ANALYSIS AND CHARACTERIZATION OF BIODIESEL PRODUCTION FROM *Glycine max* SEED OIL USING ALKALI CATALYST

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ABSTRACT

Due to the increasing energy demand and the pollution problems caused by the use of fossil fuels, the used of vegetable oils and their derivatives as alternative for diesel fuel is the best solution in the current scenario. Nowadays the use of biodiesel as a substitute for conventional diesel engine has been of great interest. This study aim to evaluate the analysis and characterization of the biodiesel production from *Glycine max* based on the yield of the oil extracted from the seed. The percentage oil content of *Glycine max* seed is found to be 17.10 %.The result of this research also signifies the identification of the optimum characterization of the physical and chemical parameters from the extracted oil and biodiesel production by transesterification using alkali catalyst such as pH, moisture content, specific gravity, kinematic viscosity at 40°C, refractive index, acid value, saponification value, iodine value, flash point, Fatty acid methyl ester (FAME) and identification of functional groups. The fatty acid methyl ester analyzed using GCMS contains palmitic acid 15.4, oleic acid 28.9, linoleic acid 54.4, arachic 1.2. The FTIR results also conform that the*, Glycine max* having 1769 cm $^{-1}$ is attributed to carbonyl (**C=O)** of typical esters which ranged 1800-1700 cm $^{-1}$.All the results of the seed oil and the biodiesel were conformed to the standard specified by USA (ASTM D6751) and European organization (EN 14214) and they have met the specified standard recommendations for suitably used in conventional diesel engines.

Keywords: Glycine max, biodiesel, transesterification, alkali, catalyst.

INTRODUCTION

Biodiesel is a renewable and biodegradable source of energy derived from various biological sources which can be used in unmodified diesel engines (Jain and Sharma, 2010). Biodiesel can be mixed in any quantity with mineral diesel to produce a biodiesel blend or can be operates in unblended form. Just like petroleum diesel, biodiesel operates in the compression ignition (diesel) engine, and essentially need very little or no engine alteration because almost their properties are similar with that of hydrocarbon diesel. Biodiesel can be kept just like mineral diesel and hence does not require separate infrastructure. The use of biodiesel in conventional diesel engines results in considerable decrease in the discharge of unburned hydrocarbons, CO, and particulates. Currently there is a huge number of prevailing biodiesel production plants worldwide and also huge numbers are under construction to supply the growing global requirement (Azam *et al*., 2005).

 Glycine max L. known as Soybean also called soja bean or soya bean, and it is an annual legume of the pea family *Fabaceae* which is grown in wide range of soil, with optimum growth in moist alluvial soils with a good organic content. As stated by United States, Department of Agriculture (USDA), that the U.S. was one of the leading manufacturer of *glycine max* oil in the world in 2006-2007. Then it was come after by Argentina, China, Brazil and India in *glycine max* oil production processes. The United State of America USA

produced 34.5 % of total *glycine max* oil in the world (United States Department of Agriculture, 2008). In extension to biodiesel production processes, a *glycine max* is very useful in producing ethanol. *Glycine max* shell contain outstanding amount of carbohydrate for ethanol production and producers prefer to use *Glycine max* shell for animal feeding because it contains high protein content (Mielenz *et al*., 2009). Although, *Glycine max* biodiesel is usually used as a blend with petro-diesel at varying ratios, it can also be utilized to fuel compression ignition engines. The results of engine emission tests showed that use of *Glycine max* biodiesel can produced less emissions of CO, HC, NO_2 and smoke than conventional diesel (Qi *et al*., 2009)**.**

Glycine max oil is a key source of edible oil in the world. With almost about 222 million tonnes, soybean is the most important oil bearing plant grows world-wide and its production is seeing a further increase in size, particularly in the United State of America USA, Brazil and Argentina (Bockey, 2006). *Glycine max* is produced without zero nitrogen. This makes soybeans foremost for the production of biodiesel as nitrogen fertilizer is one of the record energy expensive inputs in crop production. (Pimental and Patzek, 2005) studied the energy estimation for producing soybean biodiesel. They reported that 5546 kg of *Glycine max* were required for producing 1000 kg of oil and biodiesel

manufacture of soybean required 27% more fossil energy than the biodiesel fuel produced.

The main purpose of the study is to conduct an analysis and characterization of biodiesel production from *Glycine max* seed oils.

The work also considered one alternative way of producing biodiesel from vegetable oil with specific emphasis on *Glycine max* oil obtain from its seed. The aimed of this study was to characterize plant seed oil and biodiesel production from *Glycine max* seeds oil, Produce biodiesel by transesterification using alkali catalyst, analyzed the fatty acid composition of the biodiesel esterified from the plants seed oils using Gas Chromatography and Mass Spectrometer (GCMS) and identify the functional group present in the biodiesel using FTIR (Fourier Transform Infrared Spectrophotometer).

MATERIALS AND METHOD

Materials

They are classified as raw- material, chemicals, instruments and equipment.

