Leaching of Oil from Tuna Fish Liver by Using Solvent of Methyl-Ethyl Ketone

Ekstraksi Minyak dari Hati Ikan Tuna dengan Menggunakan Metil-Etil Keton sebagai Pelarut

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Abstract

Research of oil leaching from Tuna Fish Liver has been carried out by extracting of tuna fish liver in soxhlet by using methyl-ethyl ketone as solvent. Liver of fresh tuna fish is blended, put into soxhlet, and extracted at temperatures of 60°C, 65°C, 70°C, 75°C, and 80°C. After obtaining the oil, separation between solvent and oil is carried out by distillation. Oil obtained is analyzed by testing the yield, acid number, Iodine value, viscosity, and its impurities content. Yield obtained is influenced by temperature and time of leaching. Both variables indicates that the higher the variables, the more fish liver oil obtained. Maximum yield obtained is 25.552% at operating condition of leaching temperature 80°C, and leaching duration of 5 hours.

Keywords: distillation, leaching, methyl-ethyl ketone, oil, tuna fish liver

Abstrak

Penelitian mengenai ekstraksi minyak dari hati ikan tuna telah dilakukan dengan ekstraksi *soxhlet* hati ikan tuna menggunakan pelarut metll-etil keton. Hati ikan tuna dihancurkan, diletakkan dalam soxhlet, dan diekstrak pada temperatur 60°C, 65°C, 70°C, 75°C, dan 80°C. Setelah minyaknya diperoleh maka dilakukan pemisahan antara pelarut dan minyak dengan cara distilasi. Minyak yang diperoleh dianalisis dengan menguji yieldnya, bilangan asam, bilangan iodium, viskositas, dan kandungan pengotornya. Yield yang dihasilkan dipengaruhi oleh temperatur dan lamanya ekstraksi. Kedua variabel ini memperlihatkan bahwa semakin tinggi temperatur dan lamanya ekstraksi, semakin banyak minyak hati ikan yang dihasilkan. Yield maksimum yang diperoleh adalah 25,552% pada temperatur ekstraksi 80°C selama 5 jam.

Kata kunci: distilasi, ekstraksi, hati ikan tuna, metil-etil keton, minyak

1. Introduction

Fishes available in Indonesian waters consist of various types that most is consumed by resident, and made as export commodity. Among many of those fishery biological sources, one of them that could be export comodity is tuna fish. Tuna fish could be one of the best animal protein source because its flesh contains high protein, and its body and liver contains higher oil. Presently, the production of fish oil is one million tonnes, not expected to change, and for direct human use. Therefore, this research aims to investigate oil content from tuna fish liver, and characteristic of oil resulted.

Fish oil is the lipid fraction extracted from fish and fish by products (Adeniyi and Bawa, 2006). Oil is a mixture of fatty acid ester and glycerol that forms glyceride. Fish liver is part of fish body that contains high oil and fatty acid compared to the other body parts. Normally, fish liver weight is approximately 4 – 9% of fish weight with oil content is around 45 – 67%. So far, tuna fishes that are caught in Indonesian watery have not been benefitted optimally; this is probably because the limitation of information on processing technology of tuna fish.

Fat content of tuna fish is completely different from one body part to another, for example between red flesh and white flesh. Based on the fat layer, tuna fish is divided into three parts, i.e: *otoro, chutoro, akami*. *Otoro* and *chutoro* have fat content approximately 25%. Tuna flesh in fish center and has red color with fat content 14% less is called *akami*.

Tuna fish skin can be used to make intersting leathercraft industrial goods such as bag, shoes, wallet, belt, etc. whose

quality is not second to goat leather. Utilization of tuna fish skin as raw material of leathercraft industry will improve additional value of this commodity, and give bright prospect if connected to tourism industry. Tuna fish flesh has high nutrient content. Every 100 gram of bluefin tuna fish flesh contains: water 52.6%, proteins 21.4%, fat 24.6%, carbohydrate 0.1%, ash 1.3%.

Impurities in fish oil could be grouped into three parts, the first is insoluble impurities in oil (physical impurities, water, and protein), the second is impurities that is coloidal suspension in oil (phosphatide and carbo-hydrate), and the third is soluble impurities (free fatty acid, pigment, mono- and di-glyceride, compound of oxidation result) (Irianto, 2002). In order to eliminate the stench from tuna fish flesh, it could be done by heating of flesh in alkali condition, multistage salting, and cold water soaking that could reduce urea up to 50%.

