The Potential of Biodiesel Production derived from Fish Waste

Amira Farzana Samat 1, Nor Amirah Safiah Muhamad 1, Nur Aziera Abd Rasib 1, Siti Aminah Mohd Hassan 1, Khairunissa Syairah Ahmad Sohaimi 1, and Nur Izzati Iberahim 1

1 Faculty of Engineering Technology, Universiti Malaysia Perlis, UNICITI Alam Campus, 02100 Sungai Chucuh, Padang Besar, Perlis, Malaysia.

E-mail: amirafarzana@unimap.edu.my

Abstract. Petroleum based diesel is one of the largest greenhouse emitters in the worlds based on its contribution to more likely of all carbon, methane and other greenhouse emissions. Besides, the depletion of fossil fuel that indirectly increased its price has force the global oil industry not to be so dependent on the fossil fuel but instead start focusing on alternative sources. Biodiesel is recognized as a clean alternative fuel or as a fuel additive to reduce pollutant from combustion equipment. In this study, the discarded parts of mixed marine fish species were used as the raw material to produce biodiesel. Marine fish oil was extracted from the discarded part of fish and if refined through a series of pretreatment process. The refined marine fish oil undergoes esterification process to reduce the amount of free fatty acid. The oil was then transesterified with methanol and sodium hydroxide as an alkaline catalyst that will speed up the conversion of oil to methyl ester. The three process parameters considered for this study were reaction time, reaction temperature and methanol to oil molar ratio. Biodiesel obtained was then analyzed using gas chromatography (GC). Statistical analyses were performed using SPSS software. The data obtained was analyzed by using one way analysis of variance (ANOVA) repeated measure. The results obtained showed that the conversion of FAME yield is the highest at reaction time 180 minutes, reaction temperature 60˚C and methanol to oil molar ratio at 15:1 with FAME yield 80.16%, 80.03% and 80.39%. Thus, it can be concluded that the conversion of biodiesel increased as the reaction time, temperature and...
1.2. Fish waste as raw materials for biodiesel

In order to overcome this problem, there are suggestions that biodiesel must be produce by using other alternative oil resources such as non-edible oils ([2]) or from waste. Extracted oil from fish waste is an example of raw material for biodiesel production. Fish has high protein content, low saturated fat and also contains fatty acids known for its great benefit to human health. However, research show that the remaining materials that is transformed into waste exceeds 50% from the total of fish capture ([6]). Before, fish wastes are always considered as loss. Nowadays, there are several ways to utilize fish waste to produce renewable energy which are production of biodiesel, manure composting, and production of biogas and burning of fish wastes ([7]). By using fish processing industry waste as a raw material for renewable energy source, it can be turned into a business operation that gives profit to the processing team.

2. Materials and Methods

2.1. Raw materials preparation

The fish wastes were used as the source raw material for the production of fish oil biodiesel. The fish waste included viscera, fish head and other discarded part of fish were collected from the fish market. To prepare the fish oil, the fish waste/gut was treated with a series of process. The fish waste/gut (around 10 kg) were first cut into smaller sizes in order to enhance a speedy oven drying. Then, they were dried by using oven drying at temperature 70˚C for 3 days to reduce the moisture content in the fish waste. The fish wastes were then undergo further size reduction and were pounded in a mortar into finer form.

2.2. Fish oil extraction

Then, by using soxhlet extractor the fish oil was extracted with n-hexane. The solid sample (fish waste that already in finer form) was placed in a cotton cloth. Before the sample was placed in the inner tube of soxhlet, the weight of the sample (50 g) was taken and then it was fitted to a round bottom flask of appropriate size that contains the 150 ml n-hexane. Heat was applied for 3 hours at 60˚C. After 3 hours, the mixture of the solvent and the extracted fish oil was cooled at room temperature for few minutes before it was filtered to remove the remaining impurities in the mixture ([7]).

