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Engine-Compatible Biodiesel from *Leucaena leucocephala* Seed Oil

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Abstract – *Leucaena leucocephala (petai belalang) seed oil has been studied previously as a feedstock for biodiesel but no study describes its fuel properties for use in diesel engine. In this study, hexane was used to extract oil from the seeds of Leucaena leucocephala in a Soxhlet apparatus. Chemical properties of the oil were then analysed which demonstrated a relatively high FFA content of 6.0wt%, requiring the two-step method for biodiesel production through an acid-catalyzed esterification followed by alkali-catalyzed transesterification. Physico-chemical properties of the processed biodiesel such as triglyceride, diglyceride, monoglyceride, free glycerol, methanol content, ester content, carbon residue, acid value, oxidation stability, tocopherol concentration, iodine value, water content, kinematic viscosity, density, pour point, cloud point, cold filter plugging point and flash point were analysed. The obtained biodiesel showed high oxidation stability and most fuel properties complied with two international standards, European Standards (EN14214) and American Society for Testing Materials (ASTM D6751).*

Keywords: Biodiesel, *Leucaena leucocephala*, fuel properties, seed oil

1.0 INTRODUCTION

Biodiesel, a chemically modified fuel produced from plant oils, waste cooking oils and animal fats, consists of mono alkyl esters of long chain fatty acids (Phoo et al., 2014). Biodiesel exhibit fuel-like properties compatible to those of petroleum-based diesel, and are commercialized for the use in existing motor vehicles (Amini et al., 2017). Although both edible and non-edible oils can be used for biodiesel production, edible oils are widely used at the present time (Demirbas et al., 2016). Converting edible oils into biodiesel may negatively affect the balance of demand between food and fuel (Gui et al., 2008). Moser (2009) estimated that only 18% of transport diesel fuel demand would be met if production of the top twelve vegetable oils in the world were used solely for diesel transport fuel. This would result in a reduction of feedstock availability. In order to avoid such problems, many researchers have studied a wide variety of plant oils to find new and possible alternative feedstocks for biodiesel production (Azam et al., 2005; Moser, 2009; Pinzi et al., 2009).

In this study, non-edible seed oil from Leucaena leucocephala is evaluated for its potential as an alternative feedstock for biodiesel production. Leucaena leucocephala seed oil is non-edible due to the existence of mimosine, a toxic amino acid to non-ruminant vertebrates (Ilham et al., 2015). Leucaena leucocephala is a leguminous fast growing tree that populates predominantly in Mexico and Central America, but now naturalized in most of the subtropical and tropical regions around the world. L. leucocephala belongs to the Fabaceae family, with about twenty species in Leucaena genus worldwide including two named acid-tolerant hybrids (L. leucocephala Bahru and L. leucocephala Rendang) (Hughes & Harris, 1998). It is mainly known by the indigenous as Subabul in India, Ipil-ipil in the Philippines, Yin hue in China and Petai Belalang in Malaysia. Non-edible seed oils are suitable for biodiesel production as it normally could be grown on arid lands, planted as marginal crop as well as having a relatively high oil yield.

2.0 METHODOLOGY

2.1 Preparation of Materials

Leucaena leucocephala seeds were collected from University of Malaya Glami Lemi Biotechnology Research Centre, Jelebu, Negeri Sembilan, Malaysia. The seeds were washed with water and kept in an oven dryer for 5h. The seeds were ground using a Wonder® blender (WB-1). The ground seeds were then dried in an oven at 105˚C for 1h. Oil was extracted using oven dried sample with hexane in a Soxhlet apparatus at 60˚C for 8h.

2.2 Test Methodology

The fatty acid profile of *Leucaena leucocephala* seed oil was obtained using a high-performance liquid chromatography (Shimadzu LC-10AT) equipped with Cadenza CD-C18 column and refractive index detector RID-10A. The column temperature was set at 40˚C and methanol was used as a carrier solvent with a flow rate of 1ml/min. The sample was prepared according to AOCS Official Method AOCS Ce2-66 (AOCS 1993).

The free fatty acids (FFA) content in oil was determined using AOCS Official Method AOCS Cd 3d-63 (AOCS 1976). Tocopherol concentration was analysed using high performance liquid chromatography (HPLC-Shimadzu LC-10AT) equipped with Shodex Asahipak ODP-50 6D column and ultraviolet detector SPD-10A. Iodine value was measured according to Wijs method while density was determined using I-type hydrometer. Karl Fischer moisture titrator MKC-520 (Kyoto Electronics MFG. Co. Ltd.) was used to check water content and Automatic Kinematic Viscosity Measuring System AKV-201 was used to test kinematic viscosity at 40˚C.

