International Journal of Enhanced Research in Science, Technology & Engineering
ISSN: 2319-7463, Vol. 7 Issue 12, December -2018, Impact Factor: 4.059

# Extraction and Analysis of Glycerides of Ipomoea quamoclit plant found in Manipur and transesterification of the same glycerides into biodiesel followed by characterization

Md. Abdul Halim Shah[1], Dr. Naorem Sarita Devi[2]

[1]Associate Professor, Department of Chemistry, D.M. College of Science, Dhanamanjuri University, Imphal-795001, India
[2]Associate Professor, Department of Environmental Science, D.M. College of Science, Dhanamanjuri University, Imphal-795001, India

**ABSTRACT**

***Seedoil of Ipomoea quamoclit was extracted by Solvent extraction technique on the crushed kernel using petroleum ether as the solvent. The oil was purified prior to further analysis by column chromatography over silica gel (60-120 mesh) using a mixture of petroleum ether and ethyl acetate (20:1) as the eluent. The purified oil was further transesterified giving the final product known as biodiesel. The biodiesel is also known as the fatty acid methyl ester (FAME).Fatty acid methyl ester (FAME) composition of Ipomoea quamoclitdetermined by NMR, IR and GC-MS analysis. The FAME of Ipomoea quamoclitconsists of 31.34wt% of methyl palmitate (C16:O), 52.03wt % of methyl oleate (C18:1), 13.58wt% of methyl stearate (C18:O) and 3.08wt% of methyl arachidate( C20:0)***

**Keywords: Mike manbilei, cypress vine,*Ipomoea quamoclit,* transesterification, non-edible vegetable oil, Athia, Biodieseletc.**

## INTRODUCTION

A triglyceride is derived by esterification of all the three hydroxyl groups of glycerol by 3 molecules of fatty acids.

OCOR1
OCOR2
OCOR3

Glycerol Fatty acids a fat or oil (a triglyceride)

If all the R1, R2 & R3 are identical, the triester is designated as a simple glyceride and if they are not it is a mixed glyceride.

Most natural fats or oils are mixed triglycerides. Triglycerides may be saturated or unsaturated depending on whether the fatty acid component chains are saturated or contain double bonds. The carboxylic acids or fatty acids that go to form triglycerides (fat or oil molecules) have carbon chains with only even number of carbon atoms. The most common fatty acids have unbranched carbon chains of 14, 16, 18 & 20 carbons reflecting the pathway for their biosynthesis from the two carbon bulding block acetyl CoA.

Triglycerides are used as the storage form of energy and they are also important sources of energy for transport vehicles. Most natural fats or oils are mixed triglycerides which may be saturated or unsaturated. Triglycerides are used in soap industry, in the manufacture of paints, varnish, lacquers, synthetic detergents, glycerol, high molecular weight acids, vanaspati ghee, oil cloth, printing inks, hair oil, candles, polishes, medicine, drug for heart diseases and strokes, diabetes etc.

There are many wild plants which produce fruits with seeds highly rich in non-edible oils in both plain and hill areas of Manipur(India). Many of these plants have no apparent economic value. Thus such oil finds very limited commercial uses and plants producing such oils are slowly disappearing because such plants are considered not important by farmers, government, any public and private sectors etc. As a resultplantdiversity is dwindling. Moreover, it is believed that large scale production of biodiesel from edible oils may cause global imbalance to the food supply and demand market. Hence the use of non-edible oils as the feedstock for biodiesel industries will spare edible oils for use in other industries of edible products [1]. The use of biodiesel now-a-days has become important for diesel engines and is getting worldwide attention because of its renewability, biodegradability, non-toxicity and carbon neutrality [2-5]. The developed countries like Brazil, Indonesia, Malaysia, USA, UK, Canada and Germany have already started using biodiesel blended petro-diesel. Our country also desperately needed it as a substitute for petro-diesel of self-reliance [6-8]. It is in this context that identification of fatty acid constituents in glycerides is essential.

Biodiesel usually consists of methyl esters of long chain fatty acids and is made from nontoxic biological resources such as vegetable oils and animals fats by trans-esterification with methanol in presence of a catalyst [1, 9-10]. Catalysts may be acid, base and enzyme (lipase). Biodiesel has many advantages and it can contribute to both solving global warning and energy problems [1,11-13]. Hence, non-edible vegetable oils can be used as an alternative feedstock for the production of bio-diesel [14-17].

***Ipomoea quamoclit*** **Linn** (Cypress Vine), locally called **Mike manbi lei** in Manipuri belongs to the family, ***Convolvulaceae***. It is a species native to tropical regions of the New World from Northern South America to Mexico and Southern and North Eastern India including Manipur. It is an annual or perennial herbaceous, twining vine growing to 1-3 m tall. The flowers are 3-4 cm long and 2 cm in diameter, trumpet- shaped with five petals (Fig. I). It is widely cultivated as an ornamental plant throughout the tropics. The plant is considered cooling and purgative ; used in cancer and breast pain. Pounded leaves are applied to bleeding piles and as a plaster to carbuncles[18.] The seeds yield about 8.42 wt% oil after purification by column chromatography.

**(a)** ***Ipomoea quamoclitplant with fruits***

**(b) *Ipomoea quamoclit*seeds**

**Fig. 1 : *Ipomoea quamoclit*plant and seeds**

## MATERIALS AND METHODS

***Ipomoea quamoclit*** were collected from Lilong Haoreibi, Thoubal district, Manipur(India) during its availability of the season of June and July. The seeds were first cleaned and dried for 5/6 days in the sunlight, deshelled and the kernel crushed using a grinder prior to oil extraction. Methanol used was analytical grade (Merck Mumbai, India). All other solvents and chemicals used were of analytical grade and they are procured from commercial sources and used as such without to further treatment.

