



RESEARCH ARTICLE

COMPARISON STUDY BETWEEN BASE AND FREE LIPASE ENZYME CATALYSED TRANSESTERIFICATION IN THE SYNTHESIS OF BIODIESEL FROM *CARICA PAPAYA* SEED OIL.

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Abstract

The main objective of the present study was to evaluate the suitability of different catalyst usage in the production of biodiesel using papaya seed as the feedstock. Non-seasonal papaya seeds were bought from local market, oven-dried and oil was extracted with n-hexane solvent. Physiochemical analysis was done to the seed oil. Transesterification was carried out using methanol to oil in the ratio of 9:1 with base and lipase catalysts. Papaya seed yielded 34% of oil which makes it an attractive feed stock in the biodiesel synthesis. Extracted seed oil also had an Iodine value of 64.98 ± 0.97 (g I/100g), Saponification Value of 14.00 ± 9.90 (mg KOH/g), Free fatty acid content of 1.18 ± 0.41 (%) and Unsaponifiable matter of 4.55(%). Biodiesel catalysed from base catalyst gave a yield of 90% while lipase catalyst gave 50% of biodiesel. Synthesised biodiesel samples did not show any corrosion to copper stripes. Moreover, combustion energy released and cetane number determined for biodiesel synthesised using base catalysed transesterification and lipase catalysed transesterification were 1255.8J/g and 1172.08J/g and 79 and 76 respectively. Base synthesised biodiesel was analysed using GC-MS analysis and it was determined that the major fatty acid methyl esters present in the sample were Palmitic acid, Trans-13-Octadenoic acid and Oleic acid in the percentages of 73.9%, 14.4% and 10.9% respectively. Hence, the results shows that this local agricultural waste has good potential as biodiesel feedstock and base catalyst is a better catalyst than free lipase enzymes in the biodiesel production.

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Introduction:-

Today, the world depends on fossil sources such as petroleum, coal and natural gases for its energy. However, since these sources are non-renewable, they would get depleted faster as the demand for energy increases. This can ultimately lead in pricing up of these sources. Moreover, these fossil fuels are known to emit many by products such as carbon particles and gases, which cause pollution in the environment. Therefore, there has been always an interest in finding eco-friendly, clean-burning, and renewable alternative sources of energy [Agundiade & Adewole, 2014].

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Biodiesel is one of such alternative to substitute fossil fuels. Biodiesel is a type of fuel produced from renewable biological sources such as animal fats and vegetable oil. The triglycerides present in these sources are converted to biodiesel by transesterification or alcoholysis process in which the volatility and viscosity of the oil are altered to be similar to the conventional diesel. Biodiesel is known as a carbon neutral fuel because the carbon produced during the exhaust was initially present in the atmosphere [Ranganathan, *et al.*, 2008]. Due to this reason substituting them instead of fossil fuels will not only bring about an eco-friendly and pollutant free environment, but also provides a means to get rid of agro wastes and unnecessary oil.

Providing a suitable catalyst during the transesterification reaction will improve the reaction rate and yield. Although chemical catalysts such as sulphuric acid or potassium hydroxide are being used industrially in biodiesel production, due to their many disadvantages at downstream processing, they are not economical [Ranganathan, *et al.*, 2008]. On the other hand, lipases which are biological enzymes have been found to be a greener and competent substitute for chemical catalysts [Wong & Othman, 2015]. Mainly, animal lipases are being extensively studied in this regard as they are mostly exposed to several high lipid metabolism steps such as digestion, adsorption and reconstitution of fats and lipoproteins [Ribeiro *et al.*, 2011].

In this study, *Carica papaya* seed used as the feedstock to synthesise biodiesel. It's a tropical fruit belonging to the genus *Carica* of the plant family Caricaceae. Although the papaya seeds are edible, having a spicy bitter taste, they have not known to be consumed by human or used in animal feed [Medina, 2003 & Samaram, 2013]. Therefore the aim of this study was to use this agro waste to synthesis biodiesel using methanol to compare the efficiency of base and free animal lipase catalyst during the transesterification process.

Materials and Methods:-

Sample collection and extraction:-

Non-Seasonal papaya fruits were purchased. The seeds was washed, the gelatinous layer was removed and the seed was oven-dried at 60°C for three days [Wong & Othman 2015]. Dried seed was grinded, weighed and stored in falcon tubes until the extraction was done.

Extraction was carried out using n-hexane as solvent in a soxhlet apparatus at 70°C for six hours. Oil was recovered by evaporating the solvent in a rotary evaporator.

Physiochemical characterisation of oil:-

Extracted oil was characterised in terms of Iodine value, Saponification Value, Free fatty acid test and Unsaponifiable matter. These tests were conducted according to the standard methods described in [Agundiade & Adewole, 2014].

Determination of Iodine value:-

$$IV = \frac{(V1 - V2) \times C \times 0.1269 \times 100}{m}$$

Iodine value was determined as per the protocol by Syed *et al.*, 2012.

In this equation, IV represents for Iodine value, V1 and V2 are the initial and final volume of sodium thiosulfate used, C is the concentration of sodium thiosulfate and m is the mass of oil used.

