Biodiesel Production from Fish (*Cyprinus carpio*) Waste and Evaluation of Engine Performance
(Penghasilan Biodiesel daripada Sisa Ikan (*Cyprinus carpio*) dan Penilaian Prestasi Enjin)

MOHAMMED SAIFUDDIN* & AMRU N. BOYCE

ABSTRACT
As fossil energy resources are depleting quick and energy security is playing a vital role in the world economy. Quest for alternative energy sources have turned researches investigation in waste foods for next generation fuel. Biodiesel is usually considered to be environmentally friendly as it reduces greenhouse gas emission. Fish wastes rich in fatty acids and can be used as the raw material to produce biodiesel through transesterification reaction. The results showed that the seven peaks are fatty acid methyl esters, indicating all the triglycerides were successfully methylated to methyl esters. Fish based biodiesel provided a significant reduction in carbon monoxide (CO) and hydrocarbon (HC) emissions under engine loads of 15 (Nm) and required no engine modification. The viscosity of the produced biodiesel was within the range of international standards (ASTM). The biodiesel was found to contain a low base number and exhibited a lower specific fuel consumption compared to the conventional diesel. It can be concluded that biodiesel derived from waste fish oil can be considered as a potential source of commercial biodiesel.

Keywords: Biodiesel; emission; fatty acid; fish waste; fuel consumption

INTRODUCTION
The global warming is caused by using excessive fossil fuels. Therefore, renewable energy and bioresourced fuel are required for replacing fossil fuel to reduce the greenhouse gas emissions (Lin & Li 2009). Additionally, the world is on the edge of energy crisis and resulting continuous increase in global petroleum prices with adverse impacts on human life and world politics (Subbaiah & Gopal 2011). The occurrence of oil depletion, global warming and the greenhouse effect has put us on an alarming condition. In order to solve these issues, a renewable energy resource should be introduced and developed. Biodiesel is one of the most promising energy sources despite diesel-fuel. Biodiesel has been chosen as one of the main alternative fuel because of its better characteristics and advantages such as highly biodegradable (Crutzen et al. 2008). In addition, biodiesel has a low emission profile and are environmentally friendly, hence not posting dangers to our vulnerable earth.

Most of the research on biodiesel has focused on using plant based oils as feed stocks (Saifuddin et al. 2014). There has been much less study on converting animal-based oils into biodiesel. One potential source of biodiesel is fish waste. In Malaysia, fisheries industry is one of the main sectors of food supply. The Malaysian government assists the fisheries industry through the Department of Fisheries, which provides advice and technical assistance. Huge amount of fish wastes are generated every day by food industries, supermarkets and restaurants. It has been estimated that over a million tons of fish by-products are generated from the fishing industry (Wisniewski et al. 2010). Some of these products are converted into fish meal and oil, but approximately 60% is not utilized. Thus, fish by-products can be converted into biodiesel and the
utilization of fish waste assists in cleaning the environment. Otherwise, excess fat can cause land and water pollution (Bhatti et al. 2008; Tashtoush et al. 2004). Due to these reasons, fish waste is sustainable for biodiesel production and economically feasible (Wisniewski et al. 2010). Moreover, fish tissues have high content of free fatty acids and high amount of saturated fatty acids (Piccolo 2009). Fish oil derived biodiesel can reach a cetane number up to 74, meaning higher engine efficiency. It does not contribute to the net atmospheric \( \text{CO}_2 \) level and has a low emission profile.

Within several methods to produce biodiesel, transesterification is one of the most used ways to produce biodiesel (Lisboa et al. 2014). The transesterification purpose of fish oils to their methyl esters process is to get the lower viscosity of the oil (Sheinbaum-Pardo et al. 2013). Since viscosity is the most vital characteristics to assess the biodiesel quality and its effects on the operational injecting equipment. Consequently, fuel consumptions and gas emission rate are important as well. The increase of viscosity makes incomplete combustion and poorer atomization of the fuel spray (Encinar et al. 2007). A lot of research has been carried out on the production of biodiesel from plant based oil but the use of fish waste, such as *Cyprinus carpio* fish oil has not been well documented. Thus, the objectives of this study were to produce biodiesel from fish waste and to evaluate the characteristics of the biodiesel, for example to identify methyl esters present in the biodiesel, the exhaust emissions and fuel consumption by the engines.