Oil was extracted from crushed and powdered Kernel of ***Ipomoea quamoclit seeds*** in petroleum ether (b.p. 40-60$^0$C) (10ml/g) by stirring magnetically at room temp (27$^0$C) for 3.30 hours. The solvent was removed at 15$^0$C using a rotary vacuum evaporator (BUCHI Rotavapour R-200) to yield crude oil. This process was repeated 2-3 times with the seed cake using fresh solvent each time in order to extract most of the oil which was further dried by using vacuum pump. The oil was purified by column chromatography over silica gel (60-120 mesh) using a mixture of petroleum ether and ethyl acetate (20:1) as the eluent prior to trans-esterification done.

$$\%\ Oil\ content = \frac{Weight\ oil}{Weight\ of\ powdered\ \ seeds} \times 100 \qquad \text{…….. (1)}$$

The parameters of glycerides such as density, colour, refractive index, acid value, iodine value and saponification value were experimentally determined in accordance with the Association of Official Analytical Chemical Procedures [19] and these results are reported (Table 1).

$$Acid\ value\ (mg\ KOH/g) = \frac{56.1 \text{ x } V \text{ x } N}{W} \text{…….. (2)}$$

where,

$V$ = titre value (mL)

$N$ = normality of KOH solution (determined by standardizing KOH solution with oxalic acid).

$W$ = weight of test sample taken in g.

Refractive indices of purified seedoils were determined by using the Abbe Refractometer (AW-24) at room of temperature, only two or three drops of oil was required. Densities of the purified oils were determined at room temperature (32 °C). For this, a clean and empty plastic centrifuge tube was taken and weighed. Accurately 1000 $\mu$L (= 1 mL) of the liquid sample was transferred into the tube with the help of a syringe and then weighed again. Then the density is determined based on mass per unit volume of oil.

$$Iodine\ value = \frac{12.69 \text{ x } N \text{ x } (V_B - V_S)}{W} \text{…….. (3)}$$

where,

$V_B$= Volume of sodium thiosulphate solution used for the blank (mL)

$V_S$= Volume of sodium thiosulphate solution used for the oil sample (mL),

$N$ = Normality of sodium thiosulphate solution used,

$W$ = Weight of oil sample taken in g

$$Saponification\ value = \frac{56.1 \text{ x } M \text{ x } (V_B - V_S)}{W} \text{…….. (4)}$$

where,

$V_B$ = Volume of 0.5 M HCl solution used for the blank (mL)

$V_S$ = Volume of 0.5 M HCl solution used for the oil sample (mL)

$M$ = Molarfity of HCl used
$W$ = Weight of oil sample taken in g

$$\% \text{ Moisture} = \frac{W_1 - W_2}{W_1} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

Where,
$W_1$ = Initial weight of oil,
$W_2$ = Final weight of oil

**Table1: Physical parameters of *Ipomoea quamoclit as calculatdusing equation (1-5).***

| Sl No. | parameters | Observed values |
|---|---|---|
| 1 | Colour | Light yellow |
| 2 | Oil content (wt.%) | 8.42 |
| 3 | Density (g/cm$^3$) | 0.8896 |
| 4 | Acid value (mg KOH/g) | 1.370 |
| 5 | Iodine value (g $I_2$/100g) | 76.63 |
| 6 | Saponification value (mg KOH/g) | 169.57 |
| 8 | Refractive index | 1.4618 |
| 9 | Moisture (%) | 0.113 |

The purified oil was transesterified to fatty acid methyl esters (FAME) using a catalyst called Athia, a banana plants (ashes from the peels of banana fruits, variety used Musa balbisiana,20 wt% of the oil) [20]. A mixture of the oil in methanol (10 ml/1g of the oil) and the catalyst (20 wt% of the oil) was stirred vigorously magnetically at room temp (27°C) and the conversion completion of the reaction was monitored by Thin Layer Chromatography (TLC).

After completion of the reaction, the product mixture was extracted with petroleum ether. The organic layer was washed with brine, dried over anhydrous $Na_2SO_4$overnight and the solvent was removed under vacuum to yield the crude product which was further purified by column chromatography over silica gel using petroleum ether and ethyl acetate (20:1) as the eluent. The product was concentrated and evaporated to dryness on a rotatory evaporator which was further dried using vacuum pump to remove the last traces of the solvents to yield pure biodiesel. (FAME).

The composition of FAME mixture was estimated using Perkin Elmer Clarus 600GC-MS. The column used was Elite 5MS with dimension 30.0m × 250mm. The oven temp was initially held at 140$^0$C for 5 min, increased to 240$^0$C at 4$^0$C/min, and then held for 5min. The injector, transfer andsource temperatures were 250$^0$Cand 150$^0$C respectively. Carrier gas was Helium and total scan time 35 min. EI mode of ionization was applied and mass san was from 20 to 400Da. For identification of FAME library search was carried out using National Institute of Standards Technology (NIST), National Bureau of Standards (NBS) and Wiley GC-MS library. Fatty acid profile of biodiesel from ***Ipomoea quamoclit***seed oil is reported in table 2. The $^1$H &$^{13}$C NMRspectra were recorded in Carbon Deuterium Trichloride($CDCl_3$) at 300 & 75 MHz respectively using Bruker Advance III 300MHz/54mm NMR spectrometer. IR spectrum was recorded with a Perkin Elmer RX1FT-IR spectrometer as a thin film on KBr plate.

Fatty acid composition of the FAME prepared from ***Ipomoea quamoclit*** was determined by GC-MS analysis. The each peak of the gas chromatogram (Figure 2) was analysed and the fatty acid was identified using MS database. Each peak represents one fatty acid methyl ester. The three peaks in the gas chromatogram which means the presence of three different fatty acid methyl Easters. The peak at the farthest distance on the right side in mass spectrum of any fatty acid methyl ester gives the molecular weight of the fatty acid. This peak is known as molecular ion peak. Retention time is the time taken when any peak develops. Based peak means the tallest peak in the mass spectrum due to the ion with the greatest relative abundance. The peak with the greatest m/z value is likely to be the molecular ion peak.

