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Potential of Fish Wastes as Feedstock for Biodiesel

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Abstract

Fish wastes are the discarded parts include the internal organs, viscera, bones, trimmings, tails, fins and skin of fishes. These discarded portions while disposing of cause major environmental damage. Usually, the discarded parts of fishes are ground into fishmeal for livestock and aquaculture feed. This study was undertaken to explore biodiesel production based on the fatty acids composition. The fish waste sample was collected from Kota Kinabalu, Sabah fish market. The sample was drained for excess water and oven-dried at 55˚C - 60˚C for complete dryness. Crude oils were extracted in petroleum ether in Soxhlet extraction method. Methylation of the extracted crude fish oil was carried out to yield fatty acid methyl esters (FAME). The FAME was analyzed by GCMS system and the reference to NIST library was used to identify the fatty acids present in the FAME. A total of 21 fatty acids were identified that composed of 53.53% saturated fatty acids (SFA), 22.1% monounsaturated fatty acid (MUFA) and 24.37% polyunsaturated fatty acids (PUFA). The important fatty acids [myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), docosapentaenoic acid (C22:5) and docosahexaenoic acid (C22:6)] found in fish oil indicated the potentiality of biodiesel production if fish waste was stocked. The highest percentage of SFA causes higher viscosity, cetane number and density and hence these properties of biodiesel produced from the fish waste are expected to be high. Therefore, the fish waste has high potential of fatty acid in FAME to produce biodiesel through transesterification process.

Keywords

Fish Waste, Lipids, Fatty Acid, Fatty Acid Methyl Ester, Biodiesel

1. Introduction

Considerable attention has been paid to utilize renewable energy, especially bio-

fuels [1]. Biodiesel is used as an alternative to conventional petroleum diesel [2]. Edible and non-edible oils are the potential feedstock for the production of biodiesel. Edible oils include both edible animal fats, such as, beef tallow, pork lard and duck fat, and vegetable oils, such as, soybean oil, palm oil, sunflower oil, canola oil and corn oil. Non-edible oils include non-edible vegetable oils, such as Jatropha oil and Pongamia oil, waste cooking oils and waste animal fats. However, the increasing demand for edible oils and animal fats due to the increasing world population has limited their production as fuel [3]. Although biodiesel production is usually made at a larger scale by a transesterification reaction using normally vegetable oils, the high prices of edible vegetable oils and their use as food resources have become the limiting factors and hence efforts have been made to find the alternatives [4]. In recent years, non-edible feedstocks such as animal wastes have been investigated for its potential as an alternative in biodiesel production. Non-edible feedstocks are cheaper and at the same time help to mitigate environmental damage as well as improve the quality of the biodiesel [3]. Among animal wastes, fish wastes, the discarded or dressing parts cause environmental degradation both in terrestrial and aquatic ecosystem. Usually, the discarded parts of fishes are ground into fishmeal as major ingredient for livestock and aquaculture feed [5]. The majority of fish wastes are transported to fish meal plants to produce into fish meal and fish oil.

The crude fish oil extracted from fish waste can produce the highest quantity, low price and stable source of raw material for biodiesel production and at the same time reduce pollutant emissions [1]. The calorific value of fish oil is similar to that of petroleum oil [6]. There are limited studies on the fuel and combustion characteristics of biodiesel produced from marine fish oil. This is because fish oil contains approximately 90% of the energy content of diesel fuel. The conversion of fish waste into biodiesel involves transesterification process where fatty acid methyl ester (FAME) is produced. When the reaction occurs, triacylglycerols (TAG) will be transformed into fatty acid alkyl esters and usually methanol is added in the reaction [7]. Hence, the biodiesel is produce from fatty acid methyl esters (FAME) of fish oil. The presence of fatty acids in FAME such as palmitic, palmitoleic, oleic, stearic, eicosapentaenoic and docosahexaenoic acids will cause higher potential of fish waste as a feedstock for biodiesel production [8]. Thus fish wastes have the potential sources of renewable fuel with dual benefits: reduce dependence on petroleum fuel and reduce pollution load. Although there is high potential to utilize fish oil to biodiesel as an alternative to petroleum diesel, but research is limited on the fish oil properties. Furthermore, the fuel properties of the FAME found in the biodiesel are influenced by the chain length, degree of saturation and branching of the chain [7]. This study was focused to determine the fatty acids profiles in fish waste as feedstock for biodiesel production.

2. Materials and Methods

2.1. Collection of Fish Waste Sample

The fish waste sample was collected from the local wet fish market locally called

Pasar Besar, in Kota Kinabalu, Sabah, Malaysia. Various types of discarded fish wastes, such as the internal organs, viscera, bones, trimmings, tails, fins and skin were collected. Only fresh fish waste sample was collected and brought back to Borneo Marine Research Institute, Biotechnology Laboratory for further preparation.