Determination of Saponification value:-

Saponification value was determined as per the protocol by Mathur, 2016.

$$SV = \frac{(V1 - V2) \times C \times 56}{m}$$

In this equation, SV represents for Saponification value, V1 and V2 are the initial and final volume of potassium hydroxide used, C is the concentration of potassium hydroxide and m is the mass of oil used.

Determination of free fatty acid content:-

Free fatty acid was carried out as per the protocol by Zhang *et al.*, 2015

$$FFA\% = \frac{(V1 - V2) \times C \times 28.2}{m}$$

In this equation, FFA% represents for percentage of free fatty acid content, V1 and V2 are the initial and final volume of potassium hydroxide used, C is the concentration of potassium hydroxide and m is the mass of oil used.

Determination of unsaponifiable matter:-

Unsaponifiable matter was carried out as per the protocol by Hartman *et al.*, 1994.

$$UM\% = \frac{(M1 - M2) \times 100}{m}$$

In this equation, UM% the represents for percentage of unsaponifiable matter, M1 and M2 are the weight of crucible before and after evaporating the oil and m is the mass of oil used.

Transesterification:-

Base catalysed transesterification:-

Base catalysed transesterification was carried out as described by Agundiade & Adewole, 2014.

Free lipase enzyme catalysed transesterification:-

Pancreatin lipase was used for this transesterification reaction. This Lipase enzyme was purchased from Synertec Sdn. Bhd, Selangor, Malaysia. Transesterification was carried out in a conical flask as described by Facchini *et al.*, 2016.

Biodiesel yield:-

Biodiesel yield from both transesterification processes was calculated [Wong & Othman, 2015].

$$\text{Biodiesel yield} = \frac{\text{Weight of the biodiesel (g)}}{\text{Weight of the oil (g)}} \times 100\%$$

GC- MS analysis:-

GC- MS analysis (Agilent Technologies) was carried out in order to determine the percentage of different methyl esters present in the product.

Characterisation of synthesised biodiesel:-

Synthesised biodiesel was also further characterised in terms of copper corrosion stripe test, combustion test and cetane number.

Copper corrosion test was carried out according to the method described in “American Society for Testing and Materials, (2004)” [Agundiade & Adewole, 2014]. Combustion test was performed by igniting 1g of fuel in a crucible. The rise in temperature of water due to the heat transferred by the burning fuel was measured. Using the following equation heat of combustion was measured.

$$Q = m C \Delta T$$

Where;

Q = heat energy(J), m = volume of water, $C = 4.186 \text{ J.g}^{-1}.\text{°C}^{-1}$ (Specific heat capacity of water), ΔT = Rise in temperature of water ($^{\circ}\text{C}$)

Cetane number was calculated as described in [Jesikha, 2012].

$$\text{Cetane number} = 46.3 + (5458/\text{SV}) - (0.225 \times \text{IV})$$

SV represents for saponification value of synthesised biodiesel and IV represents for Iodine value of synthesised biodiesel.

Results and Discussion:-

Extraction and characterisation of oil:-

After extraction, papaya seeds were found to contain 35% oil which is in agreement with previous literature by Samaram *et al.* (2013) and Wong & Othman, (2015), where 30.4% and 34.3% of papaya oil were obtained respectively using n-hexane as solvent.

Results for various physiochemical characteristics of extracted seed oil are presented in Table 1. Papaya seed oil had an iodine value $64.98 \pm 0.97 \text{ g I/100g}$ which is similar to study done by Lee *et al.*[2011], where the papaya seed oil had an iodine value of 64.10 g I/100g . Lower iodine value means that the oil can be stored for a longer time without it undergoing oxidation. The extracted oil also had very low saponification value compare to the other studies [Lee *et al.*, 2011 & Malacrida, 2011] which means that the mean molecular weight of fatty acids is lower or not sufficient ester bonds are present in the oil [Zahir *et al.*, 2017]. Presence of free fatty acid is not preferable during transesterification reaction, especially during base catalysed transesterification where the catalyst can react with the free fatty acid which results in the formation of soap. This will lead to yield loss and difficulty in product recovery [Chai *et al.*, 2014]. This reaction can be avoided if the oil is pre-treated with the acid. Moreover, presence of unsaponifiable matter in higher amount also can affect the cloud point and flash point of biodiesel product [Agundiade & Adewole, 2014].

Table 1:-Physiochemical characteristics of *Carica papaya* seed oil

Analysis	Determined Value	Literature Values	
		1	2
Iodine value (g I/100g)	64.98 ± 0.97	64.10	79.95 ± 1.25
Saponification Value (mg KOH/g)	14.00 ± 9.90	185.0 ± 1.30	96.40 ± 1.37
Free fatty acid (%)	1.18 ± 0.41	nd ^(a)	1.27 ± 0.04
Unsaponifiable matter (%)	4.55	4.50	1.35 ± 0.14

nd^(a)= Not determined ; ¹=[Lee *et al.*, 2011]; ²=[Malacrida *et al.*, 2010]

Characterization of synthesized biodiesel:-

Comparison of biodiesel yield obtained from base and lipase catalyst:-

Base catalysed transesterification yielded 90% product recovery while lipase catalysed transesterification yielded only 50% biodiesel. A higher yield of 90-95% was expected from lipase catalysed transesterification. However, the low yield obtained could have been due to not immobilising the enzyme as free enzymes are not effective as immobilised enzymes [Ranganathan *et al.*, 2008].