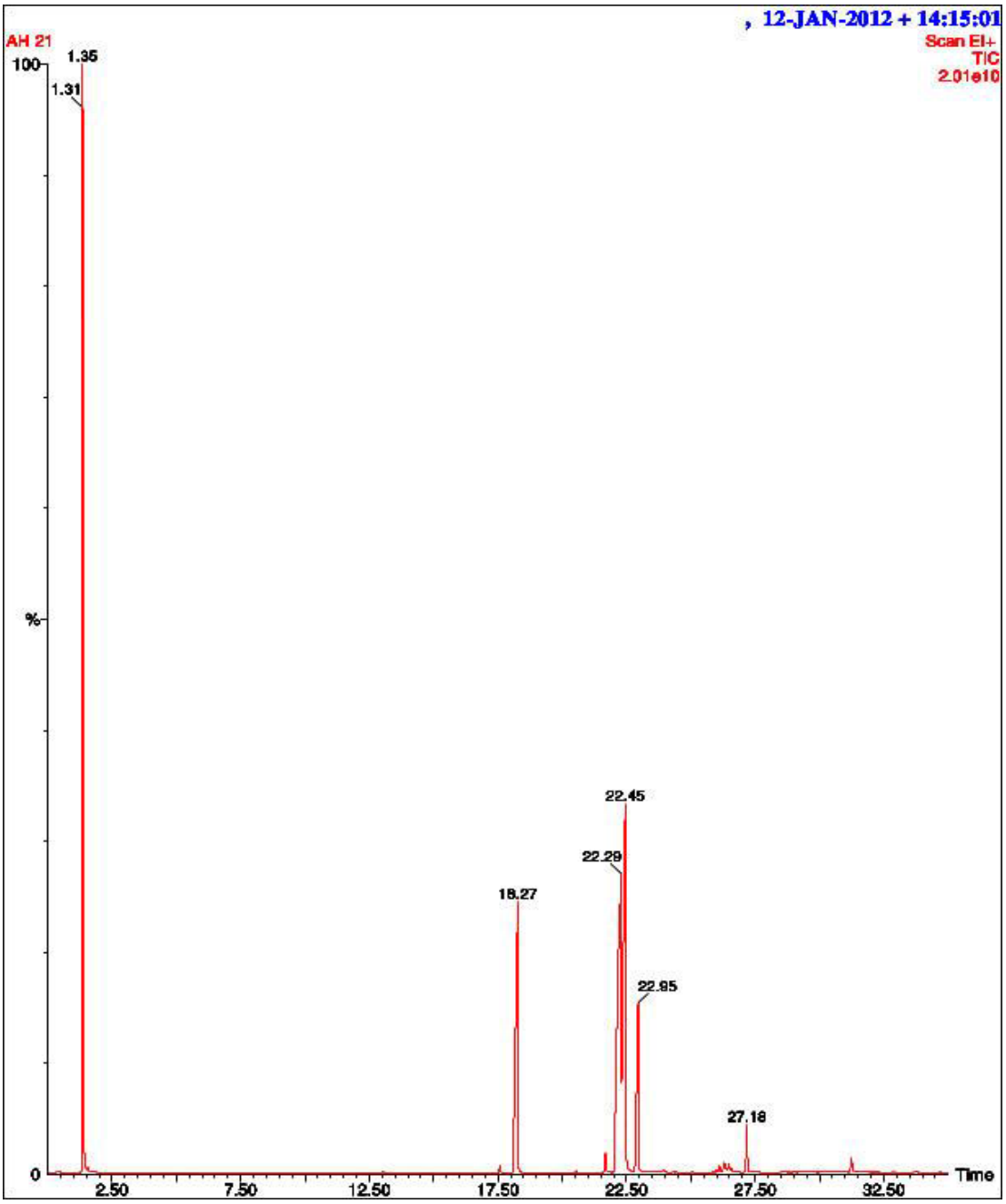


**Fig.2: Gas chromatogram of biodiesel from *Ipomoea quamoclitseed oil***

## RESULTS AND DISCUSSION

The yield of the extracted and purified glycerides from ***Ipomoea quamoclit*** was found to be 8.42wt% at the room temperature ($26^0$C) for 4hourswhile the yield transesterified glyceride known as FAME was 91.50wt% at the temperature $24^0$C for 4:30hrs. The trans-esterified products were purified by column chromatography and analyzed.

The light yellow colour of the ***Ipomoea quamoclit*** was due to the presence of natural pigments like tocopherols, carotenoides and their derivatives. The yield of the oil was moderate. Density and iodine value of ***Ipomoea quamoclit***were found to be 0.8896g/cm$^3$ and 76.63 g$I_2$/100 respectively which are comparable to those of soya bean oil and sunflower oil. The acid value of this oil was found to be 1.370 mg KOH/g which is within the limit for industrially useful oil. Saponification value was 160.57 mg KOH/g whose value is suitable for soap making and cosmetic industries. Refractive

Index of this oil was 1.4618 which is not very much different from those recorded for conventional seed oils such as palm oils (1.445-1.451), cotton seed oil (1.468-1.472), safflower oil (1.473-1.476) and soya bean oil (1.4728) at 25'C. Moisture was found to be 0.113% (low value) which is suitable good quality and contamination does not take place easily due to its low value of moisture. Low moisture content is an essential criterion for commercial oil.

**Analysis of FAME of *Ipomoea quamoclit***

*$^{1}$HNMR 300 MHz, CD Cl3):* δ 5.31-5.36 ppm, δ 3.65 ppm, δ 2.78 ppm, δ 2.30 pmm, δ 2.00-2.07 ppm, δ 1.58-1.63 ppm, δ 1.24 ppm and δ 1.30 ppm, δ 0.85-0.89 ppm.

$^{13}$C NMR (75MHz, $CDCl_3$): δ 174.33 ppm,δ 127.88 ppm, δ 128.02 ppm, δ 128.30 ppm, δ 129.72 ppm, δ 129.97 ppm, δ 130.17 ppm , δ 51.42 ppm, δ 14.07 ppm, δ 234.65 ppm.

FT-IR (thin film) : 1735.93$cm^{-1}$, *1506.41*$cm^{-1}$, 2926.01$cm^{-1}$,2758.51$cm^{-1}$, 3456.44$cm^{-1}$,1238.30$cm^{-1}$, 1166.66$cm^{-1}$, 1107.14$cm^{-1}$, 731.32$cm^{-1}$.