In Korea and Taiwan, tuna fish is costly food so that it gives possibility to be export commodity. The utilization of tuna fish liver is important part to be developed because it contains high oil content in the range of 4 to 30%. Oil of tuna fish liver contains fatty acid, vitamin A, vitamin B, vitamin C, and hydrocarbon compounds. According to Saify *et al*. (2003) as many as 11 fatty acids may be present in the oil of most fish as seen in Table 1.

Table 1. Fatty acids available in oil of tuna fish liver

No.	Fatty Acid Type	Fatty Acid Name
1.	Saturated	Lauric, Myristic, Pentadecanoic, Palmitic, Stearic acid
2.	Unsaturated: Monoenoic	Oleic acid
3.	Polyenoic	Linoleic, Linolenic, Eicosatetraenoic, Eicosapentaenoic, Docosahaxaenoic

Oil content in tuna fish liver varies depending on its type. The variation of oil content in tuna fish liver is influenced by several factors such as fish size, species, food, age, and fish condition whether laying eggs or not. Eicosapentaenoic acid (EPA) is an polyunsaturated fatty acid that is metabolically active. EPA cannot be synthesized by human body. The EPA consumption is linked to the development of brain and nervous tissue in infants. Because of dwindling supplies, fish oil may not be able to satisfy the future demand of EPA.

The utilization of tuna fish gives possibility to develop processing tuna fish liver to be fish oil, so that it could eliminate Indonesia dependency on oil fish from import, and open new vacancy. Processing of fish liver oil has methods that are principally based on two ways.
1. Oil leaching through extraction by using heat from steam (vapor).
2. Oil leaching through extraction by using organic solvent, such as acetone, n-hexane, benzene, carbon tetra chloride, etc.
3. Rendering, that is an extraction method of oil from material that is assumed consisting of oil with high water content.

Oil leaching through extraction by using steam is carried out by contacting fish liver with steam that flows from up to bottom in order to attract oil from fish liver. Then oil obtained is separated by filtering or directly with centrifugal separator. By vaporizing the residue, it will be obtained the dark oil that contains free fatty acid approximately 25 – 40% and protein 50%.

Processing of fish liver oil by extraction by using organic solvent is one of ways to improve vitamin content obtained from fish liver content. Leaching is separation process of soluble matter (solute) from a mixture by using organic solvent. Leaching is one of the most generally used methods for deter-mination of total lipids in dried fishes. This is particularly because, it is fairly simple to use and is the officially recognised method for a wide range of fat content determinations. It contacts solid with solvent to obtain mass transfer of solute. The type of leaching equipment that is often used is as follows.
1. Equipment that uses fixed bed, solvent is passed through solid that is set with fixed bed.
2. Equipment with dispersed contact, solid particle is dispersed with solvent so that relative movement occurs between solid particle of solvent, and among the solid itself.

Solvent selection in order to obtain higher extract in this operation has to be paid attention, solvent used is solvent that could dissolve desired matter in a mixture from a solid.

2. Method

Frozen tuna fish liver is weighed as much of 150 grams and then blended. Erlenmeyer flask has to be dried in oven at temperature of 110°C for an hour, then it is cooled in exicator and weighed. After weighing, sample (tuna fish liver) blended is put into soxhlet, then extracted by using methyl-ethyl ketone on electrical heater for particular time (2, 3, 4, 5, and 6 hours). Solvent could be separated by distillation. Then erlenmeyer flask is dried in oven at temperature of 110°C for an hour, cooled in exicator and weighed. Drying and weighing is repeated until constant weigh is obtained. Oil content could be calculated by using Equation 1 (Ketaren, 1986).

$$OC = \frac{(B - A)}{sample\ weigh} \qquad (1)$$

where: OC = Oil content (%)
      A = weight of empty flask (gram)
      B = weight of flask and extracted oil (gram)

Oil with the highest yield will be analyzed the peroxide value, saponification value, viscosity, moisture content, refractive index, acid number, iodine value, and impurities. Peroxide value is analysed according to AOAC (1995). Whereas the method specified by Weiss (2000) was used to determine moisture content of the fish. Abbey refractometer was used in determining the refractive index of the oil.

