Extraction of FAME from fish waste by using modified soxhlet method

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Abstract. The number of fish waste has increased rapidly over the time. Most of discards composed of head, tail and internal organs are considered to be worthless. In this research, fish waste from Pangasius Hypophthalmus (Patin fish) was being used as the raw material to produce fish oil. Therefore, the objective of this research is to evaluate the effect of different ratio solid to solvent (1:1, 1:2, 1:3, 1:4 and 1:5), temperature (70, 75, 80, 85 and 90 °C) and mixing time (1, 2, 3, 4 and 5 hours) on production of fish oil and to analyze the amount of fatty acid methyl ester (FAME) in the fish oil. Sample preparation was being made by choosing solid fat from internal organ of Patin fish. Modified Soxhlet method was used to extract the fish oil from fish waste and ethanol as the solvent. FAME from extracted fish oil was analyze by using Gas Chromatography Mass Spectrometry (GCMS). The result showed that the highest yield of oil being produced was 59.92 ± 0.926 % at the temperature of 85 °C with 1:2 of ratio solid to solvent and 5 hours of mixing time. Besides, the extracted fish oil for sample 3 contained the highest amount of FAME which is 0.4016 mg/g with majority amount of saturated fatty acid.

Keywords: Fish waste; fish oil; Pangasius Hypophthalmus (Patin fish); modified Soxhlet method; fatty acid methyl ester

1. Introduction

Fish products for human consumptions include fresh and frozen, whole and fish fillet [1]. Most of the discards composed of head, intestine, skin and bones and are considered to be worthless trash. There is no recovery into valuable products to be made. Actually, these wastes have high content of nutritive compounds like protein and fatty acid [2]. *Pangasianodon hypophthalmus* (Patin Fish) is one of the most common catfish species that contribute to Malaysian diet. The muscle and ovaries of Patin catfish are consumed while the visceral fat and liver are discarded. These tissues actually can accumulate fat and they are the potential source of extractable fish oil. Disposal of these wastes incur cost and environmental pollution.

Fish by-product such as head, skins, fat and gills are comprise about 50% of the fish body weight [3]. By-products are usually used to produce fishmeal, fertilizers and fish oil. Fish oil content depends on the fish species and tissue. Therefore, fish by-products are important sources of fish oil that could also serve as good sources of fatty acid methyl ester. Fatty acid methyl esters are a type of fatty acid ester that are derived by transesterification of fats with methanol. Fatty acid methyl ester is separated into two which are saturated fatty acid and unsaturated fatty acid. Saturated fatty acid consist of lauric acid, palmitic acid and stearic acid while unsaturated fatty acid consist of oleic acid, linoleic acid and linolenic acid.

The nutrients contain in fish waste makes it high demand in market especially fatty acid such as EPA and DHA which well known as a good supplement. Fish waste is obviously can turn into something beneficial for human being and we can save world from wasting and dumping away all the fish wastes. Therefore, by using method that suit to demand, modified Soxhlet extraction method is used to extract the fish oil that contain desired fatty acid from the fish waste.
In order to avoid wasting the fish waste and to make sure keep on supply fish oil according to high demand in market, this research is utilized fish waste to extract the fish oil. The extracted fish oil was analyzed by using GCMS to identify the amount of fatty acid methyl ester. Selection of the extraction parameters is important as it can produce high yield of fish oil that contain fewer impurities. Therefore to achieve the aims of this project, the modified Soxhlet method are used to get high yield of fish oil. The detail on operating conditions of this research were to study the different ratio of solid to solvent (1:1, 1:2, 1:3, 1:4 and 1:5), temperature (70, 75, 80, 85 and 90 °C) and mixing time (1, 2, 3, 4 and 5 hours) in order to obtain the high yield of fish oil.