Then, the obtained mixture was separated by using rotary evaporator in order to get crude fish oil. The temperature of the water bath was set at 35˚C. For each run, 200 ml of the obtained mixture of extracted fish oil and n-hexane was placed inside the rotary bottle for 15 minutes. After 15 minutes, the crude fish oil was obtained inside the rotary bottle. The fish oil extracted was then placed in a freezer ready to be used for transesterification process.

2.3. Esterification reaction of fish oil

Esterification reaction to remove free fatty acid in the fish oil was carried out as fish oil was mixed with methanol and 1.0% w/w sulphuric acid (H2SO4) that act as acidic catalyst. 100 g fish oil was weight and poured in a 2 neck round bottom flask. Methanol to oil ratio used during this process was 20:1. Thus, 100 g of fish oil with 80.91 ml of methanol was measured and was pour into a beaker together with 6 g of sulphuric acid. A beaker fill with water was placed on top of the hot plate. The 2 neck round bottom flask that was filled with 100 g fish oil was put inside the beaker. The fish oil was heated until it reached 60˚C. The mixture of sulphuric acid and methanol was added into the 2 neck round bottom flask. This esterification process was run for 3 hours. After 3 hours, the oil obtained was washed with distilled water. The distilled water was heat until the temperature reached 50˚C and the oil was added and mixed by using magnetic stirrer until bubbles formed. The mixture was poured into separatory funnel and allowed to separate into two different layers. The oil was collected and sodium anhydrous was added to remove the remaining water. The oil was then filtered to remove sodium sulphate anhydrous. The oil obtained was ready to be used in transesterification process ([8]).

After the reaction was completed, the mixture obtained was cool down for few minutes in room temperature and then was pour into separatory funnel to separate the mixture. The mixture was allowed to separate for one night. After few hours, two different layers can be observed. The upper layer was biodiesel and the bottom layer was glycerol. The bottom layer was removed and the upper layer was collected and ready for biodiesel purification process.
The oil obtained was washed with distilled water to remove the remaining methanol, unreacted catalyst and free glycerol. The distilled water was heated until the temperature reached 50˚C and the oil was added and mixed by using magnetic stirrer until bubbles form. The mixture was poured into separatory funnel and allowed to separate into two different layers. The oil was collected and sodium sulphate anhydrous was added to remove the remaining water. The oil was then filtered using filter paper to remove sodium sulphate anhydrous ([9]).

2.4. Gas chromatographic (GC) method to analyze fatty acids methyl ester
A gas chromatographic (GC) method was used to analyze fatty acids methyl ester content. The biodiesel was mixed with heptane in a ratio of 50:50. 2 ml of biodiesel and 2 ml of heptane was mix in a beaker. The mixture was then transferred into a vial by using syringe and 0.45μm whatman column filter. The 30 parameters used in biodiesel GC analysis were as follow: detector used was flame ionization detector (FID), type of column was Bp × 70 (30cm length × 0.25μm thick ×0.32mm diameter), injector temperature was 245˚C, detector temperature was also 245˚C, column temperature was 120-245˚C (4˚C min-1), carrier gas was nitrogen and its flow rate was 20 cm/min. Each run of biodiesel sample take about 35 minutes to complete ([10]).

Statistical analyses were performed using SPSS software. The data obtained was analyzed by using one way analysis of variance (ANOVA) repeated measure. Significant values were assessed with Tukey’s test and p-values less than 0.05 were considered significant.

3. Results and Discussion
Discarded part of fish waste was used as the source of raw material in this research producing crude fish oil that was converted into fatty acid methyl ester. The main problem with processing these low quality animal fats is that they contain high amount of free fatty acids that could not be converted to biodiesel using an alkaline catalyst due to the formation of fatty acids salts (soap). Soap can prevent separation of biodiesel from glycerin fraction thus lowering the biodiesel conversion yield. To overcome this problem, two-steps esterification reaction was used. First, the extracted crude fish oil that is high in free fatty acid was treated with acid-catalyzed esterification in order to reduce the free fatty acids content. Free fatty acids content in the fish oil must be less than 2% in order for transesterification reaction to take place.