Saponification value is the mg of calium hydroxide needed to bind free acid, and to saponize ester from 1 gram of compound (Sudarmadji, 1984). Saponification value is analyzed by weighing oil fish as much of 5 gram. Then, it is added by 50 ml KOH that is made of KOH 40 grams in alcohol 1 liter. After that, it is closed by back cooling. It is boiled carefully for 30 minutes. Next, it is cooled and added by two drops of phenolpthalein. Excess of KOH solution is titrated by standard solution of 0.5 N HCl. Saponification value (Sv) is calculated by using Equation 2. Acid value is analyzed by weighing oil fish as much of 10 grams. Then it is added by alcohol 96% as much of 25 ml, and heated on waterbath for 10 minutes and agitated at the same time. The solution is added by 2 drops of phenophtalein as indicator, and titrated with KOH 0.1 N. Titration is stopped until pink color is formed for 15 seconds. Acid value is determined by Equation 3.

$$SV = \frac{28.05\ x\ (blank\ titran - oil\ titran)}{oil\ weigh} \qquad (2)$$

$$Acid\ value = \frac{ml\ KOH\ x\ 0.1\ N\ x\ 56.1}{gram\ of\ oil} \qquad (3)$$

Iodine value is determined by weighing 0.1 gram fish oil, put into erlenmeyer, and added by 25 ml CHCl$_3$. Then, it is added by 25 m wijs solution, closed, and agitated slowly. It is kept in dark room for 2 hours, added 20 ml KI solution 15%, 100 ml aquadest, closed again, agitated carefully. The solution is titrated with Na$_2$S$_2$O$_3$ solution 0.1 N by using amylum as indicator. Iodine value is determined by Equation 4 (Ketaren, 1986).

$$Iodin\ value = \frac{(V_1 - V_2)\ x\ N\ x\ 12.69}{W} \qquad (4)$$

where: V$_1$ = volume of sodium thiosulphate for blank titration (ml)
      V$_2$ = volume of sodium thiosulphate for sample (ml)
      W = sample weight
      N = normality of sodium thio-sulphate
      12,69 = iodine atomic number

Impurity content is analyzed by drying filter paper at temperature of 105°C for 15 minutes, then it is cooled and weighed. Fish oil 10 gram is put into erlenmeyer, and dissolved with n-hexane. The solution is filtered with the weighed filter paper, then it is washed with n-hexane until the filter paper is free of oil. The filter paper is dried at temperature of 105°C in oven for 60 minutes, then it is cooled and weighed until its weight is constant. Impurity content (*Ic*) could be determined by Equation 5.

$$IC = \frac{(filter\ paper + oil) - (filter\ paper)}{sample\ weigh} \qquad (5)$$

3. Results and Discussion

3.1. Effect of Leaching Temperature on Yield

In this study, five different temperatures (60°C – 80°C) were investigated in order to determine the effect of temperature on the oil recovery yield. Good process is obtained while operating temperature is 80°C (Figure 1) and operating time 6 hours. There was a significant effect of temperature on oil recovery yield. Theoretically, oil content from fresh tuna fish liver is in the range of 4 – 30%, whereas research result indicates

that oil content obtained is in the range of 15,623 – 25,552%. This is caused by oil tissue on tuna fish liver that has not been crushed yet, so that after extraction finishes, fish liver still contains oil. In order to break the tissue, it can be carried out by prolonging operating time, and increasing the temperature. This decreased mass is also influenced by the amount of impurities on oil. Oil with high impurities will also have high decreased mass because impurities on oil will be lost while filtering.
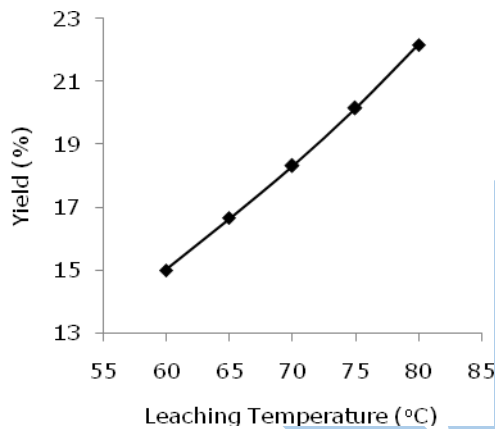

Figure 1. Correlation of leaching temperature vs. yield at operating time of 2 hours

The leaching temperature has dually opposite effects on yield with methyl-ethyl ketone leaching of tuna fish liver oil. On the one hand, an increase in temperature results in reducing methyl-ethyl ketone density, which in turn weakens methyl-ethyl ketone power to dissolve oil. On the other hand, the increase in temperature leads to an increase of volatility; therefore results in an increase of oil solubility in methyl-ethyl ketone (Wei *et al*., 2009). In this study, when the temperature was below 80°C, its effect on oil volatility was remarkable, and therefore the oil yield was increased with an increase in temperature from 60°C to 80°C (Figure 1). Small variation in leaching temperature (5°C) may change the results by 1.7 – 1.8%. However, many solvents have unfavorable response to high temperatures. An increase in temperature may increase the oil yield up to a maximum level after which the oil yield may decrease suddenly with a further increase in the temperature.

