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Production and Characterization of Biodiesel from Brebra (*M. ferruginea*) Seed Non-edible Oil

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Abstract: *Millettia ferruginea* is a leguminous tree endemic to Ethiopia. Although brebra tree produces large quantities of seeds, currently the seed application is limited. The objective of this study was to produce standard biodiesel from brebra oil. Brebra seed contained 48.5±0.99% oil. As analysis of fatty acid composition showed, 75% of the total fatty acid is accounted for by oleic acid (30.7%) and linoleic acid (44.3%). Refined oil obtained after removal of the hexane was used for biodiesel production using KOH as a catalyst. The resulting biodiesel was pure and showed excellent physical and chemical properties meeting international standards. Briefly, the specific gravity, viscosity, acid value, pH value, saponification value and iodine value of brebra biodiesel were within specification of ASTM and EN. From the total refined oil, 81.6% and 17.18% biodiesel and glycerol was recovered, respectively. Therefore, brebra seed oil has potential application for biodiesel and glycerol production.

Key words: Biodiesel, fatty acids, glycerol, *Millettia ferruginea*, transesterification

INTRODUCTION

Millettia ferruginea (Hochst) Baker (brebra in Amharic) is an important endemic tree species in Ethiopia with huge potential for agro-forestry. The tree belongs to the family Fabaceae (Leguminoeae) sub-family Papilionnodeae. To date two subspecies of *Millettia ferruginea*, namely, *Millettia ferruginea ferruginea* and *Millettia ferruginea darassana* have been recognized. Subspecies *ferruginea* is mainly found in North Ethiopia within the altitude range of 1,000 to 2,500 m above sea level, while subspecies *darassana* is mainly found in the Southern part of the country in the altitude range of 1,600 to 2,500 m. The hybrid of the two subspecies is believed to be found in the Central and Western parts of the country (Thulin, 1983).

Millettia ferruginea is the most important coffee (*Coffea arabica* L.) shade tree (Negash, 1995). In the coffee growing regions of Ethiopia, brebra is known to account for up to 22.3% of the coffee shade tree (Muleta, 2007). It is also commonly found in farmlands used to grow barley (*Hordeum vulgare* L.), enset (*Ensete ventricosum* Welw.), maize (*Zea mays* L.) and sorghum (*Sorgum bicolor* (L.) Moench S.l.) etc. As a legume, brebra fixes nitrogen and thus contribute to soil fertility and significantly improve plant growth (Hailu *et al*., 2000). In addition to its use as a shade tree in coffee plantation and its importance in improving soil fertility, traditionally brebra tree has several other uses. Some of these uses include keeping traditional bee hives, fire wood, fencing, house construction, etc.

Although brebra produce large quantities of seed, its only use known to date is as mass fishing in rivers and in some localities as an insecticide against chiggers and scabies (Stein, 1973). The compound in brebra seed responsible for these actions is known to be rotenone (Dagne *et al*., 1990), a respiratory inhibitor, toxic to cold blooded animals and mildly toxic to warm blooded animals (Ray, 1991). During preliminary analysis, brebra seed was rich in content of oil (48.5%) and protein (29.7%). This indicates that potentially brebra seed could serve as a source of oil for biodiesel production.

Currently, large scale uses of fossil fuel have a number of problems. First fossil fuel reserve of the world is finite and its excessive use could lead to rapid depletion (Campbell and Laherrere, 1998). Secondly, excessive use of fossil fuel exert a number of negative impacts on the environment, such as greenhouse gas emissions (which aggravate global warming), air pollution and acid rain (Soetaert and Vantamme, 2009). Thirdly, as the demand for petroleum increases, petroleum prices are expected to increase further sharply (Soetaert and Vantamme, 2009).

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To overcome the risk of fossil fuel depletion and minimize the impact of excessive burning of fossil fuel on the environment, it is important for mankind to look for alternative energy sources. Biodiesel derived from oil and fat has a good potential to substitute part or all petroleum derived liquid fuels used in the transport and other economic sectors.