The fuel properties of the fatty acid methyl esters produced from *Leucaena leucocephala* seed oil were analysed for triglyceride, diglyceride, monoglyceride, free glycerol, methanol content, ester content, carbon residue, acid value, oxidation stability, tocopherol, iodine value, water content, kinematic viscosity, density, pour point, cloud point, cold filter plugging point and flash point. Triglyceride, diglyceride, monoglyceride, free glycerol, methanol and ester content were analysed by a gas chromatograph (GC-2014, Shimadzu, Japan) equipped with flame ionization detector (FID) and capillary column (Omegawax, 30m x 0.25mm x 0.25µL).

The detector and injector were set at 250˚C and 260˚C, respectively. The oven program (200˚C-5min, 20˚C/min to 260˚C, kept constant 260˚C-6min) with Helium as carrier gas at 2mL/min flow-rate. Carbon residue was measured by Micro Carbon Residue Tester ACR-M3. Oxidation stability was determined by Rancimat 743 (Methrom, Herisau, Switzerland). Tocopherol, iodine value, water content and kinematic viscosity were measured using the same method with oil properties determination. Acid value was determined using AOCS Official Method AOCS Cd 3d-63 (AOCS 1976).

The pour point and cloud point tests were done on a Mini Pour/Cloud Point Tester MPC-102. Automated Cold Filter Plugging Point Tester AFP-102 was used to determine cold filter plugging point while Pensky-Martens Closed Cup Automated Flash Point Tester APM-7 was used to determine the flash point.

2.3 Two-Step Biodiesel Production Method

Both acid-catalyzed esterification and alkali-catalyzed transesterification were carried out in a round bottom flask mounted with condenser using oil bath stirrer for 1h at 60-65˚C. In acid-catalyzed esterification, concentrated sulfuric acid at 5wt% of oil and methanol at 40:1 molar ratio to oil were used. The mixture was transferred to a separatory funnel which was left to stand for a minimum of 12h to allow separation of the esterified oil (bottom layer) and the alcohol-water-acid (top layer). The esterified oil was separated and washed with distilled water. For each washing, 50vol% distilled water of the oil was used and washings were done about 5 to 7 times. The esterified oil was filtered using anhydrous sodium sulfate to remove water.

In alkali-catalyzed transesterification, methanol (6:1 molar ratio to oil) and sodium hydroxide (1wt% of oil) was mixed together until the sodium hydroxide was completely dissolved in methanol to form sodium methoxide solution. The prepared sodium methoxide solution was added to the esterified oil and stirred for 1h at 60-65˚C. After transesterification, the product mixture was transferred to the separatory funnel for a minimum of 12h to separate the biodiesel and the glycerol layer. The washing process was conducted in the same way as washing after acid-catalyzed esterification. The biodiesel was filtered and passed through anhydrous sodium sulfate to remove water. For the calibration curve of the authentic standard products, triglyceride, monoglyceride, diglyceride and methyl oleate were used. All sets of experiments were made to at least triplicate for confirmation of the yields by utilizing 6:1 molar ratio of methanol to oil, although not treated statistically. The *Leucaena leucocephala* biodiesel yield in weight percent as presented in this study refers to the percentage of yields conversion recovered based on theoretical yield. The whole process is simplified in Figure 1.