Relative percentages of fatty acid esters were calculated from the total ion chromatography by computerized integrator and results are presented (Table 2). Fatty Acid Methyl Ester (FAME) from***Ipomoea quamoclit***consists of 31.34wt% of methyl palmitate (C16:0), 52.03wt% of methyl oleate (C18:1),13.58wt% of methyl stearate (C18:0)& 3.08wt% of methyl arachidate (C20:0).

**Table2: Fatty Acid profile of FAME from *Ipomoea quamoclit***

| Entry | Retention time (min) | FAME | wt% |
|---|---|---|---|
| 1 | 18.22 | *Methyl palmitate* | 31.34 |
| 2 | 22.39 | *Methyl oleate* | 52.03 |
| 3 | 22.92 | *Methyl stearate* | 13.58 |
| 4 | 27.18 | *Methyl arachidate* | 31.08 |

The mass spectra of biodiesel from ***Ipomoea quamoclit***are shown in figure 3a to 3d. Molecular ion peaks and the basic peaks of the FAME are presented in Table 3 and they are in the expected values. The molecular ion peaks of methyl palmitate,methyl oleate, methyl strearate, and methyl arachidate at were observed at 270, 296,298,and 326 respectively as was expected.

**Table3: Molecular ion and base peaks of FAME from seed oil from *Ipomoea quamoclit***

| Entry | FAME | Molecular ion peaks (m/z) | Base peak (m/z) |
|---|---|---|---|
| 1 | *Methyl palmitate* | 270 | 74 |
| 2 | *Methyl oleate* | 296 | 55 |
| 3 | *Methyl stearate* | 298 | 74 |
| 4 | *Methyl arachidate* | 326 | 74 |