Transesterification reaction covert fatty acid into fatty acid methyl ester in the presence of methanol and sodium hydroxide (NaOH) as alkaline catalyst. The transesterification reaction takes place with methanol to oil molar ratio, reaction temperature and reaction time as the process parameters. After transesterification reaction take place, the after product, biodiesel and glycerin was separated. The desired product, biodiesel was then undergo gas chromatography (GC) analysis to determine the compound contain in fish oil biodiesel.

In this research, effect of reaction time on fatty acid methyl esters (FAMEs) yield was investigated. The reaction times used in this study during transesterification reaction for conversion of oil into FAME were 90, 120, 150 and 180 minutes. Figure 1 showed the result obtained during transesterification reaction with methanol to oil molar ratio and temperature used were constant at 15:1 and 60˚C respectively.
It was observed that the ester yield slightly increased with the increased in reaction time ([4]). FAME yield is at the lowest at reaction time 90 minutes (71.27%) and increased as the reaction time increased. This was because, after 90 minutes, methanol has not yet completed the conversion of oil to ester. So, as the reaction was stopped after 90 minutes, not all of the oil has been fully converted into esters. FAME yield continued to increase at reaction time 120 and 150 minutes with 74.65% and 76.33% respectively. The conversion of FAME yield is at the highest at reaction time 180 minutes with 80.16%. Thus, it was observed that the conversion of oil into ester increased as the reaction time increased ([3]).

The reaction temperatures used were 30, 40, 50 and 60˚C. The reaction temperature used is below the boiling point of methanol which is 68˚C. During the transesterification reaction, methanol to oil molar ratio and reaction time is kept constant at 15:1 and 180 minutes. Figure 2 showed result obtained during transesterification reaction with reaction temperature as the parameter.

Based on ANOVA analysis, there was a significant difference at the p < .000 level in FAME yield for four reaction time F (3, 8) = 77.797, p < .000. Despite reaching statistical significance, the actual difference in mean yield between groups was quite large (0.97). The Tukey HSD test indicated that the mean yield for reaction time at 90 minutes (M = 71.3, SD = 0.77) was significantly different from reaction time at 120 minutes (M = 74.6, SD = 0.91), reaction time at 150 minutes (M = 76.3, SD = 0.77) and reaction time at 180 minutes (M = 80.2, SD = 0.34). There was no significant difference in mean yield between reaction time at 120 minutes and reaction time at 150 minutes.

From Figure 2, it was observed that the ester yield increased as the reaction temperature increased. The minimum yield of ester was obtained at the temperatures 30˚C at 69.99%. The yield of methyl ester continued to increase at temperature 40 and 50˚C with 72.95% and 75.67% respectively. The highest methyl ester yield obtained was at temperature 60˚C with 80.03%. As the temperature increased, the kinetic energy also increased. The increased in kinetic energy will cause the molecules of the oil and methanol as well as sodium hydroxide to moves more vigorously and results in more contact with one another and increase the conversion rate of oil to methyl ester. Thus, FAME yield increased as the reaction temperature increased ([3]).

Based on ANOVA analysis, there was a significant difference at the p < .000 level in FAME yield for four reaction temperature F (3, 8) = 213.127, p < .000. Despite reaching statistical significance, the actual difference in mean yield between groups was quite large (0.98). The Tukey HSD test indicated that the mean yield for reaction temperature at 30˚C (M = 69.9, SD = 0.11) was significantly different from reaction temperature at 40˚C (M = 72.9, SD = 0.57), reaction temperature at 50˚C (M = 75.7, SD = 0.80) and reaction temperature at 60˚C (M = 80.0, SD = 0.22).
It was observed that FAME yield increases with increase in molar ratio. From the figure 3, it can be observed that the molar ratio of methanol to oil at 6:1 has the lowest FAME yield percentage with 75.42%. The molar ratio of methanol to oil at 9:1 and 12:1 has the slightest increase in FAME yield with 78.12% and 79.23% respectively. Molar ratio of methanol to oil at 15:1 recorded the highest conversion of FAME with 80.39%. This is because lower molar ratios mean that methanol used during transesterification reaction is also low. The lack of methanol prevents smooth conversion of oil into methyl ester thus lowering the percentage yield ([3]).