**MATERIALS AND METHODS**

**PREPARATION OF OIL SAMPLES**

Fish (*Cyprinus carpio*) waste is utilized in this study to produce biodiesel. Fish fats were collected from discarded parts of certain types of fishes in the wet market in Petaling Jaya, Malaysia. Several simple processes have to be done to prepare the crude fish oil. Firstly, 50 g fish fats were added to the flask and the temperature was increased to 60°C. Then, filtration is necessary as intestines or some parts of the fish organs might be embedded in the fish fat. Filtration was done by utilizing a filter funnel using filter paper to filter off the undesired particles. Fish oil produced is golden yellow in colour and contains some impurities such as water, fish residue and fish blood. After that, the fish oil is kept for the transesterification step (Figure 1).

**THE CONDITIONS FOR BIODIESEL PRODUCTION**

Filtered fish oil was heated in the oven for approximately 15 min at 60°C before subjecting to transesterification. Methanol was used for the transesterification reaction to produce biodiesel from waste fish oil. The molar ratio of oil to alcohol 1:6 was used in this reaction. Sodium hydroxide (NaOH) was used as a catalyst. The amount of base catalyst (NaOH) needed is 1% by weight of the oil used. Catalyst and methanol mixture was heated for 2 h at 55±2°C on a hotplate with magnetic stirrer under rotation speed of 250 rpm.

**BIODIESEL PRODUCTION**

After transesterification process, the solution was separated into two distinct layers, crude biodiesel and glycerol (Figure 2). This was due to the principle of gravity difference between both layers (Lin et al. 2009) and the solution in conical flask is transferred into a beaker for methanol to evaporate easily as beaker possesses a larger surface area.

**PURIFICATION**

There are three major steps in the purification stage, which are the separation step, washing step and lastly the drying step. Separation step is done by pouring the solution into a separating funnel and let it to settle down. After two distinct layers are formed, the lower layer that contains glycerol will be release out and left with methyl esters. Methyl esters obtained are then washed with distilled water for at least four times to remove impurities such as pigments, excess alcohol, excess catalysts, glycerol and soaps. Washing step is complete when the distilled water is clear after washing the methyl esters for several times. Distilled water is then removed. The ratio of distilled water to methyl esters is 1:1.

The last step will be the drying step. Drying step is carried out by drying up the biodiesels in the oven at 48°C and by using sodium sulphate anhydrous as well. Small amount of water will be eliminated when biodiesel is dried up in the oven. Whereas, large amount of water is removed by using sodium sulphate anhydrous. As sodium sulphate anhydrous meets with water, hard crystals will be formed. Hence the crystals can be filtered off by pouring the solution into the filter paper. Pure and dry biodiesels then prepared for analysis.

---

**FIGURE 1. Transesterification of triglyceride with methanol in presence of NaOH**

![Transesterification of triglyceride with methanol in presence of NaOH](image-url)
BIODIESEL ANALYSIS AND CHARACTERIZATION

Gas chromatography- mass spectrometer (GC-MS) is used for biodiesel analysis and the main purpose of this analysis is to identify the methyl esters present in the biodiesel produced. Biodiesel characterization was done in Tribology Laboratory of the Department of Mechanical Engineering in the Faculty of Engineering, University of Malaya. The test methods of total acid number, total base number, multi element and viscosity of biodiesel were ASTM D664, ASTM D4739, EN 14538 and ASTM D445, respectively.