Biodiesel is a liquid biofuel which are esters of long chain fatty acids and short chain alcohols. Biodiesel molecules are synthesized through direct transesterification of vegetable oils and fats with short chain alcohols (such as methanol and ethanol) in the presence of suitable catalysts (Vicente *et al*., 2004). Considerable research has been done on the production of biodiesel from edible oil sources (Shay, 1993). The important concerns about large-scale development of biodiesel are its competition with crop lands currently used for food crops and direct use of edible oil or grains for biodiesel production.

One of the most important concerns about large-scale development of biodiesel from such sources is its competition for land currently used for the production of food crops. As the available land is fixed and the demand for biofuel increases, there will be pressure on the other primary uses of land: food production and environmental preservation. As land used for biofuel production increases, land available for food production decrease and this results in an increase in food price (Sexton *et al*., 2009).

The remaining challenges are its cost and limited availability of fat and oil resources. There are two aspects to the cost of biodiesel, the costs of raw material (fats and oils) and the cost of processing. The cost of raw materials accounts for 70 to 85% of the total cost of biodiesel production cost (Noordam and Withers, 1996).

To make biodiesel production competitive and commercially attractive a sustainable supply of less expensive oil source is crucial. As a result, in many countries the use of non-edible vegetable oil for biodiesel production is becoming very popular. The use of non-edible oils avoids direct competition with food. In addition, plants bearing non-edible oil are considered capable of growing on marginal soil, land not suitable for food production (Chhetri *et al*., 2008). As a result, there is no competition between the growth of non-edible oil producing plants and crops used for food production. Currently, the technology of biodiesel production from vegetable oil feedstock is clearly defined. A sustainable supply of less expensive oil will be a crucial factor for biodiesel to be competitive commercially (Dorado *et al*., 2006).

Of the different plants tested, brebra (*Millettia ferruginea*) a leguminous tree endemic to Ethiopia, was identified as having a good potential as a source of oil. Up to the date of this study, there is no any scientific investigation conducted on brebra oil to produce biodiesel. Therefore, the main objective of this study was to develop oil refinery and biodiesel production methods from brebra seed oil and also to determine the produced biodiesel physic-chemical characteristics.

MATERIALS AND METHODS

Seed yield and harvesting: This study was conducted from September 2009 to April 2010. Harvesting process was adopted from traditional method of the society. Matured brebra (*M. ferruginea*) pods were collected around Addis Ababa from January to February, 2008 and kept under shade for about a week covered with cereal straw. The pods were then transferred to fiber sac so that when the pods dry the seeds are released by explosion into the sac. After further drying the seeds were stored at room temperature and used for analysis.

Oil extraction: Crude oil was extracted from brebra seed using two methods; mechanical press (Singh and Bargale, 2000) and solvent extraction (Govindarajan *et al*., 2009). In mechanical extraction, the oil was separated from the oil bearing seed by mechanical press and the crude oil was collected. For solvent extraction, mechanically dehulled brebra seed was grounded with a Waring blender and the fine powder mixed with hexane (1:3 ratio, w/v) and the whole content stirred for about 4 h and filtered through Whatman's No. 1 filter paper. The solvent (hexane) was recovered using Rotavapor (Buchi, Switzerland) at 150 rpm.

Oil refining process: Crude oil obtained by mechanical press and solvent extraction was mixed with hexane (1:1 ratio) and the precipitate formed was removed through filtration. To two volumes of hexane oil mixture, one volume of 96% ethanol was added and allowed to stand for 1 h in a separatory funnel for phase separation. The bottom layer containing hexane and oil was separated from the upper layer containing ethanol, phospholipids and the solvent from both phases recovered using Rotavapor.

Fatty acid analysis: The fatty acid profile was determined by standard methods (Alcantara *et al*., 2000) using gas chromatography. Standards of the methyl esters were purchased from Sigma Aldrich. The mean value of the results was recorded.