2. Methodology

2.1. Preparation of fish waste

The internal organs of the fish waste were separated with other parts of fish waste such as tail, fin and head. Only solid fat was selected from the fish waste. Then, solid fat was washed with cold water to remove the residual blood and was dried under room temperature. After the drying step was done, the dried fish wastes were ground using an electrical grinder and blend it well until becomes a fine powder. This procedure is to ensure the solid pieces are roughly equal in terms of resistance to extraction procedure since the wastes come from different parts of the fish. The fish waste powder after blend was stored in the freezer to avoid it from being rotten and only take it out for experimenting purpose.

2.2. Extraction process

The modified Soxhlet extraction method was used for oil extraction process with Ethanol was selected as a solvent. Difference with Soxhlet method, the modified Soxhlet method was not using the extractor in the extraction. The condenser was directly attached to the round bottom flask so that the solvent was directly contact to the sample and the extraction rate was increased and the extraction will not take so long. By using the extractor in Soxhlet method, the duration of extraction took several hours to have very small amount of fish oil.

8 g of fish waste was weighed every time to run the experiment with different conditions of parameter. Approximately 100 ml of ethanol was poured into the flat bottom flask that contains 8 g of fish wastes. Next, a magnetic stirrer was used to mix up the solvent and the sample thoroughly in the flat bottom flask. During the extraction process, the flat bottom flask was heated in the beaker that filled with water at the temperature of 70 °C. The flat bottom flask should immersed until the stirrer was stirred at the rotation speed which of 500 rpm. The time taken for the extraction process is 2 hours. The evaporated solvent that moved through the condenser would drip back into the flat bottom flask since the steam was converted into liquids. The extracted fish waste (solid)-solvent-oil mixture in the flat bottom flask were collected and was separated at the end of the extraction process by using centrifuge equipment. The experiment was repeated at temperature of 75, 80, 85, and 90 °C and was conducted in triplicate to obtain a better result for the fish oil extraction.

The same experimental procedures as mentioned above was repeated to study the different ratio solid to solvent. For this parameter, the volume of solvent was adjusted at 25 ml at the best temperature obtained in the first experiment. Then, the experiment were repeated with 50, 75, 100 and 125 ml of solvent.

The study of different mixing time was evaluated with the same procedure as previous experiment. The best temperature and ratio of solid to solvent from the previous experiment was selected with the range of mixing time from 1 hour to 5 hours.

2.3. Centrifugation process and solvent recovery

The mixture of solvent-oil in the flat bottom flask were collected and separated at the end of the extraction process by using centrifuge equipment. Centrifugation method was used to separate the solvent-fish oil mixture and produce two layers which are solvent (first layer) and fish oil (second layer). Then, the fish oil was sucked from the mixture by using micropipette. Next, the oil was placed for 24 hours at 80 °C in the oven to remove residue solvent in the oil. Fish oil was weighed and then stored in the chiller before analyzing process. The yields of fish oil extraction are according to a calculation described in literature [4].
2.4. Transesterification process
Fish oil was undergo transesterification process before send to GCMS for analyzing fatty acid that contain in the fish oil. First, 1 ml of fish oil was mixed with 5 ml of hexane, 4.2 ml methanol and 0.215 ml diluted HCl as the catalyst. Then, the mixture was poured in the test tube with cap and parafilm tightly to ensure no vaporization occur. The test tube was heated in the beaker that filled with water and heated it on the hot plate at temperature 80 °C with 700 rpm for 2 hours. After done the transesterification process, there are 2 layers were formed in the test tube. 1 ml of upper layer mixture was sucked and transferred by using micropipette into GCMS vial.

2.5. Fatty acid methyl ester analysis
The fatty acid methyl ester profiles in the extracted fish oil was analyzed by using Gas Chromatography Mass Spectrometry (GCMS) Agilent Technologies (G3171A, China) after each extraction process [5]. 1 μL of extracted fish oil samples for each parameter at optimum condition was injected into GCMS. The compound of fatty acid methyl ester were identified by comparing the peak areas and retention time with the standard of palmitic acid, palmitoleic acid, stearic acid and oleic acid.

