Identification omega 3 and 6 on the positions sn-2 triacylglycerol Nile tilapia fish oil by hydrolysis using lipase from Mucor miehei

M Pandiangan1,4*, J Kaban2, B Wirjosentono2 and J Silalahi3
1Postgraduate Student of Chemistry Science, Universitas Sumatera Utara, Medan, Indonesia
2Department of Chemistry, Universitas Sumatera Utara, Medan, Indonesia
3Department of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia
4Agricultural Products Technology, Faculty of Agriculture, Universitas Katolik Santo Thomas, Medan, Indonesia
*Corresponding author: maruba.pandiangan@gmail.com

Abstract. Omega 3 and 6 are unsaturated fatty acids needed by the body for cell formation and control of inflammation. In the molecules of omega 3 and 6 fats found in the position (stereospecific numbering) sn-1, 2 and 3. The position of fatty acids affects the digestion and absorption processes in the body. In the metabolism of fat in 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 fat molecules so that the potential of fish oils Nile tilapia as a source of omega 3 and 6 can be known. Fish oils Nile is extracted with the soxhletation method. The fatty acid composition was analyzed by gas chromatography (GC) which was previously esterified using BF3, for hydrolysis of fish oil 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 than in the sn-1+sn-3 position. Seen from the composition and position fatty acids of Nile tilapia oil which contain omega 3 and 6 and there is a sn-2 position, so that more is absorbed in fat metabolism in the body. Thus fish oils Nile tilapia has the potential as a source of omega 3 and 6 from one of the freshwater fish that is widely consumed by the public.

1. Introduction
Consumption of foods rich in omega 3 has been shown to be effective in reducing the risk of heart attacks. Fish oil is a rich source of omega 3 and 6 polyunsaturated fatty acids (PUFA) that provide health benefits with cardiovascular effects that have proven beneficial [1,2]. Natural fatty acids which include omega 3 fatty acids are linolenic acid (C18:3,ω-3), ericosapentaenoic acid or EPA (C20:5,ω-3), docosahexanoic acid or DHA (C22:6,ω-3), whereas for omega 6 are linoleic acid (C18:2,ω-6) and arachidonic acid or ARA (C20:4,ω-6) while the more dominant ones in fish oil are DHA, ARA and EPA [3,4]. Based on research, it is known that the sudden death rate due to heart attacks in Eskimo races is at the lowest level compared to other races in the world. This turned out to be closely related to the habits of Eskimo races who often consume a menu rich in omega 3 [5,6,7].

The use of fish as a source of omega 3 and 6 is still very low. This is because fish which are known to contain omega 3 and high omega 6 such as whales, tuna, cod, salmon, and mackerel are rare fish found in traditional markets and have relatively high prices. In addition to high prices, another obstacle
to the use of marine fish as a source of omega 3 and 6 fatty acids is the continuous and massive exploration of ocean resources will damage or disrupt biodiversity. The use of some rare types of marine fish needs to be reduced and limited by finding other alternative sources, in this case it is hoped that freshwater fish that can be cultivated have the potential to replace marine fish, one of which is cultivation Nile tilapia fish [8,9,10].

The position of fatty acids in the fat molecule (triacylglycerol = TAG) is determined by stereospecific numbering (sn) ie sn-1, 2 and 3 positions, which can affect the nutritional value of fat, because this position will affect the metabolic processes in the body. Lipase enzymes play a role in hydrolyzing fatty acids in the TAG structure when fat metabolism 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. Thus the fatty acid in sn-2 position will be absorbed properly in the process of metabolizing fat in the body [11,12]. Recently it has been found that the effect of lipid reduction from fish oil is related to the distribution/position of EPA and DHA on TAG. In addition, it seems that DHA bound to sn-2 position can reduce rat serum cholesterol and TAG levels [13,14,15].

2. Research Methods

The material used is Nile tilapia fish cultivated in cages and ponds obtained from the Medan City market. The research is conducted in January-June 2018 at the USU Laboratory and Unika Santo Thomas Laboratory. Reagents for testing the fatty acid composition are NaOH 0.5N, methanol, BF₃, saturated NaCl, n hexane, and anhydrous Na₂SO₄, for the hydrolysis process is 0.063 M CaCl, Tris-HCl buffer solution, ethanol, immobilized lipase enzyme from Mucor miehei specific to sn-1,3 position (Lipozyme® TL IM).