The amount of solvent used in fish liver leaching is constant. This is caused by the amount of solvent that has been sufficient, which is indicated by solvent used has been able to circulate. Solvent or solvent mixture used for extraction should be sufficiently

polar to remove lipids from cell constituents, but not too polar so that the solvents do not readily dissolve all triacylglyceride (Christie, 1992). Penetration of polar solvent into the cells is necessary to extract the lipid from the cell membrane and fibres that includes the phospholipids materials (Norziah *et al*., 2009). In this study, methyl-ethyl ketone solvent system was used.

Selection of leaching operating temperature is based on material type extracted. Research result indicates that the higher temperature, the more extract obtained. This is caused by the increasing solubility of oil extracted while the operating temperature increases. High heating temperature during oil leaching deactivated the enzyme and the release of free fatty acids by the lipase activity thus lowered the FFA value (Chantachum, 2000).

3.2. Effect of Leaching Time on the Yield

The influence of leaching time on the yield of tuna fish oil was indicated in Figure 2. It indicates that significant change on yield toward operating time change. In general, it could be stated that the increasing of time influences on leaching rate.
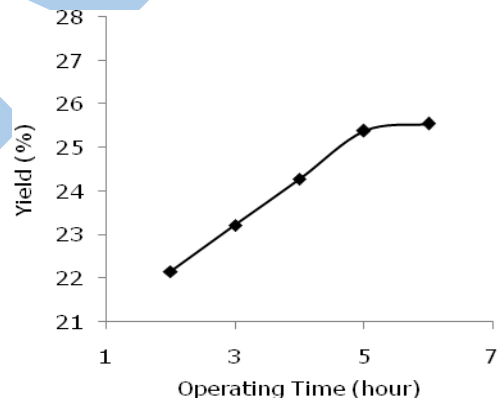

Figure 2. Correlation of operating time vs. yield at temperature of 80°C

Based on operating-time change carried out, it seems that the highest amount of the oil is obtained at leaching time of 6 hr. It was seen from the figure that the shape of the curve oil versus leaching time from 2 hr. to 5 hr. was linear. This is caused by the unsteady state, where diffusion that occurs from tuna fish liver into solvent, is still influenced by time. Within the hour from 2 hr. to 5 hr. the oil yield increased linearly from 22.162% to 25.392% with an increase in leaching time. From then to 6 hr. the oil yield increased by smaller amount in

response to increases in leaching time until a maximal oil yield of 25.552% was reached. It could be related to the reduced concentration gradient of the oil as the extraction proceeded and an increase in viscosity of the solvent; since high viscosity does not support effective extraction. It means oil yield increases in number as much of 1.15 times of initial amount. The operating time of 3 hours increases the yield as much of 1.05 times of initial amount (at 2 hour leaching).

After that, the oil yield was probably no longer dependent on leaching time. If solute concentration has not achieved saturation, operating time will also influence the amount of oil that could diffuse into solvent. The results could be explained based on the distribution of the oil within the sample. At the early stage, the oil extracted from the surface of the particles, and the solubility of the oil in methyl-ethyl ketone controls the mass transfer. In the later stage, oil from the tuna liver was leached, then the mass transfer was controlled by the diffusion of oil within the particles (Danh *et al.*, 2009). The mass transfer rate was low and oil yield increments were insignificant.

The percentage values are of 22.162 – 25.552% for oil content of tuna could be said to be in conformity when compared to standard. However, the linear relationship between the two values is being supported by the fact that an increase in the oil content of fish is usually accompanied by a decrease in the moisture content in almost linear proportion or vice versa. Finally from the graph, it can be concluded that the percentage oil yield removed from the fish are directly proportional to the time of leaching and evaporation respectively.