Reaction conditions: The reaction mixture consisting of brebra seed oil, methanol and 1% KOH (w/w) was incubated at room temperature with constant agitation for 2 h. A methanol to oil molar ratio of 6:1 was used throughout the study. Thus, a typical reaction mixture consists of 100 g brebra oil, 22.6 methanol and 1 g KOH. The reaction was carried out as one stage and two stage reaction. In the one stage reaction, the entire methanol and the catalyst were added at once. In the two stage reaction half the methanol and half the catalyst were first added and incubated for 2 h at room temperature with constant agitation. After separation of the glycerol and oil phase using separatory funnel, the remaining half of the methanol and catalyst were added to the oil phase. After 2 h incubation, the whole reaction mixture was transferred to a separatory funnel and allowed to stand overnight (Dmytryshyn *et al*., 2004). The glycerol phase was removed and the fatty acid methyl ester phase neutralized using 6 N HCl. Residual methanol was removed from the fatty acid methyl ester using Rotavapor. Residual catalyst was washed from the biodiesel using warm water (50°C) as it was reported by Alamu *et al.* (2007).

Thin layer chromatography: Progress of the reaction was monitored using analytical Thin Layer Chromatography (TLC) as follows. At time intervals 0.1 g of the reaction mixture was withdrawn and diluted with 10 mL hexane and spotted on TLC plates. The plate was developed using petroleum ether/diethyl ether/methanol/acetic acid (90:7:2:0.5 by volume) as a mobile phase. Spots were visualized by exposing the plate to iodine vapor (Rachmaniah *et al*., 2002).

Characterization of brebra biodiesel: The physico-chemical properties of the biodiesel, such as density, specific gravity, distillation, kinematic viscosity, flash point, copper strip corrosion, cetane index, color, water content, water and sediment, heat of combustion, cloud and pour points, acid value, ash and iodine value of methyl esters were determined following standard test methods (Table 1).

Table 1: Standard test methods used to characterize methyl esters

Properties	Method
Specific gravity	ASTM D1298
Density	ASTM D1298
Distillation	ASTM D 86
Flash point	ASTM D 93
Copper strip corrosion ASTM D849-09	
3 H at 100°C	ASTM D 130
Cloud point	ASTM D 2500
Pour point	ASTM D 97
Kinematic viscosity at 40°C	ASTM D 445
Cetane number	Kalayasiri *et al.* (1996)
Cetane index	ASTM D 976
Color	ASTM D 1500
Water content	ASTM D 95
Water and sediment	ASTM D 2709
Acid value	ASTM D 974
Ash	ASTM D 482
Heat of combustion	Parri (1987)

ASTM (2002)

RESULTS

Sample collection and oil extraction process: In this study, the potential productivity of seed from brebra tree was evaluated. The typical tree can produce nearly 150 kg of dry weight of pods. From 100 kg pod, 25 kg of dry seed was harvested and then a total of 37.5 kg seed per tree was produced. One hectare land can accommodate 36 trees, since a single tree canopy is extended about a diameter of 15.6 m in average. Therefore, from one hectare land it is possible to produce about 1350 kg dry weight seeds (Table 2). In average, the brebra seed is composed of 48.5% (w/w) of oil.

When equal volumes of crude oil, hexane and ethanol were mixed and allowed to stand, two phases were formed. The above phase contained ethanol, phospholipids, proteins, rotenone and other impurities. The bottom phase consisted essentially of hexane and oil. The bottom phase was separated from the upper phase and the solvent (hexane) removed using Rota evaporator leaving the refined oil. The overall refining process is shown in Fig. 1.

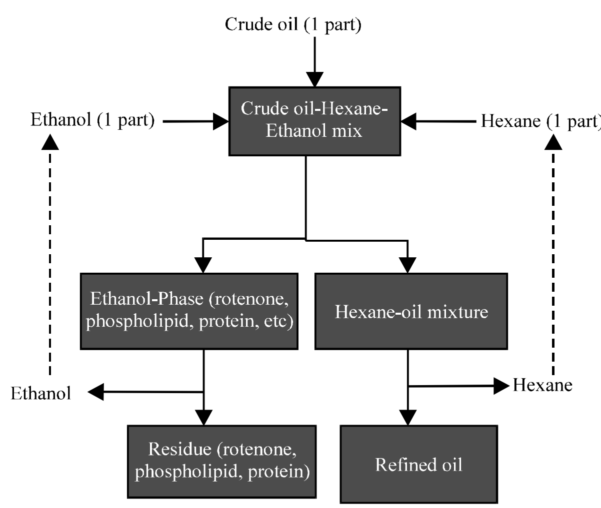

Fig. 1: Flow chart for refinery of brebra oil through the use of co-solvent refinery techniques (hexane and ethanol)