EMISSION TEST

This test was carried out in the Tribology Laboratory of the Department of Mechanical Engineering in the Faculty of Engineering, University of Malaya as well. The model of the engine is YANMAR TF120-M. The produced biodiesel was used to run the diesel engine and its emissions were analyzed using a BOSCH gas analyzer, which was used to analyze the carbon monoxide and unburned hydrocarbon. A BACHARACH gas analyzer was used to analyze the NOx emission.

The engine specifications for the YANMAR Model TF120-M are as follows; Type: 1-cylinder, horizontal, water cooled, 4 stroke diesel engine with a direct injection combustion system and natural aspiration. The cylinder bore x stroke: 92 x 99 mm with a displacement of 638 cc and a continuous rated output of 10.5 Ps/7.7kw@2400 rpm and a 1 h rated output of 12.0 Ps/8.8 kw@2400 rpm. The cooling water capacity was 2.3 L. The engine dry weight was 101.5 kg.

RESULTS AND DISCUSSION

With regard to catalyst concentration, the optimum value for alkaline transesterification is 1%. Bhatti et al. (2008) reported that as the catalyst concentration increases, the biodiesel yield increases as well until a maximum concentration of 1%. The maximum biodiesel yield is achieved using a molar ratio of 1:6 for oil:methanol. Theoretically, an oil/methanol ratio of 1:3 is enough to form methyl esters from the reaction of methanol with triglycerides. However, the reaction might be in equilibrium or backward. According to many studies, a ratio of 1:6 is sufficient for the high yield of methyl esters (Guan et al. 2008; Hossain & Boyce 2009). For the production of biodiesel more economically viable, a maximum oil:methanol ratio of 1:6 was used and it contributed to maximum conversion rate in this experiment (Table 1).

Different molecules take different periods to wash out with a solvent from the gas chromatograph which is known as the retention time and consequently this leads the mass spectrometer to find out the ionized molecules separately (Woods & Fryer 2007). This is generally happened by mass spectrometer where each molecule has been broken into ionized fragments. These fragments provide a representative spectral using their mass to charge ratio. In order to determine the identity of fatty acid methyl
esters present in the biodiesel samples, the chromatogram and mass spectra obtained from the GC-MS analysis had to be interpreted. It can be seen that there are total seven compounds that are present in fish based biodiesel (Figure 3). Details of these compounds, retention time, area (%), possible identities are shown in Table 2. Each of the pick has its unique retention time, which is important in determining the compound present in the biodiesel sample (Wisniewski et al. 2010).

All seven compounds have been identified as fatty acid methyl esters, meaning all the triglycerides were successfully methylated to methyl esters indicating a 100% conversion of TAG to fatty acid methyl esters (FAME). The 100% conversion proved that the optimum conditions used were suitable for fish based biodiesel production. Additionally, the identities and molecular structure are shown in Table 3. The chain length of fatty acids composition ranged from 12C to 18C. The fatty acids with a chain length of 18 carbons are the major type of fatty acids in Cyprinus carpio. As the chain length of methyl esters increases, the heat of the combustion/energy content of the biodiesel increases as well (Knothe 2008). However, the viscosity and cold flow properties of the biodiesel will be down due to the higher chain length of biodiesel. It can be seen that the fatty acids present in the fish oil sample was much lesser in comparison to Kminkova et al. (2001). Most of the reports in the literature showed the presence of C20 fatty acids and the absence of C12 fatty acids in fish based biodiesel. The results obtained in this study are different from Kminkova et al. (2001). Although the amount of C22 fatty acids that are present in the fatty tissues is actually negligible, with a value of 5.75%. The major fatty acids that were identified in our sample were similar to Kminkova et al. (2001) which included oleic acid followed by palmitic acid and palmitoleic acid. The table also showed that Cyprinus carpio is rich in mono-unsaturated fatty acids followed by saturated fatty acids and poly-unsaturated fatty acids.