2.2. Pre-Treatment of Sample

To ensure successful extraction of oil, the fish waste sample was treated prior to oil extraction following the steps described by Abdulkadir *et al.* [9]. The wanted parts (internal organs) were washed using distilled water for several times to removed dirt and the remaining blood. The moisture content of the fish waste sample was reduced by oven drying maintained the temperature of 55˚C - 60˚C for complete dryness. The dried sample was crushed into powder form by using a mortar and kept in sealed plastic bags and stored in the laboratory fridge until use.

2.3. Extraction of Fish Oil

The fish oil was extracted with petroleum ether using Soxhlet™ 2043. Extraction was done based on manual of Soxhlet TM 2043, where 60˚C temperature was maintained. The extracted crude fish oil was then kept in the laboratory chiller for further analysis.

Quantification of total crude lipid (%) *in sample*

The total crude lipid (%) content was calculated by using the following equations [10]:

Lipid content (%) = (mass of lipid extract)/(sample weight) × 100

2.4. Determination of Acid Value and Free Fatty Acid (FFA) Content

The acid value of the extracted fish oil was determined according to the method described by [11]. The method was based on titration of the sample, diluted with ethanol-diethyl ether mixed solvent, with KOH solution in ethanol using phenolphthalein as indicator to detect the end point.

The volume of KOH titrant used was measured and the acid value was calculated according equation as below:

$$\text{Acid value}\ \left(\text{mg KOH/g}\right) = \frac{V_{\text{KOH}} \times 5.61}{w\left(\text{g}\right)}$$

where, V_{KOH} = Volume of potassium hydroxide titrant used (mL) and w = weight of the crude oil sample (g).

The free fatty acids (FFA) content was then calculated by using following equation with conversion factor of 1.99 [12].

Free fatty acid, FFA (%) = Acid value/1.9

2.5. Fatty acid Profiling

The analysis of fatty acid composition was done by using gas chromatogra-

phy-mass-spectrometry (GCMS). Conventional solvent lipid extraction in total crude lipids sample was done by using chloroform and methanol according to Bligh & Dyer (1959) method which was modified by Yong [13]. The extracted lipid was separated into two layers, the methanol and water at the upper layer was removed while the chloroform and lipid at the bottom layer was collected. The solvent was removed using vacuum concentrator. Methylation was carried out before the analysis in order to transform the lipid into fatty acid methyl esters (FAME). As a preparation for the injection into GCMS, the transformed fatty acids or the FAME was dissolved in 3 mL of hexane and filtered through a 0.2-μm syringe filter. The FAME was then injected into GCMS system (composed of an Agilent 7890A gas chromatograph system and an Agilent 5975 mass spectrometry detector). A capillary column HP-5MS (5%-Phenyl)-methylpolysiloxane with 30 m length, 0.25 mm diameter and a 0.25-μm film thickness was used in the gas chromatograph system. The injection volume was set as 1 μL and the injector temperature was set at 250˚C. The temperature program was set to run as follows: begin at 90˚C and hold for 3 min, from 90˚C to 180˚C at 3˚C per min for 5 min, from 180˚C to 290˚C at 3˚C per minfor 15 min. The initial hold time at 90˚C for 3 min was also taken as the delay time for the mass spectrometry detector in order to preserve the sensitivity of the detector and its filament. A post-run at 290˚C for 10 min was performed for cleaning as a preparation for the next injection. The gas chromatography was set in splitless mode. Helium gas was consumed as carrier gas and maintained at 1.0 mL/min at constant flow rate. The reference to the National Institute of Standards and Technology (NIST) library was used to identify the fatty acids composition present in the FAME. The relative compositions were then computed with reference to the abundance of the compounds in chromatogram. Each analysis was carried out in triplicate (three times of injection) and a blank solvent was injected prior each sample analysis.

3. Results

3.1. Total Crude Lipid (%) Content

The total crude lipid content of 17.31% was extracted from the fish wastes by Soxhlet method. The total powdered sample of 146.55 g was used in order to obtain 25.37 g of extracted fish oil.

3.2. Acid Value and Free Fatty Acids Content

The acid value obtained in the range of 15 - 75 mg KOH/g when 0.5 g of crude fish lipid was used and with accuracy of 0.001. The average acid value (AV) was 33.83 mg KOH/g. The free fatty acids content (FFA) of the crude fish oil was then calculated to be 17%.

3.3. Fatty Acid Profiles

A total of 21 fatty acids were identified in the crude fish oil using GCMS. How-

ever, only 15 (fifteen) fatty acid had values greater than 0.5%. The fatty acid composition classified into saturated, monounsaturated and polyunsaturated fatty acids of extracted waste fish oil are shown in Table 1. Among all the identified fatty acids, seven of them are saturated fatty acids (SFA), six of them are monounsaturated fatty acids (MUFA) and another eight are polyunsaturated fatty acids (PUFA). The SFA present in the waste fish oil include myristic, pentanoic, palmitic, margaric, stearic, nonadecylic and arachidic acids. The MUFA

Table 1. The fatty acid composition (%) of crude fish oil extracted from fish waste collected from the local fish market.