Table 2: The seed production capacity of brebra (*Millettia ferruginea*) tree

Characteristics	Quantity of production
Dry weight of pods per tree (kg)	150
Dry weight of seeds per tree (kg)	37.5
No. of tree per hectare land	37
Amount of seeds per hectare land (kg)	1350

Optimization of transesterification process: Optimization of transesterification process is important to determine the time taken to accomplish transesterification reaction. It is also important to determine whether one or two-stage process is effective to produce more purified product. Refined brebra oil was used as feed stock for biodiesel production. The reaction was carried out at room temperature as one or two phase and its progress was followed using Thin Layer Chromatography (TLC) (Fig. 2).

In one phase reaction monoglycerides/diglycerides, triglycerides and fatty acid methyl esters were detected indicating that the reaction did not reach to completion. In the two phase transesterification reaction, however, only fatty acid methyl esters were detected on the TLC indicating that the reaction reached to completion. After completion of the reaction the biodiesel yield was 81.7%, with glycerol accounting for 17. 84% of the original oil and the remaining 0.56% accounted for as loss in the process (Fig. 3).

Characterization of methyl esters: Table 3 shows the chemical and physical characteristics of brebra biodiesel in relation to known standards. The specific gravity (0.88 Kh L^{-1}), viscosity (5.06 mm $^{2}sec^{-1}$), acid value (0.69 mg KOH g^{-1}), pH value (8.9), saponification value (174.95 mg KOH g^{-1}) and refractive index (1.473), of brebra biodiesel were within the ASTM standard specifications (D960-52, 1952). The iodine value of the oil was also within specification of ASTM (2002) and EN (2003).

The fatty acid composition of brebra oil was analyzed using gas chromatography. The fatty acid content of brebra oil was compared with other oils (Table 4). The total unsaturated fatty acid content (80.7%) was higher

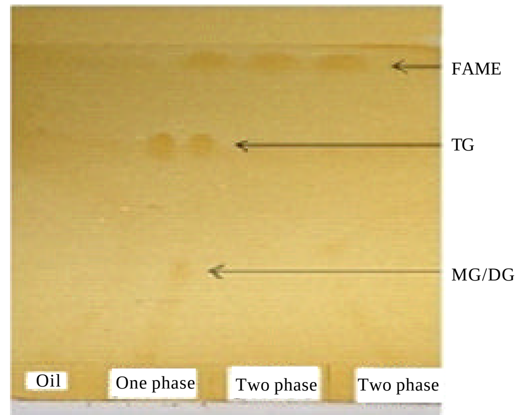

Fig. 2: Typical thin layered chromatography methanolysis of refined brebra oil in one and two phase reactions; FAME: Fatty acid methyl eater, TG: Triglyceride and MG/DG: Mono or diglyceride

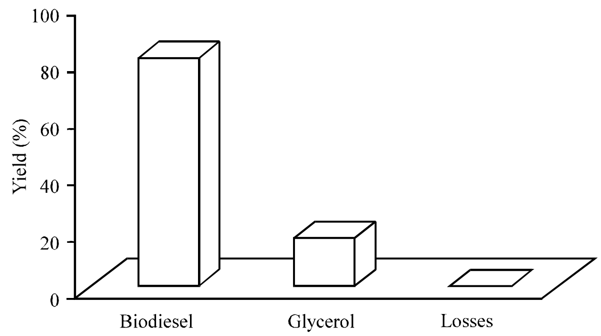

Fig. 3: Yields of fatty acid methyleaster and glycerol from brebra seed oil

Table 3: The physicochemical characteristics (mean values) of biodiesel compared to the standards of ASTM and EN

