The ratio of omega 3 and omega 6 goldfish oil from cages and ponds is (1:6.8) and (1:7.1), and still achieved the recommended ratio of (1:1) or at least (2:1), in this case omega 3 should not be too much compared to omega 6. Based on the content of fatty acid, the goldfish oils contained of omega 3 and 6, and the percentage of omega 3 and 6 at the position sn-2 too much, so it would be better in the process of absorption in fat metabolism of the body. Thus, the goldfish oil is one of the trout which is consumed by many people and it has the potential as a source of omega 3 and 6, and also it was good for improving human health.

References

[1] Mariasole D B, Hunter A M and Gray S R 2017 Fit with good fat? The role of n-3 polyunsaturated fatty acids on exercise performance *Metabolism Clinical And Experimental* 66 (2017) 45-54

[2] Itsiopoulos C, Marx W, Mayr H L, Tatucu-Babet O A, Dash S R, George E S, Trakman G L, Kelly J T, Thogoldfish C J and Brazionis L 2018 The role of omega-3 polyunsaturated fatty acid supplementation in the management of type 2 diabetes mellitus *Journal of Nutrition & Intermediary Metabolism* xxx:1-10

[3] Simopoulos A P 2016 An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity *Nutrients* 8 128: 1-17 doi:10.3390/nu8030128

[4] Peltoma E, Johnson M D and Taipale S J 2018 Marine cryptophytes are great sources of EPA and DHA *Marine Drugs* 16 3: 1-11 doi:10.3390/md16010003

[5] Spector A A and Kim H Y 2015 Thematic review series: Living history of lipids. Discovery of essential fatty acids *Journal of Lipid Research* Volume 56 2015: 11-21

[6] Jain A P, Aggarwal K K and Zhang P Y 2015 Omega-3 fatty acids and cardiovascular disease European Review for Medical and Pharmacological Sciences 19: 441-445

[7] Moharana T R, Byreddy A R, Puri M, Barrow C and Rao N M 2016 Selective enrichment of omega-3 fatty acids in oils by phospholipase A1 *Plos One* 1-14

[8] Twining C W, Brenna J T, Lawrence P, Shipley J R, Tollefson T N and Winkler D W 2016 Omega-3 long-chain polyunsaturated fatty acids support aerial insectivore performance more than food quantity *PNAS* vol. 113 no. 39: 10920-10925

[9] Mori T A 2017 Marine omega-3 fatty acids in the prevention of cardiovascular disease *Fitoterapia* Volume 123 Pages 51-58
[10] Radcliffe J E, Thogoldfish J, A.L. Bramley A L, Kouris-Blazos A, Radford B E, Scholey A B, Pipingas A, Thogoldfish C J and Itsiopoulos C 2016 Controversies in omega-3 efficacy and novel concepts for application *Journal of Nutrition & Intermediary Metabolism* 5:11-22
[11] Stark K D, Van Elswyk M E, Higgins M R, Weatherford C A and Salem N Jr 2016 Global survey of the omega-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid in the blood stream of healthy adults *Progress in Lipid Research* 63:132-152
[12] Borrelli G M and Trono D 2015 Recombinant lipases and phospholipases and their use as biocatalysts for industrial applications *Int. J. Mol. Sci.* 16 20774-20840
[13] Alfieri A, Imperlini E, Nigro E, Vitucci D, Orrù S, Daniele A, Buono P and Mancini A 2018 Effects of plant oil interesterified triacylglycerols on lipemia and human health. *Int. J. Mol. Sci.*19 104
[14] Yoshinaga K, Sasaki K, Watanabe H, Nagao K , Inoue N, Shirouchi B, Yanagita T, Nagai T, Mizobe H and Kojima K 2015 Differential effects of triacylglycerol positional isomers containing n-3 series highly unsaturated fatty acids on lipid metabolism in C57BL/6J mice *J. Nutr. Biochem*. 2015 26 431-432
[15] Ruiz-Lopez N, Stubhaug I, Ipharraguerre I, Rimbach G and Menoyo D 2015 Positional distribution of fatty acids in triacylglycerols and phospholipids from fillets of atlantic salmon (salmo salar) fed vegetable and fish oil blends *Marine Drugs* 13 4255-4269
[16] Innes J K and Calder P C 2018 The differential effects of eicosapentaenoic acid and docosahexaenoic acid on cardiometabolic risk factors: a systematic review *Int. J. Mol. Sci*. 19 532
[17] Zhang H, Shen Y, Zhang Y, Li L and Wang X 2018 Regiospecific analysis of fatty acids and calculation of triglyceride molecular species in marine fish oils *BioMed Research International* Volume 2018 Article ID 9016840 7 pages
[18] Ivanovs K and Blumberga D 2017 Extraction of fish oil using green extraction methods: a short review *Energy Procedia* 128 (2017) 477-483
[19]. Senarath S, Yoshinaga K, Nagai T, Yoshida A, Beppu F, Jayasinghe C, Devadawson C and Gotoh N 2017 Quantitative analysis of the distribution of cis-eicosenoic acid positional isomers in marine fishes from the indian ocean *J. Oleo Sci*. 66 (2) 187-197
[20] Utami P I, Rahayu W S, Nugraha I and Rochana A N 2018 Fatty acid analysis of lipid extracted from rats by gas chromatography goldfishs spectrometry method *Materials Science and Engineering* 288 (2018) 012115
[21] Association of Official Analytical Chemists 2016 *Official Methods of Analysis of AOAC International 20th Edition* (2016) Rockville MD 20850-3250 USA
[22] Akerele O A and Cheema S K 2016 A balance of omega-3 and omega-6 polyunsaturated fatty acids is important in pregnancy *Journal of Nutrition & Intermediary Metabolism* 5 (2016) 23-33
[23] Wang H and Daggy B P 2017 The Role of Fish Oil in Inflammatory Eye Diseases *Biomed Hub* 2017 2:455818
[24] Codex Alimentarius Commission 2017 *Standard for Fish Oil* CXS 329-2017 Food and Agriculture Organization of the United Nations WHO Roma Italy

Acknowledgments

The author expressed his gratitude to Rector of the Universitas Katolik Santo Thomas, Rector of the Universitas Sumatera Utara and the Ministry of Research and Technology of the Republic of Indonesia for permission to study and BPPDN education and research funding assistance at the program Doctor of Chemistry Science of Universitas Sumatera Utara.