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 [16]. Testing of physical properties: cloudy point. Testing of chemical properties: peroxide number, saponification number, free fatty acid level, iodine number [17].

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 CaCl₂ 0.063 M, 5 mL of Tris-HCl buffer solution, 100 mg of lipase, then it was incubated at a temperature of 37 ± 0.5°C 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. Then it was evaporated on top of a water bath in an evaporating dish that was known to be heavy. The fatty acid layer obtained from the evaporation results is weighed to a constant weight. If the final weight has been stabilized then the hydrolysis reaction has been perfect [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 BF₃ 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 Na₂SO₄ 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]. Flowchart of the research method as in Figure 1. The instrument used was gas chromatography (GC) Shimadzu QP 2010 ULTRA with FID detector. The column used is DB-23, 30 meters long, column temperature 40°C-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 [20].
3. Results and Discussion
The analysis uses GC through two stages, namely first esterification of fatty acids to fatty acid methyl esters to make it easier to become a gas because of the low ester vapor point. After that the separation of fatty acids in GC and obtained chromatogram which shows the number of compounds contained in oil. The chromatogram of Nile tilapia oil can be seen in Figures 2 and 3, the composition of fatty acids contained in Nile tilapia oil in Table 1.

![Figure 1. Flowchart of research method](image)

![Figure 2. Chromatogram of Nile tilapia oil from cages (a) before hydrolysis, (b) after hydrolysis](image)
Figure 3. Chromatogram of Nile tilapia oil from ponds (a) before hydrolysis, (b) after hydrolysis

Table 1. Composition of fatty acids contained in Nile tilapia fish oils before and after hydrolysis by gas chromatography

| Type of fatty acids | Carbon   | Name of fatty acids | Amount (%) | Nile tilapia oil cages | Nile tilapia oil ponds |
|---------------------|----------|---------------------|------------|------------------------|------------------------|
|                     |          |                     |            | Before hydrolysis      | After hydrolysis        |
| Saturated fatty acids |         |                     |            | Before hydrolysis      | After hydrolysis        |
| C:14-0              |          | Myristic acid       | 2.604      | 2.239                  | 3.181                  |
| C:15-0              |          | Pentadecanoic acid  | -          | 0.194                  | 0.299                  |
| C:16-0              |          | Palmitic acid       | 27.470     | 26.960                 | 27.440                 |
| C:17-0              |          | Heptadecanoic acid  | -          | 0.217                  | 0.283                  |
| C:18-0              |          | Stearic acid        | 7.105      | 6.460                  | 6.514                  |
| C:20-0              |          | Arachidic acid      | -          | 0.209                  | 0.216                  |
| C:21-0              |          | Heneicosanoic acid  | 0.719      | 0.670                  | 0.895                  |
| C:24-0              |          | Lignoceric acid     | 0.720      | 0.404                  | 0.497                  |
|                     |          | Total Saturated fatty acids | 38.618   | 37.353                 | 39.325                 |
|                     |          | Total Unsaturated fatty acids | 62.864   | 61.546                 | 62.617                 |
| Unsaturated fatty acids |       |                     |            | Before hydrolysis      | After hydrolysis        |
| C:16-1              |          | Palmitoleic acid    | 4.798      | 4.334                  | 5.080                  |
| C:17-1              |          | Cis-10-Heptadecanoic acid | -      | -                      | 0.243                  |
| C:18-1              |          | Oleic acid<sup>W-9</sup> | 34.950     | 34.930                 | 35.440                 |
| C:18-2              |          | Linoleic acid<sup>W-6</sup> | 15.990     | 15.720                 | 15.780                 |
| C:18-3              |          | γ-Linoleic acid<sup>W-6</sup> | 1.024     | 0.999                  | 0.938                  |
| C:18-3              |          | Linolenic acid<sup>W-3</sup> | 1.139     | 1.090                  | 1.288                  |
| C:20-1              |          | Ercoosenoic acid<sup>W-9</sup> | 1.493     | 1.311                  | 1.333                  |
| C:20-2              |          | Ericosadienoic acid | 0.874      | 0.758                  | 0.880                  |
| C:20-3              |          | Ericosatrienoic acid<sup>W-3</sup> | 0.903     | 0.862                  | 0.779                  |
| C:20-5              |          | Ericosapentaenoic acid<sup>W-3</sup> | 0.310     | 0.280                  | 0.250                  |
| C:22-6              |          | Docosahexanoic acid<sup>W-3</sup> | 1.383     | 1.262                  | 0.606                  |
|                     |          | Total MUFA          | 41.241     | 40.575                 | 42.096                 |
|                     |          | Total PUFA          | 21.623     | 20.971                 | 20.521                 |
|                     |          | Total Unsaturated fatty acids | 62.864   | 61.546                 | 62.617                 |
Based on Figures 2 and 3 and Table 1, it is known that saturated fatty acids and unsaturated fatty acids Nile tilapia oil from cages have considerable differences, total saturated fatty acids are 38.618%, while total unsaturated fatty acids are 62.864% which consists of MUFA 41.241% and PUFA 21.623%. After hydrolysis the total saturated fatty acids was 37.353%, while the total unsaturated fatty acids was 61.546% which consisted of MUFA 40.575% and PUFA 20.971%. In Nile tilapia oil from ponds before hydrolysis obtained total saturated fatty acids 39.325% and total unsaturated fatty acids 62.617% which consisted of MUFA 40.782% and PUFA 19.838%. Unsaturated fatty acids in Nile tilapia oil from cages and ponds was higher by 36.443% and 36.773%, compared to the total omega 6 by 3.735% and 2.293% as well as omega 9 fatty acids in Nile tilapia 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 17.014% and 16.718%.