Properties of synthesised biodiesel:-

The quality of a fuel is a crucial factor need to be considered to commercialise the synthesised biodiesel. Quality can be affected by number of physical and chemical factors. Biodiesel synthesised from both catalysts did not show any corrosion to the copper stripes. This could have been attributed to no significant amount of impurities such as glycerol, excess methanol, or even residues of catalyst were present in the tested sample that could have reacted with the copper stripes [Singh *et al.*, 2012]. However, the tested sample and time period are not adequate to conclude that the synthesised sample will not cause any corrosion in the long term use.

Biodiesel synthesised using base catalyst released higher amount of energy (1255.8J/g) than biodiesel synthesised using lipase catalyst (1172.08J/g). However, these values are lower than the conventional diesel (1674.4J/g). In order to increase the combustion energy performance of biodiesel, chemical additives such as ethanol or diethyl ether can be added [Ali *et al.*, 2016].

Cetane number was the most important criteria used in this study to analyse the synthesised biodiesel to be used as substitute to fossil fuel. It is the delay time to ignite the fuel. A higher cetane number indicates a lesser time is required to ignite the fuel. This number was determined by calculating the iodine and saponification values of the sample. Synthesised biodiesel by base and free lipase catalyst had a higher cetane value of 79 and 76 respectively compared with diesel 49-55. According to Barabás & Todoruț [n.d], pure palmitate and oleate methyl esters have cetane numbers of 81.17 and 62.39 respectively. Since palmitic methyl ester was found highest in the produced sample, the result is almost similar to the above literature value. Over all, cetane number above 47 is accepted to be employed as a fuel for diesel engine.

Gas chromatography - Mass spectroscopy spectrum analysis:-

Biodiesel transesterified with base catalyst had the highest biodiesel yield therefore, this sample was used to carry out the GC-MS analysis to investigate the percentage of different fatty methyl esters present in the biodiesel. Three significant peaks were observed in the GC-MS spectrum as depicted in Figure 1. Palmitic acid methyl esters ($C_{17}H_{34}O_2$) were present in the highest amount (73.9%). Trans-13-Octadenoic acid methyl esters ($C_{19}H_{36}O_2$) (14.4%), Oleic acid methyl ester ($C_{19}H_{36}O_2$) (10.9%) percentages were relatively low when compared to palmitic acid methyl esters. This shows that base catalyst has promoted saturated fatty acid (Palmitic acid) to undergo transesterification better than monounsaturated fatty acids (Trans-13-Octadenoic acid and oleic acid) although, oleic acid is present highest in the papaya seed oil [Malacrida *et al.*, 2011].

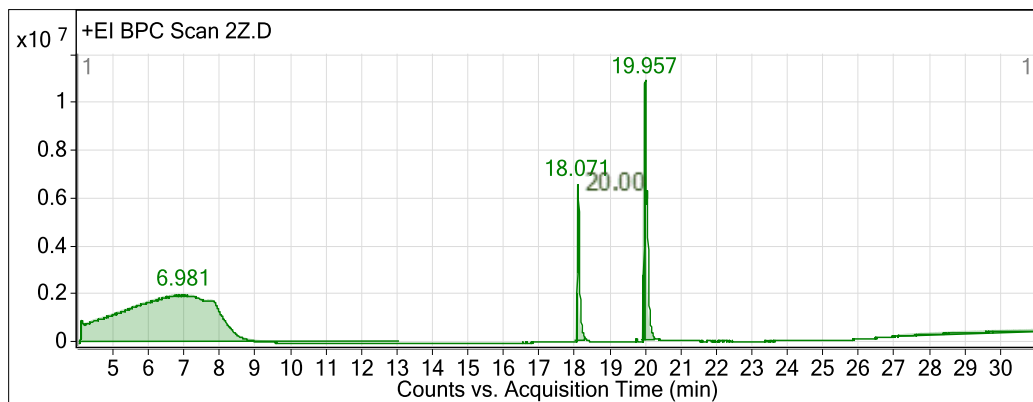


Figure 1:-GC-MS spectrum of papaya seed biodiesel

Conclusion:-

In conclusion, papaya seed has proven as a potential feed stock in the synthesis of biodiesel. Base catalyst transesterified biodiesel gave higher yield followed by higher amount of energy release and high cetane number compared with free lipase catalysts transesterified biodiesel. The main methyl esters present in the base synthesised biodiesel were Palmitic acid methyl esters, Trans-13-Octadenoic acid methyl esters and Oleic acid methyl ester. Lipase enzyme must be immobilised to enhance its activity. Synthesised biodiesel also had a higher cetane value which indicate that papaya seed biodiesel can be used as a substitute to conventional fuel.

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