3. Results and Discussions
3.1. Effect of temperature on oil yield
Since the temperature is one of the physical factors that can affect the production of fish oil [6] therefore, the current research was being carried out from temperature 70 to 90 °C. The range of this temperature was chosen in this research because the longer time for extraction is required if the temperature is lower than 70 °C whereas high temperature which is higher than 90 °C can affect the quality of oil and it can faster the formation of free radicals [7].

The result obtained in figure 1 has shown that the higher the temperature, the higher yield of fish oil produced. Lower temperature than 80 °C such as 70 and 75 °C produced less yield of fish oil compared higher temperature than 80 °C produced more yield of fish oil. During the extraction of the fish oil, when the temperature was reaching the boiling point of solvent (ethanol) which is at 78 °C, the solvent was vaporized in the condenser and drip back into the solvent-oil mixture. This is the reason of more oil was produced for the temperature above 80 °C compared to the temperature of 70 and 75 °C. The lowest yield of fish oil was obtained at 70 °C with 13.04 % and the highest yield is 30.95 ± 5.48 % at 90 °C.

![Yield of Oil versus Temperature](image)

**Figure 1.** The effect of temperature on yield of oil

Based on some research that has been made as mentioned below, there are several factors that leads to the production of fish oil on the certain amount at the certain temperature. Disruption of the cell walls of solid fat in oil-bearing cells can be promoted. This allows the oil content in the cells of solid fat to
easily escape from the cells into the extracting solvent which increases the fish oil extraction yield. Besides, Damodaran (2017) noted that proteins usually undergo irreversible denaturation when heated at 90-100 °C [7]. Denatured proteins forming a dense structure can cause inhibition of oil release. Based on the study by Yee et al. (2007), the authors also showed that the yield of extracted fish oil was increased from 60 °C to 80 °C but decreased at extraction temperature of 100 °C [8]. Decrease in yield percentage occurred was probably due to the oxidation process. High temperature can cause the oxidation process resulting in fat breakdown. The continuous increase in temperature resulted in the decrease in attained oil yields. Upon doing the extraction at even more elevated temperatures, the degradation of the oil is very much likely to occur. This leads to lower the extraction yields [9].

Based on the study by Abadi et al. (2014), the authors mentioned that the maximum yield of fish oil was recovered at 80 °C with percentage of 60 % yield of fish oil. The yield of fish oil was also increased after 80 °C but there is not much different in terms of percentage obtained from the extraction [10]. In other research conducted by Navin et al. (2017), the authors produced 60 % yield of fish oil at the temperature of 80 °C [11]. Therefore, on this current study, 85 °C was chosen as the optimum temperature because the production of oil was high and it does not over the limit of 90 °C to avoid the quality of fish oil produced which being affected by free radicals.

3.2. Effect of ratio solid to solvent on oil yield
For the study on different effect of ratio solid to solvent, the volume of ethanol was varied while the mass of solid fat was remained for each extraction to elucidate 1:1, 1:2, 1:3, 1:4 and 1:5 of ratio solid to solvent on yield of oil.

As shown in figure 2, the largest percentage of oil yield was 46.26 ± 0.78 % with ratio of 1:2 by using 50 ml solvent while the smallest percentage of oil yield was 19.74 ± 8.76 % with ratio of 1:5 by using 125 ml solvent. The yield of fish oil decreased at the ratio of 1:3 and a little bit increased at the ratio of 1:4. The result obtained for this research is in contrast with the result obtained from the literature review. In Fatemeh et al. (2014) research, increasing of oil extraction content was obtained from 2.9711 g for 150 ml of solvent volume to 3.0084 g for 250 ml solvent volume. The result showed that the percentage of fish oil yield had a very small increment by increasing the volume of solvent used. Therefore, it can be said that increasing the ratio of solid to solvent also increases the yield of fish oil. This is because the concentration gradient between the solid and the liquid phase becomes greater which favors good mass transfer and increased extraction efficiency [12]. Besides, according to Tan et al. (2011), high ratio of solid to solvent will increase the concentration gradient and rate of diffusion [13]. The high ratio allows greater extraction of solids by solvent. These results are in fact consistent with the mass transfer principles which the concentration gradient between solid and solvent becomes the driving force for mass transfer.