Fatty Acids	Chemical Name	%
	Saturated Fatty Acid (SFA)	
C14:0	Myristic acid	2.58 ± 0.11
C15:0	Pentadecanoic acid	0.91 ± 0.03
C16:0	Palmitic acid	30.60 ± 3.1
C17:0	Margaric acid	2.45 ± 0.04
C18:0	Stearic acid	12.83 ± 1.47
C19:0	Nonadecylic acid	1.27 ± 0.05
C20:0	Arachidic acid	1.89 ± 0.03
	Total saturated fatty acids	**53.53 ± 4.92**
	Monounsaturated Fatty Acid (MUFA)	
C16:1n7c	Palmitoleic acid	4.17 ± 0.12
C16:1n7t	Palmitelaidic acid	1.14 ± 0.11
C17:1n10	cis-10-Heptadecenoic acid	0.38 ± 0.02
C18:1n9c	Oleic acid	12.15 ± 0.55
C18:1n9t	Elaidic acid	3.68 ± 0.33
C20:1n9c	Eicosenoic acid	0.58 ± 0.11
	Subtotal	**22.1 ± 1.24**
	Polyunsaturated Fatty Acids (PUFA)	
C18:2ω6	Linoleic acid	1.11 ± 0.23
C20:2ω6	Eicosadienoic acid	0.18 ± 0.05
C20:4ω3	Eicosatetraenoic acid (ETA)	0.40 ± 0.17
C20:4ω6	Arachidonic acid	5.89 ± 1.13
C22:4ω6	Docosatetraenoic acid	1.32 ± 0.13
C22:5ω6	Docosapentaenoic acid (n − 6 DPA)	1.05 ± 0.40
C22:5ω3	Docosapentaenoic acid (n − 3 DPA)	1.99 ± 0.35
C22:6ω3	Docosahexaenoic acid (DHA)	12.43 ± 2.52
	Subtotal	**24.37 ± 4.98**
	Total Unsaturated Fatty Acids	**46.47 ± 6.22**

All values are expressed as mean ± standard deviation.

include palmitoleic, palmitelaidic, cis-10-heptadecanoic, oleic, elaidic and eicosenoic acids, while the PUFA include linoleic, eicosadienoic, eicosatetraenoic (ETA), arachidonic, adrenic, docosapentaenoic (n − 6 DPA), docosapentaenoic (n − 3 DPA) and docosahexaenoic (DHA) acids. The extracted waste fish oil contained higher concentration of SFA (53.53% ± 4.92%) as compared to the concentration of MUFA (22.1% ± 1.24%) and PUFA of 24.37% ± 4.98% (**Table 1**).

4. Discussion

The lipid content of crude fish oil in this study was 17.31%. The percentage was contributed by the internal organs of the combination of different fish species. Only the internal organs of small fishes were allowed for collection due to its low market demand, while the internal organs of big fishes such as tuna, mackerel etc have high market demand as the big fishes were sold as a whole without removing their internal organs. The lipid content varies with the species of fish and their environmental factors [9]. The lipid content of fish waste in the present study was different from all the listed used by other authors [9], where fish carcasses were used in their analysis instead of the internal organs only.

Esterification is the central reaction to reduce the levels of FFA, but concentrations of catalyst are important in the process (**Table 2**). The feedstock that has higher acid value, then esterification process requires extra amount of methanol. While the catalyst concentration increases, acid value of Jatropha oil normally decreases. So using different amounts of catalyst concentration, FFA to methanol ratio 1:50 at 63˚C - 64˚C, the acid value of Jatropha oil able to reduced from 17.0 mg-KOH/g-oil to below 8.1 mg-KOH/g-oil in 1 hr [14]

Generally the vegetable oil and waste with low level of FFA are used in commercial biodiesel production. In this study, the FFA of 17% was determined from the crude fish oil, which considered higher than the acceptable limit (less than 1%). It was reported that waste containing yellow grease and brown grease are attractive feedstocks for biodiesel synthesis even though the containing a FFA level are 15% and 33% respectively [15]. In such situation the fish waste oil that used in this study, could be one of the potential feed stock candidate due to

Table 2. Comparison of total crude lipid (%) contents of different fish species with present study.