Based on ANOVA analysis, There was a significant difference at the p < .000 level in FAME yield for four methanol to oil molar ratio F (3, 8) = 51.239, p < .000. Despite reaching statistical significance, the actual difference in mean yield between groups was quite large (0.95). The Tukey HSD test indicated that the mean yield for methanol to oil molar ratio at 6:1 (M =75.4, SD =0.43) was significantly different from methanol to oil molar ratio at 9:1 (M =78.1, SD = 0.27), methanol to oil molar ratio at 12:1 (M =79.2, SD = 0.64) and methanol to oil molar ratio at 15:1 (M =80.4, SD = 0.63). There were no statistically significant different in mean yield between methanol to oil molar ratio at 9:1 and methanol to oil molar ratio at 12:1 and also between methanol to oil molar ratio at 12:1 and methanol to oil molar ratio at 15:1.

Fatty acid methyl ester (FAME) analysis by gas chromatography (GC) shows the presence of palmitic acid methyl ester (C-16:0) at retention time 10.38, stearic acid methyl ester (C-18:0) at retention time 12.10 and 30.95, oleic acid methyl ester (C-18:1) at retention time 17.48 and eicosanoic acid methyl ester (C-20:0) at retention time 30.95 in the fish oil biodiesel. These fatty acids influence the amount of cetane number of diesel fuel. Figure 4 showed the chromatogram obtained from gas chromatography (GC) analysis and Table 1 showed the list of compound presence in the fish oil biodiesel.

![Figure 3: FAME yield versus methanol to oil molar ratio.](image)

**Figure 3:** FAME yield versus methanol to oil molar ratio.

![Figure 4: Chromatogram from GC analysis.](image)

**Figure 4:** Chromatogram from GC analysis.
Table 1: List of compound in fish oil biodiesel.

| Peak | Retention time | Carbon number | Compound                      |
|------|---------------|---------------|-------------------------------|
| 3    | 10.38         | C-16:0        | Palmitic acid methyl ester    |
| 5    | 12.10         | C-18:0        | Stearic acid methyl ester     |
| 6    | 12.44         | C-18:1        | Oleic acid methyl ester       |
| 7    | 17.48         | C-20:0        | Eicosanoic acid methyl ester  |
| 8    | 30.95         | C-18:1        | Oleic acid methyl ester       |

4. Conclusion

The present study indicates that instead of dumping, utilization of discarded part of fish waste as a low cost feedstock is a better option. Animal fats characterized by high free fatty acids (FFAs) have been deemed suitable as a feedstock in biodiesel production. The two-step transesterification process was used in order to convert oil with high FFAs into methyl esters. The first step in this process which is acid catalyzed esterification reduced the FFA content of the oil until the FFA content is less than 2%. Then, alkaline catalyst transesterification process is used to convert the products of esterification process into methyl esters and glycerol. The effects of methanol to oil molar ratio, reaction temperature and reaction time were analyzed in this researched. From this research, it was proven that methanol to oil molar ratio, reaction temperature and reaction time greatly affects the conversion yield of biodiesel. Fatty acid methyl ester (FAME) yield increased as the methanol to oil molar ratio, reaction temperature and reaction time increased. FAME yield has the highest percentage when methanol to oil molar ratio, reaction temperature and reaction time were the highest which are 15:1 for methanol to oil molar ratio, 60°C for reaction temperature and 180 minutes for reaction time. Overall, this two-steps transesterification method reduced the production cost and is a suitable method for conversion of oil that is high in free fatty acids into biodiesel.

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