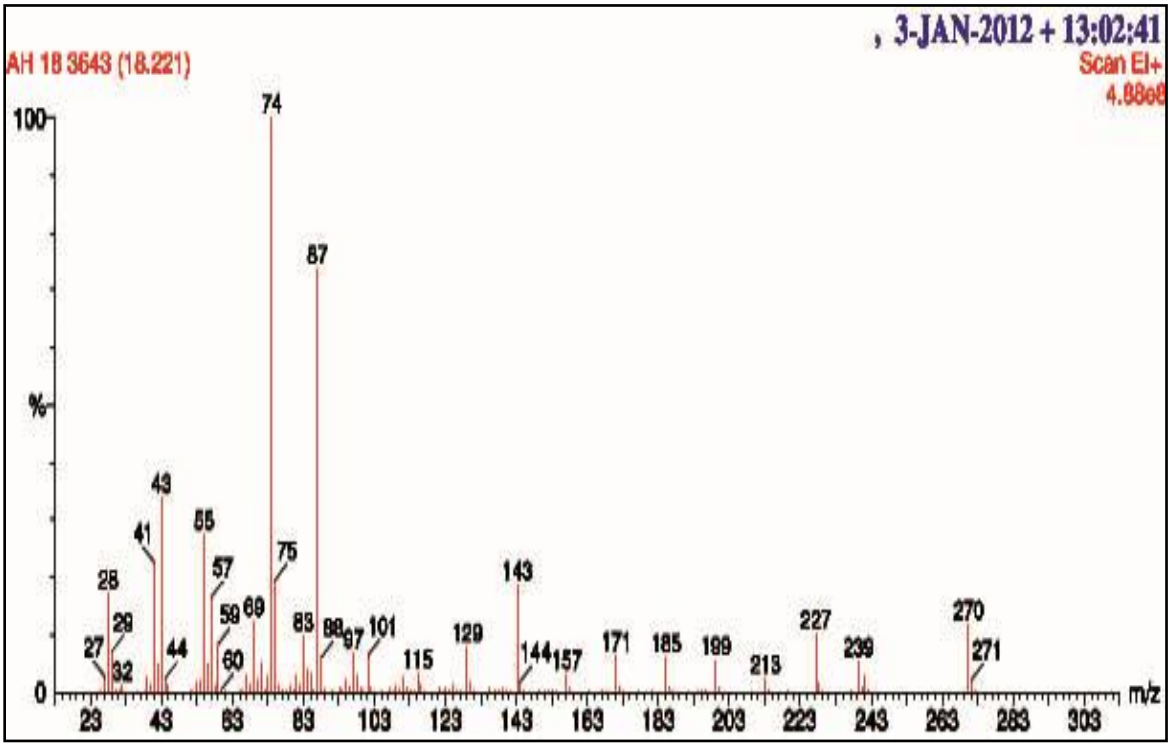


**Fig. 3a : Mass spectrum of methyl palmitate**

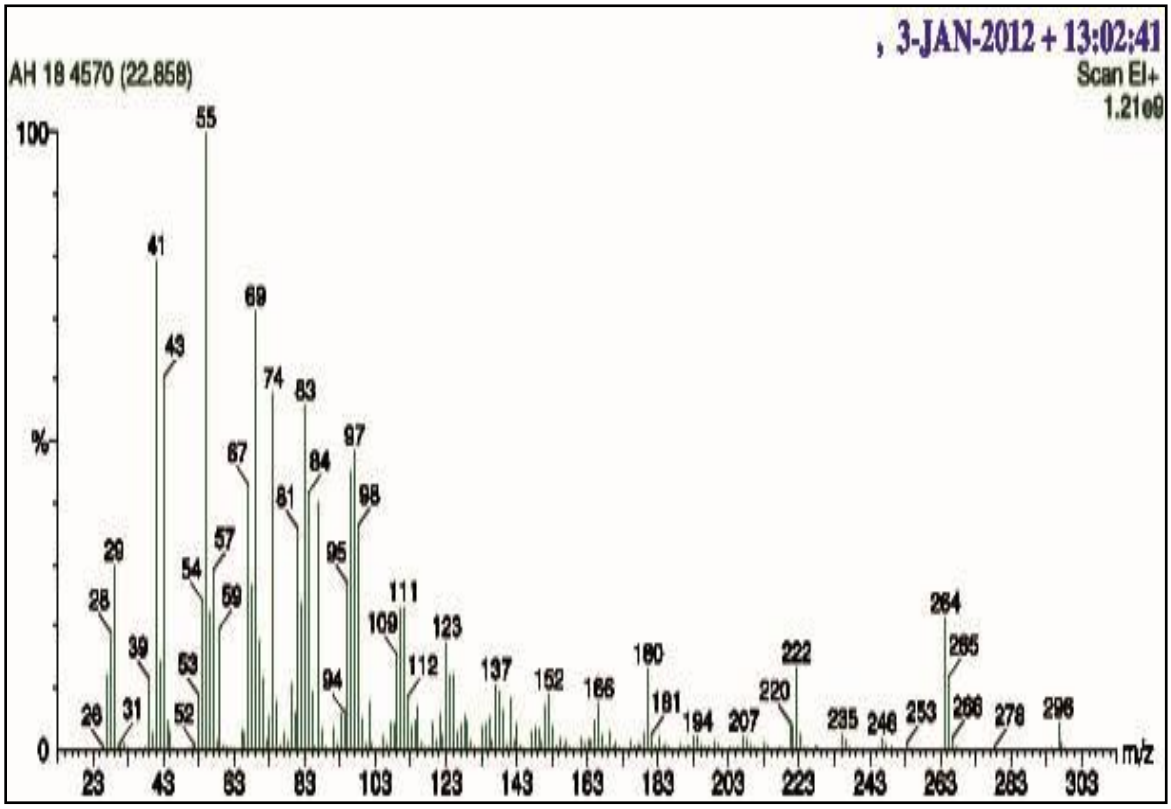


**Fig. 3b: Mass spectrum of methyl oleate**

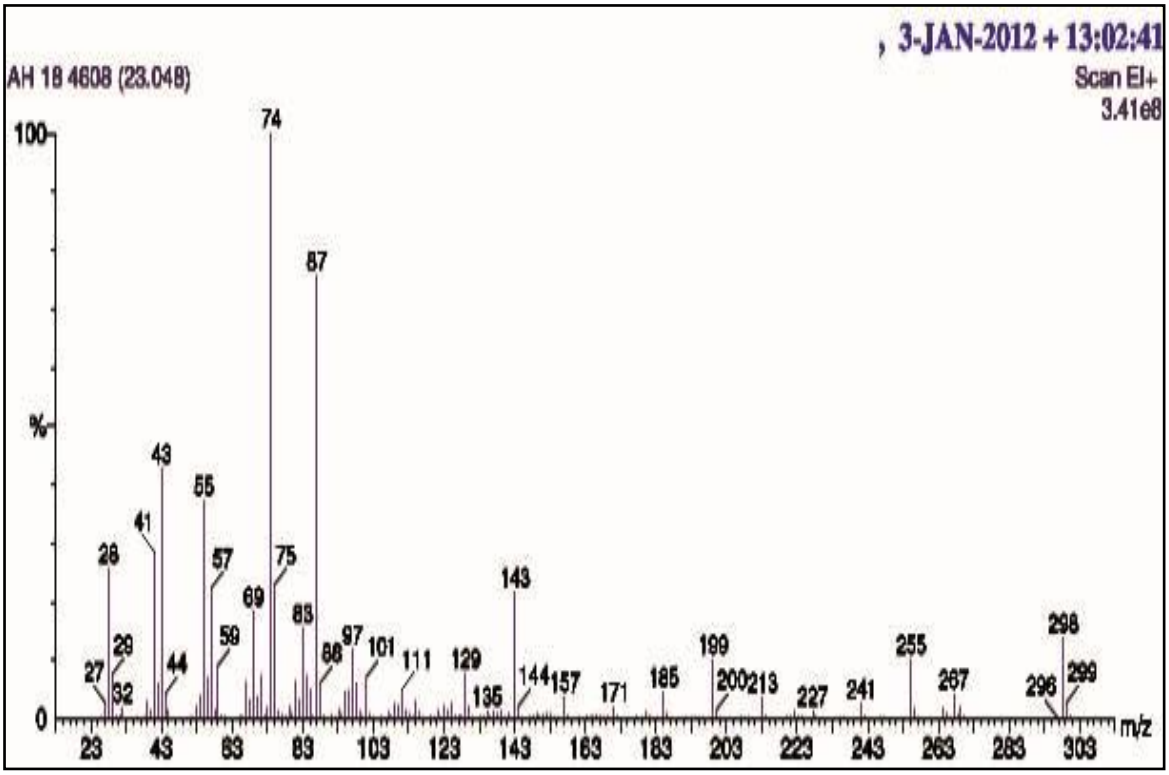


**Fig. 3c: Mass spectrum of methyl stearate**

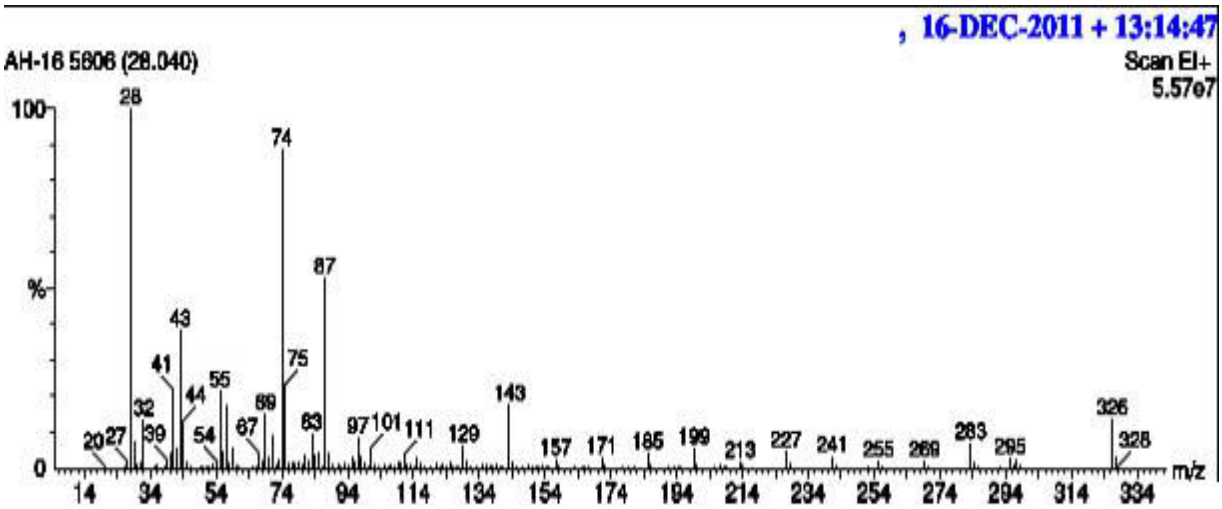


**Fig-(3d) Mass spectrum of methyl arachidate.**

The 1H NMR spectrum of biodiesel from Ipomoea quamoclitseedoil is shown in Fig.4. The multiplet at $\delta$ 5.33-5.35 ppm suggests the olefinic protons (–CH=CH–). A singlet signal at $\delta$ 3.65 ppm is representing methoxy protons of the ester functionality fatty acid chain. The signal at $\delta$ 2.78 ppm indicates the bis-allylic protons (–C=C–CH2–C=C–) of the unsaturated fatty acid chain. The triplet at $\delta$ 2.29 ppm (t, 3J=7.5 Hz) may be due to the ∝-methylene protons to ester (–CH2–CO2Me). The ∝-methylene protons to double bond (–CH2–C=C–) is seen as a multiplet at $\delta$ 1.99 - 2.01 ppm is absent. The $\beta$-methylene protons to ester (CH2–C–CO2Me) also appear as a mutliplet at $\delta$ 1.58 - 1.63 ppm. The multiplet at $\delta$ 1.24 - 1.30 ppm is due to the protons of backbone methylene of the long fatty acid chain. The terminal methyl protons (C–CH3) at $\delta$ 0.88 ppm appear as a mutliplet. The 13C NMR spectrum of biodiesel from Ipomoea quamoclitseedoil is shown in Fig.5. $\delta$ 174.32 ppm suggests the carbonyl carbon of the ester molecular and the olefinic carbons appear at $\delta$ 129.73 and 130.19 ppm. The signal at $\delta$ 51.44 ppm is due to methoxy carbons of esters. The methylene and methyl carbons of fatty acid moiety appear in the range from $\delta$ 14.08 to 34.09 ppm.

The IR spectrum of biodiesel from ***Ipomoea quamoclit***seedoil (Fig.6), a sharp signal at 1726.39 cm-1 is indicative of strong absorption by ester carbonyl stretching frequency. Strong and sharp signals at 2914.44 and 1702.27 cm-1 are due to C–H stretching frequencies. The absorbance at 3167.12 cm-1 indicates the =C–H stretching frequency. The bands at 1234.44, 1166.93 and 1049.28 cm-1 are expected for C–O–C stretching vibrations. The observation of an absorption peak at 732.95 cm-1 suggests the CH2 rocking.

