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

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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 BF3, 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), docosahexaenoic 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...
the total fatty acids in natural fish oil. Esters 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, BF₃, saturated NaCl, and Na₂SO₄ 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° - 250 °C, the rate of temperature rise of 20 °C/min, detector temperature of 260 °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.
Characterization of physico-chemical properties of fish oil
Mas fish
Mas fish oils
Hydrolysis of fatty acids with lipase enzymes
Mucor miehei
(Lipozyme® TL IM)
Esterification using BF3
Fish oil production by extraction with solvent
Fatty acid analysis with GC
GC analysis (omega 3 and omega 6 fatty acids distribution at sn-2 position)

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 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 [19,20].

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 esterified 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.

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

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 (linoleic 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 (ericosenoic 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 | Myritic 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 | Steric acid | 4.022 | 5.686 | 4.543 | 5.415 |
|                    | C:21-0 | Henecosanoic 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 acid 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 | Ericsadienoic 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 | Ericsapentenoic 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 much 47.502% which consisted of oleic acid 46.030% and ericosatrienoic acid 1.279%, and after hydrolysis as much 47.169% consisting of oleic acid 45.890% and ericosatrienoic acid 1.311%. Likewise, goldfish oil from ponds omega 3 fatty acids before hydrolysis as much 2.752% which consisted of linolenic acid 1.155%, ericosatrienoic acid 0.549%, EPA 0.495%, DHA 0.553%, and after hydrolysis as much 2.272% consisting of linolenic acid 1.120%, ericosatrienoic acid 0.396%, EPA 0.380%, DHA 0.376%. Omega 6 fatty acids before hydrolysis as much 18.463% consisting of linolenic acid 17.960%, γ-linoleic acid 0.503% and after hydrolysis as much 16.258% consisting of linoleic acid 15.870%, γ-linoleic acid 0.388%. Omega 9 fatty acids before hydrolysis as much 48.200% consisting of oleic acid 46.890% and ericosatrienoic acid 1.310%, and after hydrolysis it was 48.012% which consisted of 46.780% oleic acid and 1.232% ericosatrienoic 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 | 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 | C10-Heptadecanoic  | 0.425  | 0.098   | 0.425  | 0.049   |
| C:18-1 | Oleic acid W<sub>9</sub> | 45.890 | 0.140   | 46.780 | 0.110   |
| C:18-2 | Linoleic acid W<sub>6</sub> | 16.120 | 1.730   | 15.870 | 2.090   |
| C:18-3 | γ-Linoleic acid W<sub>6</sub> | 0.446  | 0.355   | 0.388  | 0.115   |
| C:18-3 | Linolenic acid W<sub>3</sub> | 1.057  | 0.131   | 1.120  | 0.035   |
| C:20-1 | Ericosenoic acid W<sub>9</sub> | 1.279  | 0.193   | 1.232  | 0.078   |
| C:20-2 | Eicosadienoic acid | 0.780  | 0.006   | 0.518  | 0.206   |
| C:20-3 | Eicosatrienoic acid W<sub>3</sub> | 0.482  | 0.135   | 0.396  | 0.153   |
| C:20-5 | Eicosapentaenoic acid W<sub>3</sub> | 0.434  | 0.122   | 0.380  | 0.115   |
| C:22-6 | Docosahexanoic acid W<sub>3</sub> | 0.465  | 0.302   | 0.376  | 0.177   |

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-linolic acid 0.355%, and while omega 9 at sn-2 position was 47.169%, namely oleic acid 45.890%, ericosenoic 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-linolic acid 0.388%, and sn-1+sn-3 was 2.090% ie linoleic acid 2.090%, γ-linolic 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 2.271%.

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%| 2.752%| 18.463%| (1:6) | (1:6.7) |
| Position sn-2        | 2.438% | 16.566%| 2.272%| 16.258%| (1:6.8) | (1:7.1) |
| Position sn-1+sn-3   | 0.690% | 2.085% | 0.480%| 2.205%| (1:3) | (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 physical 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 physical characteristics of goldfish oil

| Characteristics | Unit | Amount | Goldfish oil cages | Goldfish oil ponds |
|-----------------|------|--------|--------------------|--------------------|
| Physical properties |  |  |  |  |
| Cloudy point    | °C  | 51.50  | 54.50  |
| Total solid     | °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 54.50°C.

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 linoleic 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.

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**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.