Fuel property	Standard test method (ASTM D6751)	Brebra biodiesel	Specification USA	Specification EU
Calorie or energy content (MJ kg^{-1})	-	37.63	35.00	*
Kinematic viscosity, at 40°C (mm^2 sec^{-1})	D445	5.06	1.9-6.0	3.5-5.0
Specific gravity at 60°F (kg L^{-1})	D1298	0.88	0.88	0.860-0.900
Density at 15°C (g mL^{-3})	D1298	0.89	*	0.86-0.90
Density at 2°C (g mL^{-3})	D1298	0.89	*	0.86-0.90
Water content (v/v)	D95	0.04	≤0.03	*
Water and sediment (v/v%)	D2709	0.04	≤0.05max	0.03
Boiling point (°C)	D86	305	315 to 350	Max 360
Flash point (°C)	D93	145.80	≥120	≥130
Cloud point (°C)	D2500	26	-3 to 12	*
Pour point (°C)	D97	21	-15 to 10	*
Cetane number	D613	52	≥47	≥51
Cetane index	D976	46.80	≥45	≥45
Iodine value (gI_2/100 g)	-	104.48	-	≤120
Saponification value (mg KOH g^{-1})	-	174.95	100-120	-
pH value	-	8.9	9	9
Refractive index	-	1.473	-	-
Color	D1500	1.5	≤3	*
Copper strip corrosion 3 H at 1000°C	D130	1b	≤3b	Class 1
Acid number (mg KOH g^{-1})	D664	0.69	0.80 max	0.5
Ash (wt.%)	D482	0.2	0.02	*

*Not determined, -: Not given

Table 4: Fatty acid composition of brebra oil in comparison with other oils

Fatty acids	Brebra seed oil	*Idesia polycarp* fruit oil (%)[a]	Rapeseed oil (%)[b]	*Jatropha curcas* oil (%)[c]	Karanja oil (%)[d]	Soybean (%)[e]
Saturated						
C16	7.2±0.1	15.06	3.6	16.0 (max)	11.65	12.9
C18	2.5±0.1	1.18	1.5	6-7.0	7.5	3.7
C20:0	0.9±0.1	-	-	-	-	-
C22:0	7.4±0.0	-	-	-	-	-
C24:0	2.2±0.0	-	-	-	-	-
Total	20.2±0.1	16.24	5.1	22-23	19.15	16.6
Unsaturated						
C16:1	-	6.5	-	1-3.5	-	0.1
C18:1	30.3±0.2	5.5	61.6	42-43.5	51.59	22.2
C18:2	44.7±0.2	70.6	21.7	33-34.4	16.64	52.9
C18:3	1.6±0.0	1.1	-	>4.5	-	7.9
C20:1	2.4±0.1		-	-	-	
C22:1	0.2±0.0	-	-	-	-	-
Total	79.8±0.0	83.7	83.3	80-85.9	68.23	83.1

[a]Yang *et al.* (2009), [b]Chhetri *et al.* (2008), [c]Tapanes *et al.* (2008), [d]Naik *et al.* (2008), [e]Holser and Harry-OKuru (2006)

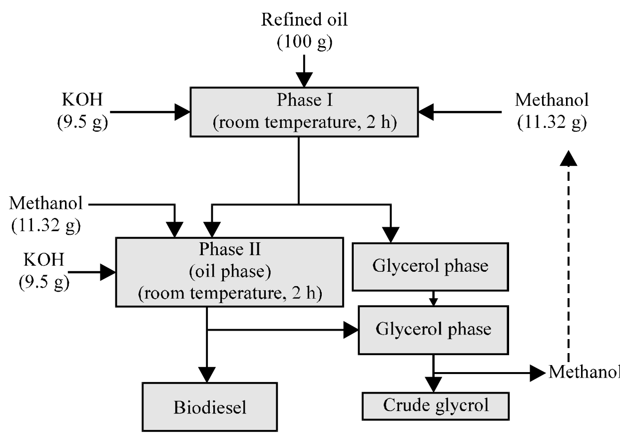

Fig. 4: Flow chart of biodiesel production through two phases of transesterification reaction processes

than the total saturated fatty acids (20.2%) of berrebera oil. Among polyunsaturated fatty acids, oleic (30.3%) and linolenic (44.7%) accounts 75% of the total fatty acids. Flow chart of biodiesel production through two phases of transesterification reaction is shown in Fig. 4. Transesterification reaction takes place at room temperature. The methanol recovered in this batch can be reused for transesterification of next batch of biodiesel production. If the glycerol produced is further refined, it can serve as feedstock for pharmaceutical and cosmetic industry.