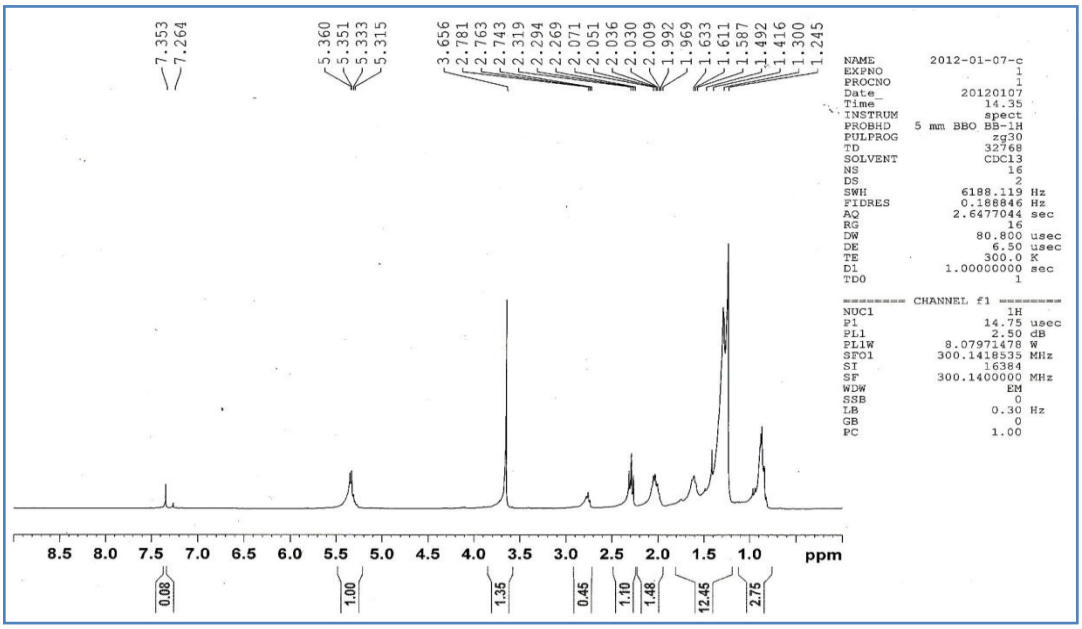


**Fig.4: $^{1}$H NMR spectrum of biodiesel from *Ipomoea quamoclit*seedoil**

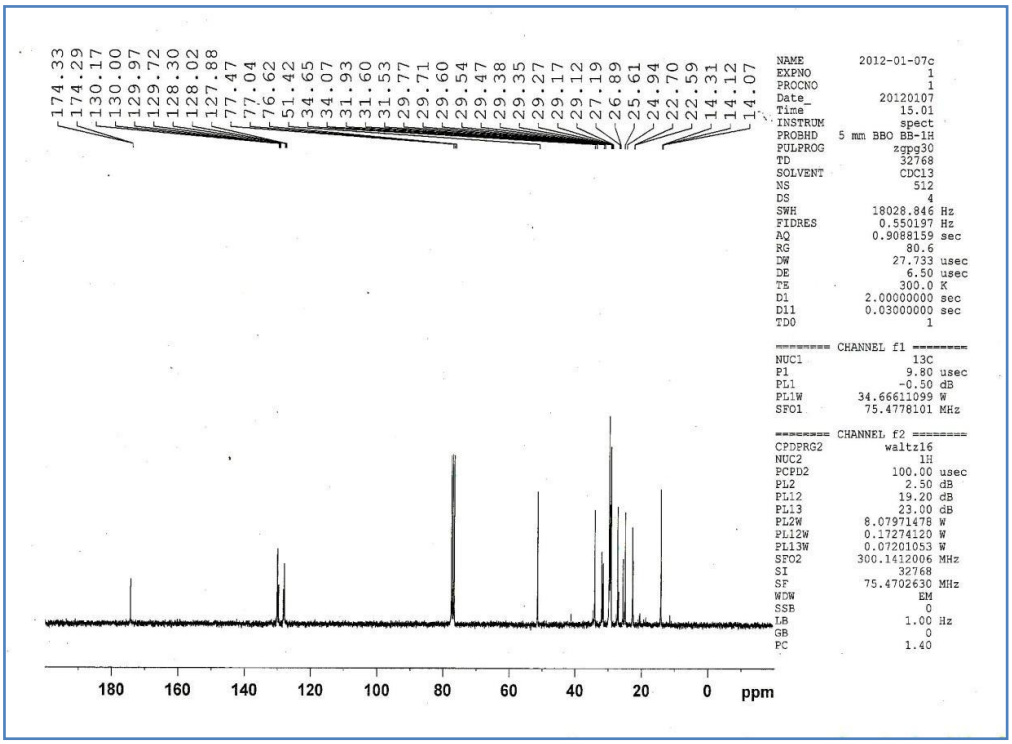


**Fig. 5: $^{13}$C NMR spectrum of biodiesel from *Ipomoea quamoclit* seedoil**

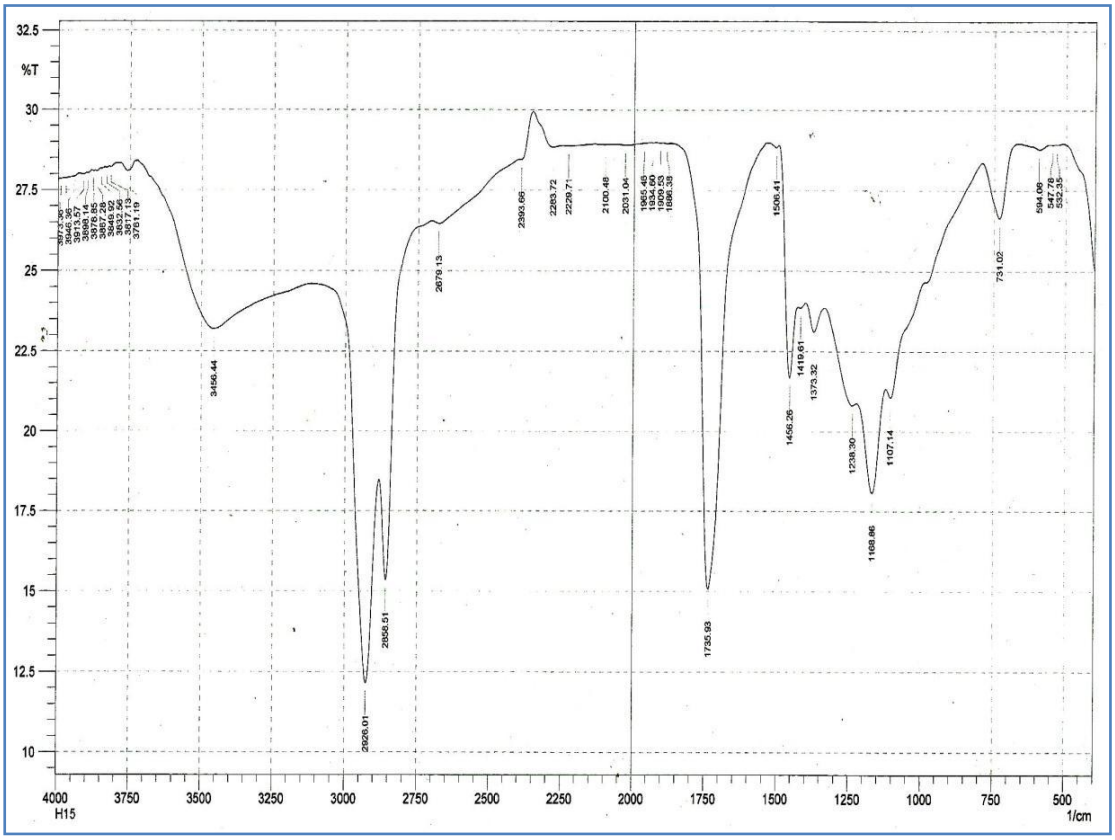


**Fig.6: IR spectrum of biodiesel from *Ipomoea quamoclit***

**Theoretical determination of IV, SN and CI of FAMEs**

Three important physical properties of biodiesel, viz. iodine value ($IV$), saponification number ($SN$) and cetane index ($CI$) were performed applying theoretical calculation based upon fatty acid profile shown in the Table IV. The IV, $SN$ and $CI$ of FAMEs were calculated using equations (6), (7) and (8) respectively.