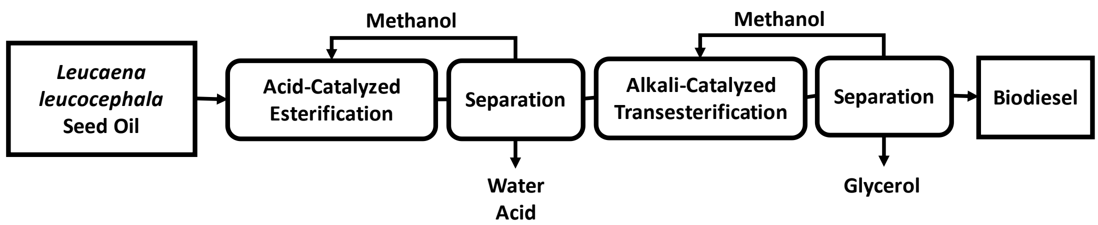

Figure 1: Two-step biodiesel production method used in this study

3.0 RESULTS AND DISCUSSION

3.1 Seed Oil Fatty Acid Profile and Physico-Chemical Properties

The fatty acid composition and physico-chemical properties of oil extracted from *Leucaena leucocephala* seeds are shown in Tables 1 and 2, respectively. It can be considered as a good source for oil as the Soxhlet extraction yielded approximately 7.2wt% of oil yield. It was reported in other studies that the *Leucaena leucocephala* seeds oil yield is the range of 6.4-7.5wt% (Nehdi et al., 2014). The oil yield from *Leucaena leucocephala* seeds in this study is relatively lower than other non-edible oils; Jatropha (20 wt%), Karanja (30%) and Mahua (35%) but those are non-native species and need to be domesticated in Malaysia (Demirbas et al., 2016). It could also be seen in Table 2 that the seed oil being used contains high amount of free fatty acids at 6.0wt% and 65ppm water. Many researchers verified that the FFA and water contents in oils were significant factors affecting the alkali-catalyzed transesterification. The FFA content in the oil should be less than 5.0wt% in order for alkali-catalyzed transesterification to work and all materials should be substantially anhydrous (Tiwari et al., 2007; Ghadge & Raheman, 2005; Clements & Gerpen, 2004). Therefore, two-step biodiesel production method; acid-catalyzed esterification and alkali-catalyzed transesterification is the one suitable for this study.

Table 1: Fatty acid composition of *Leucaena leucocephala* seed oil

Fatty Acid[a]		**Seed Oil (wt%)**	**Free Fatty Acid (wt%)**
Myristic acid	14:0	0.1	-
Palmitic acid	16:0	14.2	1.2
Stearic acid	18:0	6.2	1.2
Arachidic acid	20:0	2.3	-
Behenic acid	22:0	2.2	-
Palmitoleic acid	16:1	1.0	-
Oleic acid	18:1	22.0	3.6
Linoleic acid	18:2	50.7	-
Linolenic acid	18:3	1.3	-
Total	-	100	6.0

[a] The former number represents the one of the carbons in the hydrocarbon chain while the latter the number of the double bond in fatty acid.

Table 2: Physico-chemical properties of *Leucaena leucocephala* seed oil

Properties	**Unit**	**Seed Oil**
Oil yield	wt%	7.2
Free fatty acid	wt%	6.0
Acid value	mgKOH/g	6.2
Tocopherol content	ppm	1468
Iodine value	g I_2/100g	110
Density, 15˚C	kg/m^3	920
Water content	ppm	65
Kinematic viscosity, 40 ˚C	mm^{2}s^{-1}	38.2

3.2 Biodiesel Fuel Properties

GC results demonstrated that the fatty acid methyl esters (FAME) content in the ester layer was up to 97.2wt% (Table 3) and as an example, one chromatogram showing FAME mixture is depicted in Figure 2. In addition, the fuel properties of *Leucaena leucocephala* FAME for use as biodiesel is shown in Table 4. Triglyceride, diglyceride, monoglyceride, free glycerol, total glycerol, methanol content, ester content, acid value, density, iodine value, water content and oxidation stability complied with EU and US standards. Kinematic viscosity conformed to both EU and US standards.

Table 3: FAME composition of *Leucaena leucocephala* biodiesel

FAME		wt%
Myristic acid methyl ester	14:0	0.1
Palmitic acid methyl ester	16:0	14.2
Stearic acid methyl ester	18:0	6.2
Arachidic acid methyl ester	20:0	2.3
Behenic acid methyl ester	22:0	2.2
Palmitoleic acid methyl ester	16:1	1.0
Oleic acid methyl ester	18:1	22.0
Linoleic acid methyl ester	18:2	49.1
Linolenic acid methyl ester	18:3	0.1
Total	-	97.2

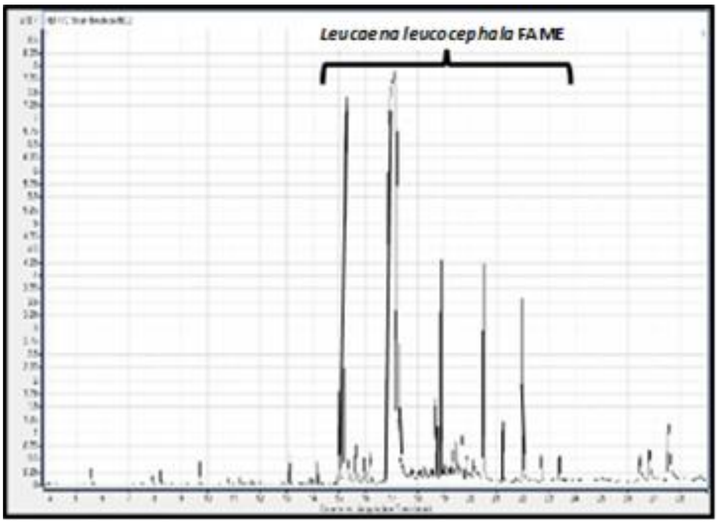

Figure 2: Chromatogram of *Leucaena leucocephala* FAME

Completion of the transesterification reaction appears to be the most important step in producing good-quality biodiesel. In this study, the total glycerol measures the amount of unconverted or partially converted fats and by-product glycerol present in the fuel. Total free glycerol in *Leucaena leucocephala* FAME could not be detected (0.0wt%), thus, implying high quality biodiesel that would never cause negative effects on the diesel engine, such as clogging of fuel filters, fouling fuel injectors, and forming sediment at the bottom of fuel storage tanks. According to the ANP legislation, 0.02wt% of free glycerol is the maximum quantity permitted (Monteiro et al., 2008). Triglyceride, diglyceride, monoglyceride and methanol content were not in significant values or not detected which may be due to its value being lower than the minimum value that could be identified by the refractive index detector.