DISCUSSION

In average, the brebra seed is composed of 48.5% (w/w) of oil. The oil yield of brebra seed in this study is nearly in agreement with the oil content of castor (50%) and sesame (50%) seed oil but greater than oil content of groundnut (42%), rapseed (37%), palm kernel (36%), mustard (35%), sunflower (32%), palm fruit (20%), soybean (14%) and cotton seed (13%) in weight (Agriculture Research Data Book, 2002).

Although triglycerides are the major components of vegetable oils, other components, like phospholipids, proteins, colouring materials, sugars, waxes and other impurities are also present (Douglas *et al*., 1977). For some industrial applications these impurities could interfere in the production processes. Crude oil from brebra contained about 4.4% protein, 7.2% phospholipid and appreciable amount of rotenone. Some of these impurities might be responsible for the inferior quality of the biodiesel prepared from crude brebra seed oil. The refinery processes developed in this study could allow fractionation of at least three important products from brebra crude oil: refined (pure oil), rotenone and phospholipid. The oil can have important application for the production of biodiesel. Rotenone, phospholipids, proteins and other impurities recovered from the upper ethanol phase could find important applications as well. For example, rotenone could be used as an insecticide or to kill unwanted fish. Phospholipids could find important application in pharmaceutical, cosmetic and food industries. It can also find various industrial uses such as paints, textiles, lubricants and waxes.

In this study, a two-stage process was chosen as it removes the majority of the mono-, di-and tri-glycerides in the first stage and those remaining could be taken out in the second stage, resulting in a more purified product.

The major problem associated with the use of pure vegetable oils as fuel for diesel engines is caused by the high fuel viscosity in compression ignition. Transesterification is the best technique applied to solve the problems encountered with the high fuel viscosity. Viscosity of brebra oil was reduced from 40.59-5.063 CSt through transesterification. Brebra oil biodiesel viscosity is similar to the viscosity of biodiesel derived from rapeseed (4.15 CSt), *Idesia polycarpa* (4.12 CSt) and soybean (4.1-5.75 CSt) (Yang *et al*., 2009). The viscosity of biodiesel in this study is in line with specification/ standard of USA (ASTM, 2002) and EU (En, 2003) biodiesel.

To bring the viscosity and other properties of the biodiesel to an acceptable range, the fatty acid composition of the oil plays an important role (Knothe and Steidley, 2005). To date *Jatropha curcas* is considered to have the best known source of non-edible oil for biodiesel production (Pramanik, 2003). One of its interesting features is that two fatty acids, oleic acid (18:1) and linoleic acid (18:2), account for about 75% of the fatty acid content of the oil. Brebra oil is also in many ways similar to *Jatropha* oil where 75% of its oil consists of oleic and linoleic acids (Tapanes *et al*., 2008). The only difference is that in *Jatropha* oleic acid accounts for up to 43.5% (Tapanes *et al*., 2008) of the oil while in brebra 44.7% of the oil consists of linoleic acid. The fatty acid composition of brebra biodiesel was also comparable with other oil sources (Naik *et al*., 2008; Yang *et al*., 2009).