The higher the total acid number (TAN) value, the higher amount of free fatty acids produced and hence more corrosive towards the automotive parts of engine (Hossain & Mazen 2010). As it can be seen in Table 4, TAN was non-detectable and the total base number (TBA) was 0.04 mg KOH/g. The non-detectable condition of TAN can be due to the low chemical reactivity of the biodiesel produced. The TBA value obtained was very low compared to the ASTM standard. The value of TBA could be due to

| Parameters                  | Value |
|-----------------------------|-------|
| Temperature                 | 55°C  |
| Retention time              | 2 h   |
| Catalyst concentration      | 1% (w/v) |
| Alcohol oil ratio           | 6:1   |
| Stirring speed              | 250 rpm |

| Total 7 peaks for fish based biodiesel | Retention time (min) | Methyl esters | Area (%) | Total (%) |
|---------------------------------------|----------------------|---------------|---------|-----------|
| 1                                     | 12.157               | C₁₆H₂₅O₂      | 9.94    |           |
| 2                                     | 16.498               | C₁₅H₃₀O₂      | 6.84    |           |
| 3                                     | 20.170               | C₁₇H₃₄O₂      | 5.58    |           |
| 4                                     | 20.577               | C₁₇H₃₄O₂      | 19.70   | 96.96     |
| 5                                     | 23.767               | C₁₉H₃₆O₂      | 14.28   |           |
| 6                                     | 23.886               | C₁₉H₃₆O₂      | 40.64   |           |
| 7                                     | 24.330               | C₁₉H₃₈O₂      | 3.02    |           |
the nature of biodiesel that are chemically un-reactive or there was too little alkaline present in the biodiesel.

Viscosity can also be known as dynamic viscosity, which is the ease of the fluid to flow well. Viscosity is important parameter in measuring the quality of biodiesel since it will affect the operation of fuel injection in biodiesel engines. Biodiesel with low viscosity may results in the leakage or increased wear of the fuel injection pumps due to insufficient lubrication (Subbaiah & Gopal 2011). Whereas the high viscosity of biodiesel will lead to the high tendency of fuel to cause deposition in engine, hence results in low efficiency of engine. According to the ASTM standard, the range of viscosity of biodiesel is 1.9-6.0 (cSt) at 40°C. The viscosity of the biodiesel sample with the maximum conversion conditions was 5.47 cSt at 40°C, which falls well within the standard range of ASTM limits (Table 4). Excess viscosity will lead to poor atomization of fuel and low viscosity will cause power loss due to the injection pump and injector leakage (Srivastava & Prasad 2000). Thus, the fish based biodiesel meets the international standards and can be used as fuel.

During the combustion process, carbon dioxide is formed in the presence of sufficient oxygen from the air and as well as from the fuel itself (Agarwal 2007). However, carbon monoxide is also produced due to the incomplete combustion of fuel in the combustion chamber of the engine. As can be seen in Table 5, conventional diesel produced a higher amount of carbon monoxide than fish based biodiesel. Probably the lower cetane number of the petrodiesel results in the lower tendency of itself to autoignite and hence the higher emission of carbon monoxide. Furthermore, a higher viscosity and poorer atomization tendency can also lead to higher emissions of carbon monoxide. Biodiesel has the advantage of having a high cetane number and this probably contributed to the lower carbon monoxide emission (Saifuddin et al. 2014). Carbon monoxide emissions from petrodiesel are always higher than of the biodiesel. It could also possibly be because biodiesel itself has approximately 11% of oxygen content which will enhance the completion of its combustion (Ramadhas et al. 2005).