In Nile tilapia oil from cages of omega 3 fatty acids before hydrolysis as much 3.735% which consisted of linolenic acid 1.139%, ericosatrienoic acid 0.903%, EPA 0.310%, DHA 1.383%, and after hydrolysis as much 3.494% consisting of 1.090%, ericosatrienoic acid 0.862%, EPA 0.280%, DHA 1.262%. Omega 6 fatty acids before hydrolysis as much 17.014% consisting of linoleic acid 15.990%, γ-linoleic acid 1.024% and after hydrolysis as much 16.719% consisting of linoleic acid 15.720%, γ-linoleic acid 0.999%. Omega 9 fatty acids before hydrolysis as much 36.443% which consisted of oleic acid 34.930% and ericosenoic acid 1.311%. Likewise, Nile tilapia oil from ponds omega 3 fatty acids before hydrolysis as 2.923% which consisted of linolenic acid 1.288%, ericosatrienoic acid 0.779%, EPA 0.250%, DHA 0.606% and after hydrolysis as 2.695% consisting of linolenic acid 1.222 %, ericosasatennoac acid 0.767%, EPA 0.190%, DHA 0.516%. Omega 6 fatty acids before hydrolysis as much 16.718% consisting of linolenic acid 15.780%, γ-linoleic acid 0.938% and after hydrolysis as 16.283% consisting of linolenic acid 15.400%, γ-linoleic acid 0.883%. Omega 9 fatty acids before hydrolysis as much 36.773% consisting of oleic acid 35.440% and ericosenoic acid 1.333%, and after hydrolysis it was 36.147% which consisted of oleic acid 34.840% and ericosenoic acid 0.767%.

The position of unsaturated fatty acids in triacylglycerol of Nile tilapia oil is presented in Table 2.