![Yield of Oil versus Ratio Solid to Solvent](image)

**Figure 2.** The effect of ratio solid to solvent on yield of oil
In this current research, ratio of solid to solvent of 1:2 is the best ratio and 50 ml of solvent is convenient with the amount of solid fat in order to produce high yield of fish oil. By using 50 ml of solvent with 8 g of solid fat, it can increase the extraction of fish oil. 50 ml of solvent can extract higher fish oil from 8 g of solid fat compared by using higher amount of solvent to extract the fish oil. The used of high volume of the ethanol as a solvent is not good for oil extraction especially in the production of FAME. Since alcohols like ethanol are hygroscopic and could easily absorb water from the atmosphere, the oil produced are mostly sensitive to contamination by water, hence reduced the oil yield with increasing the solvent to solid ratio. This can also relate with the research that had done by Abadi et al. (2014) which by increasing the volume of solvent for fish oil extraction, it does not give a big different of yield of fish oil but just small increment only [10]. Hence, it could be concluded that optimum ratio of solid to solvent to get high yield of fish oil is with ratio of 1:2 by using 50 ml of ethanol. Increased the volume of solvent could not obtained high yield of fish oil but could increase the cost. Both trial had shown large composition in fish oil amount for 50 ml consumption of solvent. Therefore, 50 ml solvent could extract the fish oil better rather than used large volume of solvent.

3.3. Effect of mixing time on oil yield

Mixing time is one of the essential parameters for obtaining high quality fish oil. In oil extraction, mixing time needs to be considered because lack of heat supply time leads to lower oil production. In this research, time at 1 hour until 5 hours was elucidated to determine the optimum extraction conditions that could produce maximum extraction yields of fish oil.

Based on the result obtained in figure 3, the yield of fish oil increased by the time increased. The oil yield increased dramatically from 1 hour to 2 hours of fish oil extraction and slightly increase when the fish waste was heated more than 2 hours. This trend of results is because of the diffusivities of the oil and solvent increases as the time increase which resulted in high fish oil yield [14]. The lower yield of fish oil obtained was 27.64 ±10.93 % while the highest yield was 59.92 ± 0.92 %. According to Abadi et al. (2014), the authors obtained high yield of oil within 4 hours of extraction. The optimum extraction time obtained from this study was 5 hours. This result may influenced by the types of part from the fish waste used for oil extraction. In Abadi et al. (2014), the authors used powdered fish waste that comprised with other parts of fish waste such as head, fin and tail that may lead lower production of fish oil until certain time [10]. Solid fat from fish waste was taken as a sample to extract the fish oil. Fat contain higher oil and can undergo extraction process until more than 5 hours to produce more fish oil.

![Yield of Oil versus Mixing Time](image)

**Figure 3.** The effect of mixing time on yield of oil
In addition, the oil yield showed correlation between the efficiency of solvent extraction process and the effect of different heating duration on the oil content. Solvent takes time to react with the fish waste to produce oil. So, the longer mixing time, the better the solvent can react with the fish waste and extract a lot of fish oil. Che Sulaiman et al. (2017) had approved that prolonged exposure of the sample in the solvent will allow sufficient time for the desired compounds to migrate into the solvent [15]. It could be related with the increasing of production of fish oil over the time. However, until at certain time the fish waste was stop producing oil to extract due to its limitation of oil contain in the fish waste. Besides, from the observation by Che Sulaiman et al. (2017), the maximum mixing time that could produce high yield of fish oil maybe at the time of 6 to 7 hours and prolonged the mixing time more than 7 hours would produce low yield of fish oil [15]. The longer the mixing time, the lower the oil yields that could be extracted from fish waste. This result agrees to the finding reported by Mani et al. (2007) that any further increase passes 6 hours in extraction time did not increase the oil yield of fish waste [14].