| Compounds | Structural formula |
|-----------|-------------------|
| Dodecanoic acid, methyl esters (methyl laureate) | |
| Tetradecanoic acid, methyl esters (methyl myristate) | |
| 9- Hordecenoic acid, (Z)-, methyl esters (methyl palmitoleate) | |
| Hordecanoic acid, methyl esters (methyl palmitate) | |
| 9,12 Octadecadienoic acid (Z, Z)-, methyl esters (methyl linoleate) | |
| 9-Octadecanoic acid (Z)-, methyl esters (methyl oleate) | |
| C16-tetjanoic ac id, methyl esters (methyl stearate) | |

| Characteristics | Value | ASTM standard |
|-----------------|-------|---------------|
| Total acid number (TAN) | No detectable | <0.8 |
| Total base number (TBN) | 0.04 (mg KOH/g) | 10 - 15(mg KOH/g) |
| Viscosity (at 40°C) | 5.47 (cSt) | 1.9 - 6.0 (cSt) |
Unburned hydrocarbon emission can also be known as partially oxidized hydrocarbon emission. Its emission can be caused by two main factors. It could increase due to fuel injection that occurred too early, causing an increased delay time and hence more fuel can contact with the relatively cool cylinder wall, or the combustion process in the engine occurring too late, causing an insufficient time for complete combustion. Biodiesel fueled engine showed a lower production of unburned hydrocarbon compared to conventional diesel. This is probably because biodiesel has a higher cetane number leading to faster evaporization and autoignition (Wu et al. 2014). All these factors enhance the complete combustion of biodiesel and hence reduce the production of unburned hydrocarbon. It was found that fish oil biodiesel showed lower hydrocarbon emission when compared with the waste palm cooking oil biodiesel or petrodiesel (Saifuddin et al. 2014). This is due to the fish based biodiesel has high combustion properties.

NOx content is an important characteristic of engine fuel. NOx emission formation is highly temperature dependent. The amount of NOx emission is directly related to the exhaust gas temperature and engine combustion chamber temperatures. As the exhaust gas temperature increases, NOx emission also increases. Hence, biodiesel fuel engines have a higher potential to emit more NOx because the exhaust gas temperature increases as the biodiesel concentration increases (Ramadhas et al. 2005). Waste cooking palm oil biodiesel showed lower NOx emission compared to the other samples because it possesses lower exhaust gas temperature (Saifuddin et al. 2014).

Specific fuel consumption (SFC) is an engineering term that is used to describe the fuel efficiency of an engine design with respect to thrust output (Durkovic & Damjanovic, 2006). Based on a study of Hossain and Mazen (2014), petrodiesel fuel showed a higher fuel consumption compared to biodiesels. The results shown that fish oil biodiesel showed a lower SFC (0.581 mL/s) in comparison with conventional diesel (0.588 mL/s). The SFC value for waste cooking palm oil and sunflower oil biodiesel were 0.613 mL/s and 0.612 mL/s, respectively (Saifuddin et al. 2014). Fish based biodiesel has the lowest SFC probably due to its higher calorific value in comparison with the other samples.

Multi element analysis showed that the level of phosphorus was higher in fish based biodiesel than the ASTM standard whilst the magnesium, calcium and sodium content were within the range of ASTM standard (Figure 4). Phosphorus is a poison for the catalytic converters in the exhaust system of a diesel engine and it increases the emission of CO, CO₂, SO₂, and HC (Korn et al. 2007). The presence of excess phosphorus can be due to the incomplete refining of fish oil and the presence of proteins in the produced biodiesel (Arvanitoyannis & Kassaveti 2008). It is most probably that there are proteins in the fish (Cyprinus carpio) waste leading to excess level of phosphorus in derived biodiesel. The influence of phosphorus towards the engine is cumulative. The very small amount of phosphorus contamination over the significant amount of biodiesel consumed by an engine can cause unexpected deterioration to the catalytic converter system. The concentration of calcium (Ca) in the biodiesel sample was 7.00 ppm, magnesium (Mg), 11.00 ppm and sodium (Na), 6.00 ppm and they were lower than the ASTM standard. These elements can lead to the formation of ash after the combustion. Deposition of ash in the engine makes the engine dirty and reduces the engine performance. Na is associated with the formation of ash within the engine and calcium soaps are responsible for injection pump sticking. Sodium hydroxide is utilized as catalysts, and magnesium and calcium are used as absorbents in the production of biodiesel. These elements should be removed through the biodiesel production process. These residual metals can form deposits in fuel injection system components and poison emission control after-treatment systems. Sodium as well as calcium and magnesium, may also be present as abrasive solids or soluble metallic soaps (Mittelbach & Enzelsberger 1999). Since the level of magnesium, calcium and sodium contents are lower than the ASTM standard; hence it did not exhibit deleterious effects on engine parts.