| Carbon | Name of fatty acids                        | Amount (%)       |
|--------|-------------------------------------------|------------------|
|        |                                           | Sn-2, Sn+Sn3     |
|        |                                           | Nile tilapia oil cages | Nile tilapia oil ponds |
| C:16-1 | Palmitoleic acid                          | 4.334, 0.464     | 4.635, 0.445          |
| C:17-1 | Cis-10-Heptadecanoic acid                 | -                | -                    |
| C:18-1 | Oleic acid W.9                            | 34.930, 0.020    | 34.840, 0.600         |
| C:18-2 | Linoleic acid W.6                         | 15.720, 0.270    | 15.400, 0.380         |
| C:18-3 | γ-Linoleic acid W.6                       | 0.999, 0.025     | 0.883, 0.055          |
| C:18-3 | Linolenic acid W.3                        | 1.090, 0.049     | 1.222, 0.066          |
| C:20-1 | Ericosenoic acid W.9                      | 1.311, 0.182     | 1.307, 0.026          |
| C:20-2 | Ericosadienoic acid                       | 0.758, 0.116     | 0.860, 0.020          |
| C:20-3 | Ericosatrienoic acid W.3                  | 0.862, 0.041     | 0.767, 0.012          |
| C:20-4 | Ericsapentaenoic acid W.3                 | -                | -                    |
| C:20-5 | Docosahexanoic acid W.3                   | 0.280, 0.030     | 0.190, 0.060          |
| C:22-6 | Palmitoleic acid                          | 1.262, 0.121     | 0.516, 0.090          |
|        | Total Omega 3                             | 3.494, 0.241     | 2.695, 0.228          |
|        | Total Omega 6                             | 16.719, 0.295    | 16.283, 0.435         |
|        | Total Omega 9                             | 36.241, 0.202    | 36.147, 0.626         |
From Table 2 it can be seen in Nile tilapia oil from cages total fatty acids omega 3 at sn-2 position was 3.494% consisting of linolenic acid 1.090%, ericosatetraenoic acid 0.862%, EPA 0.280%, DHA 1.262%, while in position sn-1+sn-3 was 0.241% ie linolenic acid 0.049%, ericosatetraenoic acid 0.041%, EPA 0.030% and DHA 0.121%. Omega 6 fatty acids at sn-2 position were 16.719% consisting of linoleic acid 15.720%, γ-linolic acid 0.999%, and in sn-1+sn-3 was 0.295% ie linoleic acid 0.270%, γ-linolic acid 0.025%, while omega 9 at sn-2 position was 36.241%, namely oleic acid 34.930%, ericosenoic acid 1.311%, and at sn-1 + sn-3 position were 0.202% ie oleic acid 0.020%, ericosenic acid 0.041%. In Nile tilapia fish oil from ponds total omega 3 fatty acid at sn-2 position was 2.695% consisting of linolenic acid 1.222%, ericosatetraenoic acid 0.767%, EPA 0.190%, DHA 0.516%, while in sn-1+sn-3 position was 0.228% ie linolenic acid 0.066%, ericosatetraenoic acid 0.012%, EPA 0.060% and DHA 0.090%. Omega 6 fatty acids at sn-2 position was16,283% consisting of linoleic acid 15.400%, γ-linolic acid 0.767%, and sn-1+sn-3 was 0.435% ie linoleic acid 0.380%, γ-linolic acid 0.055%, while omega 9 at sn-2 position was 36.147% namely oleic acid 34.840%, ericosenic acid 1.307%, and sn-1+sn-3 was 0.626% ie oleic acid 0.600%, ericosenoic acid 0.012%.

Judging from the position of omega fatty acids in triacylglycerol of Nile tilapia oil from cages and ponds, it can be concluded that the dominant omega fatty acids in sn-2 position are omega 9 as much 36.241% and 36.147%, then omega 6 as much 16.719% and 16.283%, next omega 3 as much 3.494% and 2.695%.

The ratio/comparison of omega 3 and 6 fatty acids from Nile tilapia oil obtained from the analysis by gas chromatography can be seen in Table 3.

| Sample                     | Nile tilapia oil cages | Nile tilapia oil ponds |
|----------------------------|------------------------|------------------------|
|                            | Omega 3 (n-3)          | Omega 6 (n-6)          | Ratio/Comparison (n-3/n-6) | Omega 3 (n-3) | Omega 6 (n-6) | Ratio/Comparison (n-3/n-6) |
| Before hydrolysis          | 3.735%                 | 17.014                 | (1:4.5)                    | 2.923%        | 16.718%        | (1:5.7)                     |
| Position sn-2              | 3.494%                 | 16.719                 | (1:4.7)                    | 2.695%        | 16.283%        | (1:6)                       |
| Position sn-1+sn-3         | 0.241%                 | 0.295%                 | (1:1.2)                    | 0.228%        | 0.435%         | (1:2)                       |

Based on Table 4 obtained a significant ratio/comparison of Nile tilapia oil. For Nile tilapia oil from cages obtained ratio/comparison of omega 3 and omega 6 before hydrolysis, namely (1:4.5), and in sn-2 position, namely (1:4.7). Whereas in the sn-1+sn-3 position the ratio/comparison is very small, namely (1:1.2). On Nile tilapia oil from ponds obtained ratio/comparison of omega 3 and omega 6 before hydrolysis, namely (1:5.7), in sn-2 position namely (1:6) and in sn-1+sn-3 position namely (1:2). 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 [21,22]. Excessive intake of omega 3 can cause adverse effects on enzymatic activity and effects on membrane permeability. Excessive intake of omega 6 if it exceeds the ratio/comparison (n-6: n-3) that is (20:1) can trigger the pathogenesis of inflammation, increase the risk of cancer, vision damage, autoimmune neurodegenerative diseases. Ratio/comparison of omega 3 and omega 6 to fish oil before hydrolysis and in the position sn-2 and sn-1 + sn-3 is still in the range of ratio/comparison reference so that it still meets the requirements [23].