3.3. Physico-Chemical Properties of Tuna Fish Liver Oil

In order to determine the stability and quality of fish oil extracts, some quality assessment was conducted. Quality analysis is carried out in two groups: firstly to check the fundamental parameters which includes the peroxide value, moisture, appearance (color), impurities, FFA, soap, and iodine value (EFSA, 2010). And secondly, to determine refining procedures which ususally focusses on the evaluation of peroxide value and *p*-anisidine value. The peroxide and *p*-anisidine values are the most reliable chemical methods for rancidity measure-ments of fish oils. The oil was analysed by carrying out some physical and chemical tests on it. These results are indicated in Table 2. There are significant differences in qualities of tuna oil because of different leaching methods.

One of the most widely used tests for oxidative rancidity, the peroxide value, is a measure that expresses in milliequivalents of active oxygen the quantity of peroxide contained in 1000 g of the substance (Council of Europe. (2004). Milli-equivalents of peroxide per kg of fat (meq/kg) are measured by titration with iodide ion. The number of peroxides present in the oil is an index of their primary oxidative level and its tendency to go rancid. Peroxide value could influence lipase activity. Primary oxidation processes in oil mainly form hydroperoxides, which are measured by the peroxide value. Oxidation process takes place if contact occurs between oxigen and oil. The peroxide value could increase with increases in temperature (Rabiei *et al.*, 2011). This is because of the initial propagation stage of lipid autooxidation phenomenon with chain reaction leading to increases in peroxide formation. In order to obtain lower peroxide value, experiments should be carried out at lower temperature.

The peroxide value determination on tuna fish liver oil obtained in this research (13.39 meq/kg sample) was higher than the recommend range which could be because of oil oxidation.

Table 2. Physico-chemical analysis of extracted tuna fish liver oil

No.	Analysis	Tuna Fish Liver Oil
1.	Peroxide Value (meq/kg)	13.39
2.	Saponification Value (mg KOH/g)	147
3.	Viscosity (cp)	2.307
4.	Moisture Content (%)	2.42
5.	Refractive Index	1.485
6.	Impurity Content (%)	1.57
7.	Acid Value (mg KOH/g)	0.729
8.	Iodine Value	49.111

The peroxide value was within the recommended range of 3 – 20 meq/kg indicating that fish oil is still good and was oxidized. It also indicates oil age. If unsaturated fatty acid is oxidized thus the unsaturated fatty acid will form peroxide and will cause the oil smells rancid. In general, the lower the peroxide value, the better the quality of the oil and its state of preservation (CDR, 2008). However, peroxide value could decrease as secondary oxidation products appear. Most people will require a peroxide value of less than 10 in marine oils, but peroxide value may need to be close to 2, depending on the market. Peroxide values above 10 – 20 meq/kg develop rancid tastes and smells (Connell, 1995). The oxidized oil leads to potential dangers to the physiological and biochemical functions of the body (Mukherjee and Mitra, 2009). The change in peroxide value is indicator of lipid oxidation (oxidation increases the peroxide value) (Ahmed and Mahendrakar, 1996). However, lower value can still be achieved because of the breakdown of oxidized products. Reduction 21% in peroxide value in oils could use bagasse as absorbent (Wannahari and Nordin, 2012).

The peroxide value test is a good way to measure the amount of primary oxidation products in fresh oils. Oils with significant levels of peroxides may still be odorless if secondary oxidation has not begun. If oxidation is more advanced, the peroxide value may be relatively low but the oil will be obviously rancid. As oil goes rancid, triacylglyceride is converted to fatty acid (FA) and glycerol which increases acid number (Memon *et al.*, 2010). Rancidity in oils causes undesirable chemical changes in flavor, color, odor, and nutritional value of oil. Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by alkali condition. The saponification value of tuna fish oil obtained in this study was lower (147 mg KOH/g) than standard value for fish oil (180 - 200 mg KOH/g) (AOCS, 1992). Saponification value may be contributed by unsaponifiable matter present in the leaching material such as impurities, sterols, glyceryl ethers, hydrocarbons, fatty alcohols, etc. Refining the fish oil will help in removing the impurities and reduce the oxidation products; however, it would add the cost of production.

The acid value quantifies the amount of acid present. It is the mass of potassium hydroxide in mg that is required to neutralize 1 g of chemical substance (Wrolstad *et al.*, 2005). The acid value (which is twice the free fatty acid (FFA) value) measures how many fatty acids (a component of oil) are cleaved from their parent molecules. The acid content in an elementary fat or oil is given by the quality of free fatty acids deriving from the triglycerides developing hydrolytic rancidity. This alteration occurs under unsuitable conditions of treatment and preservation of the fats and thus the acidity represents a basic indicator of the genuineness of the product. Determining the acid value is important because it is still a reliable parameter for oil quality (Food and Agriculture Organization, 1986). Increase in acid value is generally associated with the lipase activity originating from microorganism or biological tissue (Boran *et al.*, 2006). The lower the acid value the better the quality of oil. The acid value of the oil was found to be 0.729 mgKOH/g. The recommended range of acid value is less than 5 mgKOH/g for fish oil. The obtained acid value was in the recommended range.