### TABLE 5. Fuel consumption and greenhouse gas emission analysis

| Sample       | Load (Nm) | Speed (rpm) | CO (Vol%) | HC (ppm) | NOx (ppm) | Fuel consumption (mL/s) |
|--------------|-----------|-------------|-----------|----------|-----------|------------------------|
| Fish biodiesel | 15        | 2000        | 0.027     | 18.00    | 525.00    | 0.581                  |
| Diesel       | 15        | 2000        | 0.029     | 25.33    | 515.40    | 0.588                  |

**FIGURE 4.** Elements concentration of the produced biodiesel and their respective ASTM standard.
CONCLUSION
From this study, the following conclusion can be summarized. The GCMS analysis shows that there are seven peaks, indicating seven compounds. All of the seven peaks are fatty acid methyl esters indicating all the triglycerides are methylated producing 100% fatty acid methyl esters. The identities of fatty acid methyl esters are: methyl laurate, methyl myristate, methyl palmitate, methyl palmitoleate, methyl stearate, methyl oleate and methyl linoleate. The chain length of the fatty acids present in *Cyprinus carpio* ranged from 12 carbon to 18 carbons with 18C fatty acids as the major fatty acids of 51.94%. Characterization studies on TAN, TBN, viscosity and multielement analysis showed that the quality of biodiesel produced met most of the ASTM standards except for the phosphorus content and TBN value. It can be concluded that fish (*Cyprinus carpio*) waste can be considered as a potential source of biodiesel and the produced biodiesel exhibited lower fuel consumption compared to conventional diesel.

ACKNOWLEDGEMENTS
The authors greatly acknowledge the financial assistance by the Ministry of Science, Technology and Innovation (MOSTI), Malaysia.