Fish oil is one of the nutrients that contain fatty acids rich in benefits because it contains about 25% saturated fatty acids and 75% unsaturated fatty acids. One method that is 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 is 33.33%: 33.33%: 33.33%. Δ = [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 [17]. 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 fish 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 |
| Nile tilapia oil from cages  | 38.618 (5.28)                     | 41.241 (7.91)       | 21.623 (11.71) | 24.90 |
| Nile tilapia oil from cages is hydrolyzed | 37.353 (4.02) | 40.575 (7.24) | 20.971 (12.35) | 23.61 |
| Nile tilapia oil from ponds   | 39.325 (5.99)                     | 42.096 (8.76)       | 20.521 (12.81) | 27.56 |
| Nile tilapia oil from ponds is hydrolyzed | 37.602 (4.27) | 40.782 (7.45) | 19.838 (13.49) | 25.21 |

Based on Table 5 the composition of fatty acids in Nile tilapia oil from cages, Nile tilapia oil from cages is hydrolyzed, Nile tilapia oil from ponds and Nile tilapia oil from ponds is hydrolyzed consisted of consecutive SFA of 38.618%, 37.353%, 39.325%, and 37.602%, MUFA respectively of 41.241%, 40.575%, 42.096% and 40.782%, then PUFA respectively 21.623%, 20.971%, 20.521% and 19.838%. Compared to the ideal composition of fish oil, the total deviations respectively were 24.90%, 23.61%, 27.56% and 25.21%. This data states that the nutritional value of tilapia 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.

Table 5. Physical and chemical properties of Nile tilapia fish oil

| Characteristics | Unit  | Amount Nile tilapia oil cages | Amount Nile tilapia oil ponds |
|-----------------|-------|-------------------------------|------------------------------|
| Cloudy point    | ºC    | 70.2                          | 69.8                         |
| Peroxide number | meq/kg | 2.66                          | 2.81                         |
| Saponification  | mg KOH/g | 29.45                        | 31.04                        |
| Free fatty acid | %     | 3.19                          | 3.07                         |
| Iodine number   | mg/100g | 15.45                        | 16.26                        |

This cloudy point test is conducted to determine the presence of contamination by foreign materials or mixing oil. This cloudy point is 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 Nile tilapia oil from cages and ponds are 70.2 and 69.8ºC.

Table 5 shows that the rate of peroxide from the average yield of Nile tilapia fish oil from cages and ponds was 2.66 and 2.81 meq/kg. This shows that the rate of peroxide from Nile tilapia 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 Nile tilapia cages and ponds was 29.45 and 31.04 mg KOH/g, indicating lower than the SNI standard which is 196-200 mg KOH/g. The low saponification value indicates that the formation of fatty acids is longer in the chain so that it has a large molecular weight and a small saponification rate.

From Table 5 obtained levels of oil free fatty acids Nile tilapia cages and ponds respectively 3.19 and 3.07%, 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. The iodine number of Nile tilapia oil from cages and ponds was 15.45 and 16.26 mg/100g respectively, which showed a lower value than the iodine number standard according to SNI 04-7182-2006 which was 45-46 mg/100g. It can be concluded that low iodine numbers indicate that the oil contains low unsaturated fatty acids.