The refractive index of this study (1.473) is in close agreement with 1.465 (Aremu *et al*., 2007), 1.462 (Akintayo and Bayer, 2002) and 1.468 (Akpan *et al*., 2006) of castor, akee pulp and cashew nut seed oils, respectively. The saponification value of the oil in this study was 174.95 mg KOH g^{-1}. This was lower than the values for some common oils like castor seed oil (185.83) (Akpan *et al*., 2006). However, this saponification value is within the same range of some edible oils reported by Eromosele *et al*. (1994). Moreover, saponification value (174.95) of the oil in this investigation is almost within the range of (175-187) (ASTM, 2002) specification for oils. The oil under investigation has very low acid value of 0.39 mg KOH g^{-1} when compared with cashew nut oil (0.82 mg KOH g^{-1}) (Aremu *et al*., 2007), refined castor oil (0.869) and crude castor oil (1.148) (Akpan *et al*., 2006), plukenetia conophoora (11.5 mg KOH g^{-1}) as reported by Akintayo and Bayer (2002).

The specific gravity of brebra biodiesel was found to be within the limit specified for biodiesel fuel in USA (D6751:0.88), Europe (EN14214: 0.86-0.90), Austria (ONC1191: 0.85-0.89), Czech Republic (CSN656507: 0.87-0.89), Germany (DINV51606: 0.875-0.90), Sweden (SS155436: 0.87-0.90) and Italy (UNI10635: 0.86-0.90) (Chitra *et al*., 2005). The specific gravity recorded for the brebra biodiesel was higher than the values obtained for the fossil diesel (Alamu *et al*., 2007). The level of agreement recorded in specific gravity for the brebra biodiesel is an important pointer to suitability of the biodiesel as diesel fuel substitute. Moreover, fuel performance indicator values such as cetane number, heating values, fuel storage and transportation of brebra biodiesel are correlated with specific gravity of biodiesels from different oil sources (Ajav and Akingbehin, 2002). In general, specific gravity and density of biodiesel was reduced after transesterification of the oil. According to Clark (1988), low relative density is indicator of good ignition properties of fuels. Higher pour point, cloud point, flash point and boiling point obtained for brebra biodiesel is comparable to conventional petroleum based diesel. The net heat of combustion of biodiesel obtained in this study was found to be consistent with earlier findings of biodiesel from rapeseed, canola, beef tallow and soybean (Abigor *et al*., 2000).

The flash point of brebra biodiesel (145.8°C) was higher than flash point of petrodiesel (69.7°C). According to this result, brebra biodiesel is non-inflammable and safer for handling in wide range of environment. This is because the minimum flash point requirement is 120°C (according to ASTM) and 130°C (according to EU) standards. The acid value (0.69) of brebra biodiesel was found to be below the maximum limit of the standard specification (max 0.8, ASTM D 664). Above 331°C, brebra biodiesel was cracked and form smocks. To recover above 90% biodiesel, it is recommended to use vacuum distillation method (ASTM D 1160). This may be attributed to the presence of high amount of polyunsaturated fatty acids of the biodiesel under investigation.

The cost of raw materials is often the biggest obstacle to the large scale commercialization of biodiesel. For example, if edible vegetable oils are used for biodiesel production up to 70-85% of the cost is accounted for by the raw material (Noordam and Withers, 1996). If biodiesel is produced from non-edible vegetable oils and one or more byproducts of commercial importance are also generated, the production cost of biodiesel is expected to lower substantially (Dorado *et al*., 2006). Glycerol (the co-product of biodiesel) is widely used as feedstock for pharmaceutical, cosmetic and chemical industry as well as animal feed supplement (Min *et al*., 2010; Cerrate *et al*., 2006).

With regard to economical and environmental prospects, production of brebra biodiesel may help to reduce the amount of currency expenditure of the imported petroleum and address environmental problems. It can provide and improve the quality of multiple energy services: cooking fuel, heat, electricity and transportation fuels. Thus, the result of this study is significant to open new market for brebra producers and also creates job opportunity for the society thereby contributing a lot for economic development of any country. It can grow in wide range of altitude and soil type (Thulin, 1983).

CONCLUSION

Brebra oil can find important application for the production of biodiesel. Co-products, such as glycerol, phospholipid and rotenone which should be removed during refinery process, are expected to lower the production cost of biodiesel. Biodiesel can provide the quality of multiple energy services: cooking fuel, heat, electricity and transportation fuels. Thus, brebra biodiesel may help to reduce the amount of currency expenditure for the imported petroleum and address environmental problems.

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