Fish sample	Lipid content (%)
Trunk fish (*Mormyrops deliciosus*)	30.22 [9]
Silver cat fish (*Bagrus docmac* niger)	6.72 [9]
Tilapia (*Tilapia dagati*)	14.52 [9]
Cat fish (*Clarias anguillaris*)	17.93 [9]
Shawa (a type of herring)	24.02 [9]
Fish waste (this study)	17.31 (current study)

its availability, low cost resources and other properties like composition of fatty acids. The high FFA content in the fish waste indicated that pretreatment step which is esterification prior to transesterification is necessary. Pretreatment stages, acid-catalyzed esterification integrated with water separation, are necessary to minimize the acid and water content to less than the threshold limits set by the subsequent alkali catalyzed transesterification [15]. Feedstock with high free fatty acid will react in transesterification reaction undesirably with the alkali catalyst thereby forming soap. Many researchers used two-stage acid and alkali-catalyzed transesterification. In the first stage, esterification of FFA present in feedstock is carried out using acid to decrease the FFA level to less than 1%. In the second stage, transesterification of the neutral feedstocks is performed using an alkaline catalyst [16]. The fish waste as feedstocks can generate processing problems in the standard biodiesel production because the alkali-catalyzed system is very sensitive to water, free fatty acids and other impurities. So, better to have pretreatment steps for fish waste to reduce the acid and water concentrations below an optimum limit, such as., FFAs less than 1% and water less than 0.5%. Therefore, fish wastes as feedstock for biodiesel production have to face problem of high concentrations of FFAs, water, and other impurities.

The molecules of biodiesel refer to fatty acids methyl ester (FAME) that is produced from vegetable and animal oils and fats through chemical processes such as transesterification and esterification with methanol [2]. The structural features such as chain length, degree of unsaturation, and branching of the chain can affect the physical and fuel properties of the fatty esters that comprise the biodiesel [7]. The important fuel properties that could be influenced include kinematic viscosity, cetane number, oxidative stability, density and heating value [7]. These properties would determine the fuel efficiency, although these analyses were not conducted in present study.

In this study, seven saturated fatty acids (14:0, 15:0, 16:0, 17:0, 18:0, 19:0 and 20:0) composed 53.53% of the total fatty acid in the waste fish oil. The kinematic viscosity of biodiesel increases with chain length and degree of saturation of fatty acids [7]. Therefore, the kinematic viscosity of biodiesel produced from the waste fish oil is expected to be high. Higher viscosity will lead to lower fuel efficiency [17]. In order to solve the problem due to high viscosity, transesterification reaction is needed to reduce the viscosity of oil and this reaction transforms the triglycerides (TG) contained in the oil into a mixture of alkyl-esters, as biodiesel [18].

Similarly the cetane number also increases with the chain length and degree of saturation and decreases with branching of carbon chain [19]. Hence, the cetane number of biodiesel produced in this study is expected to be high. High cetane number of fuel will lead to higher fuel efficiency as it lowers ignition delays and particulate emissions and hence ensure good cold start behavior and allow the engine to run smooth [20]. In this study, the amount of SFA (53.53%) was higher compared to that of unsaturated fatty acids (22.1%) and 24.37% polyunsaturated fatty acids (PUFA). Higher unsaturation will lead to poorer oxidative sta-

bility [21]. It was also reported that biodiesel feedstocks having 2% PUFA were expected to have very high oxidative stability, in contrast, biodiesel feedstocks having over 50% PUFA were expected to have poor oxidativestability. The abundance (24.37%) and richness of PUFA (C18:2ω6, C20:2ω6, C20:4ω3, C20:4ω6, C22:4ω6, C22:5ω6, C22:5ω3, C22:6ω3) in the crude fish oil is expected to have reduced the oxidative stability of biodiesel produced.

The composition of was relatively higher SFA (53.53%) than unsaturated fatty acids (46.67%). Density increases with the number of carbon atoms and the degree of saturation [22]. Hence, the density of fatty acid methyl esters (FAME) in the crude fish oil is expected to be high. In general, the density of biodiesel is slightly higher than those of petroleum diesel [21]. SFA have lower mass energy content (MJ/kg) than unsaturated fatty acids [23]. Lower mass energy content will have lower heating value. Hence, the heating value of biodiesel produced in this study is expected to be low. Biodiesel has lower heating value than petroleum diesel, as biodiesel has higher oxygen content and thus lower mass energy content [23]. The essential fatty acid methyl esters (FAME) that were reported in most of the fish oil biodiesel include myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), docosapentaenoic acid (C22:5) and docosahexaenoic acid (C22:6) [8]. In fact all these fatty acids were identified in fish waste oil that used in present study.

5. Conclusion

Fish wastes as feedstock for biodiesel production have to solve the problem of high concentrations FFAs, water, and other impurities. The FAME of the waste fish oil also contains significant amount of all type fatty acids, essential for biodiesel production. The similarity of the fatty acids in FAME content gives a high potential of the fish waste to be used as feedstock for the biodiesel production.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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