3.4. Fatty acid methyl ester (FAME) profile of fish oil

Amount of FAME in extracted fish oil for all parameters with optimum value were being analyzed. There are 3 samples that were analyzed by GCMS in this research. Sample 1 was obtained from the optimum oil yield based on the temperature of 85 °C, sample 2 was obtained from the optimum oil yield based on the ratio of solid to solvent of 1:2 and sample 3 was obtained from the optimum oil yield based on the mixing time of 5 hours.

Based on Table 1, palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0) and oleic acid (C18:1) were identified in the fish oil. However, amount of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) cannot be detected in the sample after a lot of methods and trials were introduced. This happened maybe because of the amount of EPA and DHA in the fish oil is very small and cannot be detected by GCMS. The result showed that four types of FAME were existed in the fish oil in sample 1 which were generally composed of palmitic acid and stearic acid (saturated fatty acid) and palmitoleic acid and oleic acid (unsaturated fatty acid). However, in sample 2 only saturated fatty acid of palmitic acid and stearic acid can be detected. The extracted fish oil for sample 1, sample 2 and sample 3 contained greater amount of saturated fatty acid (SFA) which are 0.1642 mg/g, 0.0228 mg/g and 0.2722 mg/g respectively compared to amount of unsaturated fatty acid (UFA) which are 0.0871 mg/g for sample 1 and 0.1294 mg/g for sample 3. All of the samples showed that SFA which is palmitic acid and stearic acid were dominant in the fish oil. Sample 3 showed the highest amount of palmitic acid (0.2087 mg/g) and sample 2 showed that the highest amount of stearic acid (0.1048 mg/g). Besides, based on Ho and Paul (2009) research, the authors found that palmitic acid was the highest amount in Pangasius sp. [16]. In comparison between sample 1, sample 2 and sample 3, the amount of FAME of sample 3 was the highest which is 0.4016 mg/g followed by sample 1 (0.2513 mg/g) and sample 2 (0.0228 mg/g).

| FAME               | Amount of FAME (mg/g) |
|--------------------|-----------------------|
|                    | Sample 1 | Sample 2 | Sample 3 |
| Palmitic Acid (C16:0) | 0.0919  | 0.0228  | 0.2087  |
| Palmitoleic Acid (C16:1) | 0.023   | n.d.*   | 0.0185  |
| Stearic Acid (C18:0)  | 0.0723  | 0.1048  | 0.0635  |
| Oleic Acid (C18:1)   | 0.0641  | n.d.*   | 0.1109  |
| Total Amount SFA     | 0.1642  | 0.0228  | 0.2722  |
| Total Amount UFA     | 0.0871  | n.d.*   | 0.1294  |
| Total FAME           | 0.2513  | 0.0228  | 0.4016  |
4. Conclusion
The result showed that the highest yield of oil being produced at the temperature of 85 °C, 1:2 ratio of solid to solvent and 5 hours of mixing time was 59.92 ± 0.92 %. Besides, fish oil in all parameters approved that fatty acid methyl ester in Patin fish contains of palmitic acid, palmitoleic acid, stearic acid and oleic acid. All of the samples showed that Patin fish contain high saturated fatty acid in which are palmitic acid and stearic acid. The highest total FAME is 0.4016 mg/g in sample 3 followed by 0.2513 mg/g in sample 2 and 0.0228 mg/g in sample 1. The result obtained from this research was accepted to be used to produce high yield of fish oil from Patin fish and can approve that Palmitic Acid exist with high amount in Patin fish oil.

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