4. Conclusion

The results showed that fatty acids omega 3 and 6 on Nile tilapia oil fat molecules were found more in sn-2 position than in sn-1+ sn-3 position. Omega 3 in sn-2 position in Nile tilapia fish oil from cages and ponds respectively 3.494% and 2.695% consist of linolenic acid, ericosatrienoic acid, EPA and DHA. Omega 6 at sn-2 position is 16.719% and 16.283% consists of linoleic acid and γ-linoleic acid. The ratio/comparison of omega 3 and omega 6 Nile tilapia from cages and ponds is (1:4.7) and (1:6),
and still meets the recommended ratio of (1:1) or at least (2:1), in this is not too much omega 3 compared to omega 6. Viewed from the womb Nile tilapia oil fatty acids contain omega 3 and 6, and the percentage of omega 3 and 6 on sn-2 position is more so that it is better in the process of absorption in fat metabolism in the body. Thus Nile tilapia fish, one of the freshwater fish that is widely consumed by the public, has the potential as a source of omega 3 and 6, which is very good for improving human health.

Acknowledgments
The author conveyed his gratitude to the Ministry of Ristekdikti RI and the Rector of the Universitas Sumatera Utara for funding education and research BPPDN in Doctoral Program of Chemistry Science, Universitas Sumatera Utara.

References
[1] Mariasole D B, Hunter A M, Gray S R 2017 Metabolism Clinical And Experimental 66 45-54
[2] Itsiopoulos C, Marx W, Mayr H L, Tatuçu-Babet O A, Dash S R, George E S, Trkman G L, Kelly J T, Thomas C J, Brazionis L 2018 Journal of Nutrition & Intermediary Metabolism 1-10
[3] Simopoulos A P 2016 Nutrients 8 (128) 1-17
[4] Peltoma E, Johnson M D, Taipale S J 2018 Marine Drugs 16 (3) 11
[5] Radcliffe J E, Thomas J A L, Bramley A L, Kouris-Blazos A, Radford B E, Scholey A B, Pipingas A, Thomas C J, Itsiopoulos C 2016 Journal of Nutrition & Intermediary Metabolism 5 11-22
[6] Stark K D, Van Elswyk M E, Higgins M R, Weatherford C A, Salem N Jr 2016 Progress in Lipid Research 63 132-152
[7] Mori T A 2017 Fitoterapia 123 51-58
[8] Mattimu J A, As’ad S, Nurdin M A, Bahar B 2016 International Journal of Sciences Basic and Applied Research (IJSBAR) 29 (3)165-170
[9] Ayisi C L, Zhao J L 2017 Turkish Journal of Fisheries and Aquatic Sciences 17 405-415
[10] Stoneham T R, Kuhn D D, Taylor D P, Neilson A P, Smith S A, Gatlin D M, Chu H S, O’Keefe S F 2018 Plos One 11 1-14
[11] Alfieri A, Imperlini E, Nigro E, Vitucci D, Orrù S, Daniele A, Buono P, Mancini A 2018 Int J Mol Sci 19 104
[12] Shahidi F, Ambigaipalan P 2018 Annual Review of Food Science and Technology 9 345-381
[13] Yoshinaga K, Sasaki K, Watanabe H, Nagao K, Inoue N, Shirouchi B, Yanagita T, Nagai T, Mizobe H, Kojima K 2015 J Nutr Biochem 26 431-432
[14] Ruiz-Lopez N, Stubhaug I, Ishpharraguee I, Rimbach G, Menoyo D 2015 Marine Drugs 13 4255-4269
[15] Innes J K, Calder P C 2018 Int J Mol Sci 19 532
[16] Ivanovs K, Blumberga D 2017 Energy Procedia 128 477-483
[17] Association of Official Analytical Chemists 2016 Official Methods of Analysis of AOAC International 20th Edition (2016) Rockville MD 20850-3250 USA
[18] de Araújo M E M B, Campos P R B, Alberto T G, Contesini F J, Carvalho P O 2016 Brazilian Journal of Microbiology 47 (4) 1006–1011
[19] Senarath S, Yoshinaga K, Nagai T, Yoshida A, Beppu F, Jayasinghe C, Devadawson C, Gotoh N 2017 J Oleo Sci 66 (2) 187-197
[20] Zhang H, Shen Y, Zhang Y, Li L, Wang X 2018 BioMed Research International 2018 Article ID 90168407
[21] Janssen C I F, Killian A J 2013 Progress in Lipid Research 53 1-17
[22] Akerele O A, Cheema S K 2016 Journal of Nutrition and Intermediary Metabolism 5 23-33
[23] Wang H, Daggy B P 2017 Biomed Hub 2017 24 55-61
[24] Codex Alimentarius Commission 2017 Standard for Fish Oil CXS 329-2017 Food and Agriculture Organization of the United Nations WHO Roma Italy