REFERENCES
Agarwal, A.K. 2007. Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines. *Progress in Energy and Combustion Science* 33(3): 233-271.
Arvanitoyannis, I.S. & Kassaveti, A. 2008. Fish industry waste: Treatments, environmental impacts, current and potential uses. *International Journal of Food Science & Technology* 43(4): 726-745.
Bhatti, H.N., Hanif, M.A. & Qasim, M. 2008. Biodiesel production from waste tallow. *Fuel* 87(13): 2961-2966.
Crutzen, P.J., Mosier, A.R., Smith, K.A. & Winiwarter, W. 2008. N2O release from agro-biofuel production negates global warming reduction by replacing fossil fuels. *Atmospheric Chemistry and Physics* 8(2): 389-395.
Durkovič, R. & Damjanović, M. 2006. Regression models of specific fuel consumption curves and characteristics of economic operation of internal combustion engines. *Facta Universitatis-Series: Mechanical Engineering* 4(1): 17-26.
Encinar, J.M., González, J.F. & Rodríguez-Reinares, A. 2007. Ethanolysis of used frying oil. Biodiesel preparation and characterization. *Fuel Processing Technology* 88(5): 513-522.
Guo, G., Kusakabe, K., Sakurai, N. & Moriyama, K. 2009. Transesterification of vegetable oil to biodiesel using acid catalysts in the presence of dimethyl ether. *Fuel* 88(1): 81-86.
Hossain, A.B.M.S. & Boyce, A.N. 2009. Biodiesel production from waste sunflower cooking oil as an environmental recycling process and renewably energy. *Bulgarian Journal of Agricultural Science* 15: 312-317.
Hossain, A.B.M.S. & Mazen, M.A. 2010. Effects of catalyst types and concentrations on biodiesel production from waste soybean oil biomass as renewable energy and environmental recycling process. *Australian Journal of Crop Science* 4(7): 550-555.
Kminkova, M., Wintrova, R.E.N.A.T.A. & Kucera, J. 2001. Fatty acids in lipids of carp (*Cyprinus carpio*) tissues. *Czech Journal of Food Sciences* 19(5): 177-180.
Knothe, G. 2008. Designer biodiesel: Optimizing fatty ester composition to improve fuel properties. *Energy & Fuels* 22(2): 1358-1364.
Korn, M., Santos, D.S., Welz, B., Vale, M.G., Teixeira, A.P. & Limaa, D. 2007. Atomic spectrometric methods for the determination of metals and metalloids in automotive fuels - A review. *Talanta* 73: 1-11.
Lin, C.Y. & Li, R.J. 2009. Fuel properties of biodiesel produced from the crude fish oil from the soapstock of marine fish. *Fuel Processing Technology* 90(1): 130-136.
Lisboa, P., Rodrigues, A.R., Martín, J.L., Simões, P., Barreiros, S., & Paiva, A. 2014. Economic analysis of a plant for biodiesel production from waste cooking oil via enzymatic transesterification using supercritical carbon dioxide. *The Journal of Supercritical Fluids* 85: 31-40.
Mittelbach, M. & Enzelsberger, H. 1999. Transesterification of heated rapeseed oil for extending diesel fuel. *Journal of the American Oil Chemists' Society* 76: 545-550.
Piccolo, T. 2009. Framework analysis of fish waste to bio-diesel production-aquafrica-case study. *Aquatic 39(338): 1965884.
Ramadhas, A.S., Muraleedharan, C. & Jayaraj, S. 2005. Performance and emission evaluation of a diesel engine fueled with methyl esters of rubber seed oil. *Renewable Energy* 30(12): 1789-1800.
Saifuddin, M., Goh, P.E., Ho, W.S., Moneruzzaman, K.M. & Nasrulhaq-Boyce, A. 2014. Biodiesel production from waste cooking palm oil and environmental impact analysis. *Bulgarian Journal of Agricultural Sciences* 20: 186-192.
Sheinbaum-Pardo, C., Calderón-Irazoqué, A. & Ramírez-Suárez, M. 2013. Potential of biodiesel from waste cooking oil in Mexico. *Biomass & Bioenergy* 56: 230-238.
Srivastava, A. & Prasad, R. 2000. Triglycerides-based diesel fuels. *Renewable and Sustainable Energy Reviews* 4(2): 111-133.
Subbaiah, V.G. & Gopal, R.K. 2011. An experimental investigation on the performance and emission characteristics of a diesel engine fuelled with rice bran biodiesel and ethanol blends. *International Journal of Green Energy* 8(2): 197-208.
Tashtoush, G.M., Al-Widyani, M.I. & Al-Jarrah, M.M. 2004. Experimental study on evaluation and optimization of conversion of waste animal fat into biodiesel. *Energy Conversion and Management* 45(17): 2697-2711.
Wisniewski, J.A., Wiggers, V.R., Simionatto, E.L., Meier, H.F., Barros, A.A.C. & Madureira, L.A.S. 2010. Biofuels from waste fish oil pyrolysis: Chemical composition. *Fuel* 89(3): 563-568.
Woods, G.D. & Fryer, F.I. 2007. Direct elemental analysis of biodiesel by inductively coupled plasma-mass spectrometry. *Analytical and Bioanalytical Chemistry* 389(3): 753-761.
Wu, Y.P., Huang, H.M., Lin, Y.F., Huang, W.D. & Huang, Y.J. 2014. Mackerel biodiesel production from the wastewater containing fish oil. *Energy* 70: 1-6.

Institute of Biological sciences
Faculty of Science
University of Malaya
50603 Kuala Lumpur
Malaysia
*Corresponding author; email: saifuddin@um.edu.my

Received: 26 September 2016
Accepted: 4 March 2017