The presence of water in biodiesel is also harmful as it could accelerate the growth of microbe colonies that can clog fuel systems. However, the water content of the resulting biodiesel is low at 76ppm. Acid value of the biodiesel was 0.27mgKOHg^{-1} with 0.07wt% of carbon residue. Thus, the lower acid value and carbon residue suggested that there is a lower predisposition for incomplete combustion when *Leucaena leucocephala* FAME is utilized. A high iodine value can give significant effects on NOx emissions (Graboski et al., 2003). In this study, the iodine value of *Leucaena leucocephala* FAME was 101.7gI_2 (100g)$^{-1}$ therefore indicating minimal NOx emission. Viscosity, on the other hand, is the major reason of conversion of fats and oils are to biodiesel. The kinematic viscosity (K.V.) of *Leucaena leucocephala* FAME was significantly lower than that of its oil which was 4.8 mm^{2}s^{-1} and could lead to proper atomization of fuel in the combustion chamber of the engine.

Oxidation stability is one of the most important fuel properties of biodiesel. This is influenced by the fatty acid profile of plant oils. Higher amounts of unsaturated fatty acids show that there are more double bonds which are vulnerable to oxidative attack and therefore could lead to a lower oxidative stability (Knothe, 2005). *Leucaena leucocephala* FAME has a high oxidation stability at 12h due to high amount of antioxidants in its plant oil (Nehdi et al., 2014). Pour point, cloud point and cold filter plugging point (CFPP) of *Leucaena leucocephala* FAME were -3˚C, 1˚C and -1˚C respectively. It could be suggested that it is better to be used in tropical countries. The flash point of *Leucaena leucocephala* FAME was 118˚C, which complies with the European and American standards. The flash point is inversely proportional to fuel volatility (Hoekman et al., 2012).

Table 4: Properties of *Leucaena leucocephala* FAME for use as biodiesel in comparison with international standards[a]

Properties	Method	Unit	FAME (Biodiesel)	EU (EN14214)	US (ASTM D6751-07)
K.V. (40°C)	D445	mm^2/s	4.8	3.5-5.0	1.9-6.0
Carbon residue	D4530	wt%	0.07	$\leq$0.30	$\leq$0.05
Pour point	D2500	°C	-3	-	-
CFPP	D6371	°C	-1	-	-
Flash point	D93	°C	118	$\geq$101	$\geq$130
Cloud point	D6749	°C	1	-	-
Oxidation stability	EN11442	h	12	6	-
Ester content	EN14103	wt%	97.2	>96.5	-
Monoglyceride	EN14105	wt%	0.02	<0.80	-
Diglyceride	EN14105	wt%	0.04	<0.20	-
Triglyceride	EN14105	wt%	n.d.[b]	<0.20	-
Total glycerol	EN14105	wt%	0.00	<0.25	<0.24
Water content	ISO12937	ppm	76	<500	<500
Acid number	EN14105	mg(KOH)/g	0.27	<0.50	<0.50
Iodine value	EN14111	g(I_2)/100g	101.7	<120	-

[a] CEN (2003); ASTM (2007); [b] n.d. – not detectable

4.0 CONCLUSIONS

Physical and chemical properties of *Leucaena leucocephala* seed oil were investigated to evaluate it as a potential alternative feedstock for biodiesel. In terms of oil content, it is good enough to be used as a feedstock for biodiesel production. *Leucaena leucocephala* seed oil can also prevent competition with food utilization as it is non-edible and currently left unused. The FFA and water contents of oil were 6.0wt% and 65ppm, respectively. Therefore, acid-catalyzed esterification followed by alkali-catalyzed transesterification was necessary for biodiesel production. Fuel properties of biodiesel produced from *Leucaena leucocephala* oil also complied with most of the properties listed in the international standards, proving its compatibility for use in diesel engine. However, carbon residue and flash point only complied with EN14214 standard. With high oxidation stability, biodiesel from non-edible *Leucaena leucocephala* seed oil would have a longer storage time, thus, potentially be a sustainable feedstock for biodiesel production.

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