Glycine max seed, Ethanol BDH Chemical England, Methanol BDH England, petroleum ether JHD China, Sodium hydroxide Titan Biotech LTD India, Potassium Hydroxide Qualikems, Hydrochloric Acid E.Merck Germany, Potassium Iodide Qualichems, Carbon tetra chloride, Sodium thiosulphate , Diethylether ,Round bottom flask Water bath, Table top Bunsen burner, Soxlet extractor, GC-MS and FTIR.

Collection of sample

Matured seeds of *Glycine max* are bought locally in the market. The sample is collected from Damaturu, and it's were identified by the department of Biological Science at Yobe State University. They were decorticated manually. The cleaned seeds were dried in the sun for 24 hours and then dried in an oven at 105 ºC to a constant weight to reduce the moisture content.

Extraction of the oil

The dried seed is ground into smaller particle size distribution by using an electrical grinder. The total oil content of seed was determined by Soxhlet extraction. About 2 kg of *Glycine max* of ground seed placed in an extraction thimble at different time intervals and 250 ml Petroleum ether in 500 ml round bottom flask was refluxed using a Soxhlet extractor. The temperature of the Petroleum ether was maintained at 40 ˚C.

Moisture Content determination

The seed sample was measured (m_1), and oven dried at 105 ºC for 3hrs. After the 3 hrs, the samples were removed from the oven, placed in desiccator for 30 min. to cool, then removed and re-measured (m_2). The percentage moisture in these seeds was calculated (Akpan *et al.*, 2006) as in below:

$$\text{Moisture} = \frac{(m_1 - m_2) \times 100}{M_2} \%$$

M_1 = Original mass of the sample before drying
M_2 = mass of the sample after drying

Calculation of Oil Content (yield)

The percentage of oil was calculated from the Equation below

$$\% \text{ of oil} = \frac{(M_2 - M_1) \times 100}{M}$$

Where, M = mass of sample,
m_1 = mass of the beaker with glass ball,
m_2 = mass of the beaker with glass ball and oil,
$m_2 - m_1$ = mass of oil. (Doan, 2004)

pH Value   determination

Two (2) gram of sample was poured into 25 ml glass beaker and 13 ml of hot distilled water was added to each sample and stirred slowly. The mixtures were then allowed to cool in a cold-water bath to 25 °C. The pH meter was standardized with buffer solution and then inserted into the sample and pH value was measured (Akpan *et al.*, 2006).

Specific Gravity

Empty density bottle of 5 ml capacity was weighed (D_0), filled with oil, and then stopper inserted and reweighed (D_1). The oil was exchanged with water after washing and drying the bottle and weighed (D_2). The expression for specific gravity is:

Specific gravity = $\dfrac{(D_1 - D_o) \times 100}{D_2 - D_o}$ = Mass of the substance / Mass of an equal volume of water
(Akpan *et al.*, 2006).

Refractive Index

A few drops of oil samples were moved to glass slide of the refractometer (ATAGO Co. Ltd. Japan). Through the eyepiece of the refractometer, the dark portion viewed was adjusted to be in line with the intersection of the cross, In this case the pointer on the scale pointed to the refractive index and values were recorded (Akpan *et al.*, 2006).

Kinematic Viscosity

Viscometer of flow time above 200 seconds was elected (Cannon Fenske Opague, Bransted international , Glass capillary viscometer), charged with the sample by inverting the tube's thinner arm into the liquid sample . Suction force drawn up to the upper timing mark of the viscometer, then the instrument was turned to its normal vertical position.

The viscometer was held by metal holder and inserted in a water bath at 40 °C for approximately 10 min. to allow the sample to reach bath temperature. The suction force was then applied to the thinner arm to draw the sample slightly above the upper timing mark. The afflux time by timing the sample free flow from the upper timing mark to the lower timing mark was recorded (Akpan *et al.*, 2006).

Acid Value

25 ml of diethyl ether and 25 ml of ethanol were mixed in a 250 ml beaker, and then added to 10 g of oil contained in a 250 ml conical flask and add few drops of phenolphthalein. The mixture was titrated with 0.1 M NaOH to the end point with regular shaking, a dark pink color appears and the volume of 0.1 M NaOH (V_o) was recorded. Free Fatty Acid

(FFA) was calculated (Akpan *et al*., 2006; Kyari, 2008) as in below:

V_o/W_o X 2.82 X 100 100 ml of 0.1 M NaOH = 2.83 g of oleic acid

W_o = sample weight

Then, acid Value = FFA x 2

Saponification Value

2 g of oil sample was measure into a conical flask and 25 ml of 0.1N ethanolic potassium hydroxide (KOH) was added. The blend was constantly stirred and allowed to boil gently for 60 min. A reflux condenser was placed on the flask containing the mixture. Few drops of phenolphthalein indicator were added to the warm solution and titrated with 0.5 M Hydrochloric acid HCl to the end point until pink color of the indicator is disappeared. The procedure was the same used for other samples and blank. Saponification value was calculated (Akpan *et al*., 2006; Kyari, 2008) as in below:

S.V = 56.1 x $N(V_0-V_1)/M$

V_0 = volume of the solution used for blank test

V_I = volume of the solution used for determination

N = Actual normality of the HCl used

M = Mass of the sample

Indicator method was used as specified by ISO 3657 (1988).