Results are shown in Table 4.

$$IV = \sum(254 \times D \times Ai)/MWi. \ldots\ldots\ldots\ldots\ldots\ldots (6)$$
$$SN = \sum(560 \times Ai)/MWi. \ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$
$$CI = 46.3 + \frac{5458}{S} - 0.225R. \ldots\ldots\ldots\ldots\ldots\ldots (8)$$

Where, $D$ = number of double bonds in the ith component
$Ai$ = percentange of the ith component in the chromatogram
$MWi$ = molecular weight of the ith component of the FAME in the oil
S = saponification number ($SN$) as calculated by the equation [7]
$R$ = iodine value ($IV$) as calculated by equation (6)

***Table 4. Experimentally and theoretically as* calculated *IV , SN, CIof FAME Profile Carthamus tinctorius* plant**

| **Name of the oil plant** | $IV$ (g/100g) | $SN(mg\ KOH\ /\ g)$ | $CI$ |
|---|---|---|---|
| *Carthamus tinctorius* | 44.64 | 193.20 | 64.51 |

## CONCLUSION

The yield of the extracted and purified glyceride from ***Ipomoea quamoclit*** was found to be 8.42wt% at the temperature **$26^0$C within 3.30 hours** while the yield of transesterified glyceride known as fatty ester (FAME) was 91.50wt% at

temperature $24^0$C within 4:30 hours. The colour, density, acid value, iodine value, saponification number, refractive index and the moisture of the ***Ipomoea quamoclit*** were found to be light yellow, 0.8896g/cm3, 1.370 mg KOH/g, 76.63g $I_2$/g, 169.57mgKOH/g, 1.4618 and 0.113wt% respectively.

The biodiesel from ***Ipomoea quamoclit*** after extraction and purification by column chromatography was prepared by heterogeneous transesterification process and analysed for its fatty acid methyl esters composition using IR, NMR and GC-MS. This study found that FAME from ***Ipomoea quamoclit*** consists of 31.34wt% of methyl Palmitate (C16:0), 52.03wt% of methyl Oleate (C18:1), 13.58wt% of Methyl SterateC18:0) and the 3.08wt% of methyl arachidate (C20:0). The molecular ion peak of methyl palmitate, methyl oleate, methyl stearate, and methyl arachidate were observed at 270, 296,298 and 326 respectively as was expected. The Iodine value (IV), Saponification number (SN) and Cetane Index (CI) of ***Ipomoea quamoclit*** were calculated experimentally and theoretically and were found to be 44.64 (g/100 g), 193.20 (mg KOH/g) and 64.51 respectively.

## REFERENCES

[1]. Leung DYC, Wu X, Leung MKH, **2010.** A review on biodiesel production using catalyzed transesterification. Applied Energy.87(4), 1083-1095.
[2]. Moser BR, Knothe G, Vaughn SF, Isbell TA, *2009*. Production and evaluation of biodiesel from field pennycress *(Thlaspiarvense L.)* Oil. Energy & Fuels, 23(8), 4149-4155.
[3]. Satyarthi JK, Srinivas D, RatnasamyP, **2009**. Estimation of free fatty acid content in oils, fats, and biodiesel by 1H NMR spectroscopy. Energy & Fuel, 23 (4) , 2273-2277.
[4]. Knothe G. 2010. Biodiesel Derived from a Model Oil Enriched in Palmitoleic Acid, Macadamia Nut Oil. Energy & Fuels, 24 (3): 2098-2103.
[5]. Li E., Rudolph V., 2008. Transesterification of vegetable oil to biodiesel over MgO-functionalized mesoporous catalysts. Energy & fuels, 22 (1), 145-149.
[6]. Diwani G.I.E., S.A.E. Rafei, S.I. Hawash, S.A.E. Enin, 2011. Der Chemical Sinica 2; 12-25..
[7]. Aliyu A., Godwin O., Hamza A., 2011. Biodiesel production from waste soybean oil. Der Chemica Sinica 2; 286-298.
[8]. Rejila S., Vijaya kumar N., Jayakumar M., 2012. Biodiesel production from waste soybean oil. Asian J. Plant Sci. Res., 2: 123-128.
[9]. Zhang Y., Dube M.A., McLean D., Kates M., 2003. Biodiesel production from waste cooking oil: Process design and technological assessment. Bioresource Technol., 89: 1-16.
[10]. Zabeti M., Ashri W.M., Daud W., Aroua M.K., 2010. Biodiesel production using alumina-supported calcium oxide: An optimization study. Fuel Process Technol, 91 (2), 243-248.
[11]. Satyarthi J.K., Srinivas D., Ratnasamy P., 2009. Estimation of Free Fatty Acid Content in Oils, Fats, and Biodiesel by 1H NMR Spectroscopy. Energy & Fuels 23 (4): 2273-2277.
[12]. Knothe G., 2014. Energy Fuels, Cuphea oil as a potential biodiesel feedstock to improve fuel properties. Journal of Energy Engineering, 140(3): A5014001.
[13]. Srivastava A., Prasad R., 2000. Triglycerides-based diesel fuels. Renewable and Sustainable Energy Reviews., 4 (2): 111-133.
[14]. Hussain, H., Mamadalieva, N.Z., Hussain, A., Hassan, U., Rabnawaz, A., Ahmed, I. and Green, I.R., 2022. Fruit peels: Food waste as a valuable source of bioactive natural products for drug discovery. Current Issues in Molecular Biology, 44(5): 1960-1994.
[15]. Aliyu A., Lomasahaka E., Hamaza A., 2012. Production of biodiesel via NaOH catalyzed transesterification of mahogany seed oil. Advances in Applied Science Research., 3 (1): 615-618.
[16]. Aliyu A, Adoyi O and Hamza A, **2012**. Binary blends of petro diesel with biodiesels derived from soyabean and groundnut oils. Adv. Appl. Sci. Res. 3:611-614.
[17]. Zabeti M., Ashri W.M., Daud W., Aroua M.K., 2009. Activity of solid catalysts for biodiesel production: A review. Fuel Process Technology, 90 (6): 770-777.
[18]. S M Haque and B Ghosh. In vitro completion of sexual life cycle : production of R1 plant of *Ipomoea quamoclit*. *Propag. Ornam. Plants*. **2013**, 13, 19 - 24.
[19]. Horwitz, W., 2010. Official methods of analysis of AOAC International. Volume I, agricultural chemicals, contaminants, drugs/edited by William Horwitz. Gaithersburg (Maryland): AOAC International, 1997.
[20]. DC Deka, S Basumatary , Biomass Bioenergy 2011, 25, 1797-1803.