In this study, frozen homogenate was used to extract fish oil which might be reason for deterioration of oil quality. The characteristic and properties of fish liver oil are strongly influenced by the process and raw material (EFSA, 2010). According to Aubourg and Medina (1999), the free fatty acids have been produced during the frozen storage of fish as a result of enzyme catalyst. This effect was increased with time and temperature of the leaching and with the FFA content (Miyashita and Takagi, 1986) and leading to a high proportion of polyunsaturated FFA (Aubourg *et al.*, 1996). In order to avoid the extensive release of FFA, freshly prepared sample should be used. The result of free-fatty-acid content is 0.365%. The objective of this free-fatty-acid determination is to know the oil quality. The oil is in good quality if the oil has low free-fatty-acid content. Analysis result obtained indicated that tuna fish liver oil extracted is the oil in good quality.

The iodine value ("iodine number" or "iodine index") measures the number of reactive double bonds present in an oil. The iodine value was found to be 49.11 I_2/100 g of the sample as against the standard value of between 120 - 180 I_2/100 g of the sample. The extracted oil from precooked skipjack tuna head by cooking contained 122 - 174 g/100 g iodine value (Chantachum *et al.*, 2000). The whole process of methyl-ethyl ketone leaching was carried out at lower temperature (80°C),

which led to a lower oxidation rate compared to extraction oil by cooking (95°C). The iodine value of the oil implies that few of the double bonds in the oil has been saturated, giving the oil wider applications. A higher iodine value number indicates more double bonds in the sample and therefore that greater care will be needed to slow down oxidation. Iodine value can be up to 185 for fish oil. Iodine value is not a measure of quality but is an indicator of oil composition.

The lower values for acid and peroxide indicate the oil was oxidized and appeared to be light in color. The light color may be because of the presence of products resulting from hemoglobin degradation (Batista *et al.*, 2009). The appearance of the oil was reddish brown because of the prolonged heating period and the employed solvent extraction method which often oxidizes the oil and produces a reddish color (Hall, 1992). In the solvent extraction procedure, the samples were not subjected to high temperature. Subjecting lipids to high temperature was found to induce lipid peroxidation (Mishra and Singhai, 1992). To handle this, Palanisamy *et al*. (2011) stated that bleaching can be used to remove minor constituents (color pigments, free fatty acid, peroxides, odor, and non-fatty materials) from oils.

Experimental analysis was conducted on the oil extracted from each of the samples of fishes and the results in Table 2 indicates that the percentage moisture content of species was 2.42%. This signifies that the oil will require low extraction time. Moisture content of the fishes is a reflection of its oil content, this is because species with higher moisture content yield high amount of oil when leached. Moisture is usually used as indicators of nutritional value of fish. The result of the characterization carried out for the oil in Table 2 indicates that the oil has a refractive index of 1.485 which is outside the range of standard value of 1.4 – 1.473 for fish oil. The significance of the result is that, the oil obtained from the fish is denser than water. Hydrolysis of fish was greatly reduced upon sterilization (De Koning, 1999). Since we did not sterilize the samples nor study them under aseptic conditions, it is possible that some microorganism contamination might occured during sample leaching.

4. Conclusion

In order to obtain the maximum oil yield, the optimum condition of leaching has to be determined. Experimental results indicated that the yield obtained was dependent very much on the linear form of leaching temperature and time. The higher the extraction temperature and time, the more the tuna fish oil obtained. The relatively good result is obtained at extraction temperature of 80°C and extraction time of 5 hours. At the relatively good condition, yield obtained from oil of tuna fish liver is 25.552%, saponification value of 147 mg KOH/g, acid number of 0.7293, iodine value of 49.17, and viscosity of 2.34 cp. The oil was evaluated and some of its physical and chemical properties were determined. Some of these analytical results obtained were outside the normal range. Based on the research result on the oil, it could be suitable for applications in pharmaceutical and food industries. Optimization of this method is necessary to get the highest oil yield.

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