Iodine Value

0.4 g of oil sample was measured into a conical flask and 20 ml of carbon tetra chloride (CCL_4) was added to dissolve the oil. Then 25 ml of Dam's reagent was added to the mixture using a safety pipette in fume chamber. Stopper was inserted and the content of the flask was vigorously swirled. The flask was placed in the dark for 2 and half hrs. Then, 20 ml of 10 % aqueous potassium iodide (KI) and 125 ml of water were added using a measuring cylinder. The solution was titrated with 0.1 M sodium thiosulphate ($Na_2S_2O_3$) solutions until the yellow color almost disappeared. Few drops of 1 % starch solution indicator was added and titration continued by adding thiosulphate drop wise until blue coloration disappeared after vigorous shaking. The same procedure was used for blank test and other samples. The iodine value (I.V) is given by the expression:

I.V = 12.69 x $C(V_1-V_2)/M$

C = Concentration of sodium thiosulphate used

V_1 = Volume of sodium thiosulphate used for blank

V_2 = Volume of sodium thiosulphate used for determination

M = Mass of the sample (Akpan *et al*., 2006; Kyari, 2008)

Flash point

The flash point of biodiesel is measured by flash point tester which consists of 80 ml closed copper cup, heater, and a source that gives continuous sparks. The source that gives continuous sparks consists of a battery connected to small engine, disporater, coil, and spark plug. The engine is used to rotate a disporater, which is used to fractionate the current to electrical pulses. A coil is used to amplify the electrical pulses, and spark plug is used to create sparks inside the cup. Biodiesel sample is heated and the vapor accumulated inside the cup, at the moment that the vapor was sufficient to ignite the flash light noticed, and the temperature measured.

Transesterification procedure, the process involves three steps

1. **Preparing the Methoxide Solution**
   i. Measure 24 ml methanol and place into 250 ml conical flask. Close the lid and tighten securely.
   ii. Measure 2 grams NaOH alkali catalyst and quickly add it into the methanol, minimizing its exposure to air. Recap the flask with the lid and tighten securely.
   iii. With enough time, the catalyst will dissolve in the methanol, with gentle agitation which makes all of the solid catalyst dissolved in the methanol before proceeding

2. **Transesterification**
   i. Measure 100 ml of oil and place in a 250 ml beaker.
   ii. Using hot water bath, heat the oil to 55 °C though Methanol boils at about 64 °C
   iii. Place oil into the 250 ml conical flask labeled with the type and quantity of feedstock, catalyst, and methanol used.
   iv. Quickly add the methoxide solution to the flask of oil. Use caution when re-opening the flask of methoxide. Ensure that the lid is securely tightened.
   v. Shake the flask vigorously for at least 10 minutes.

3. **Settling/separation**
   i. The mixture is allowed to settle overnight for completion reaction.
   ii. The settled reactant mixture would emerge two layers, the higher layer as biodiesel and traces of glycerin etc. and the bottom layer as glycerin and gums.
   iii. The glycerin was eliminated from the biodiesel preparation unit by opening the tap on the bottom of the separating funnel.
   iv. Add 100 ml of hot water at approximately 40° C per liter of biodiesel with consistence shaking and then allowed to settle to separate two layers for nearly 5-6 hrs.
   v. Repeat step above at least three times to remove traces of glycerin and soap from the biodiesel yield. (Penugonda and Venkata, 2012)

Determination of Fatty Acid Composition

This involved analyzing the chemical composition of the biodiesel oil sample of *glycine max*.

Procedure; Transesterification of fatty acids to Fatty Acid Methyl Esters (**FAMES**): The 0.5 g of biodiesel oil sample was refluxed with 5 ml of 0.5 N potassium hydroxide methanolic solutions for 5 min. After the reflux, 10ml hexane was added to it and mixed using vortex mixer, then centrifuged at 3000 rpm for 5 min. After the centrifugation, the resultant solution was subjected to GC MS analysis.

The GC-MS analysis of oil sample was performed using Agilent 7890B GC System, fitted with a 30 m x 250 μm x 0.25 μm Rtx-5MS capillary column; maximum temperature325 °C, coupled to Agilent 5977A MSD. Ultra-high purity helium (99.99%) was used as carrier gas at a constant flow rate of 1.0

mL/min. The injection, transfer line and ion source temperatures were 250 ^{0}C, 230 ^{0}C and 280 °C respectively. The ionizing energy was 70 eV. Electron multiplier voltage was obtained from autotune. The temperature of the oven is programmed from 45 °C (hold for 2 min) to 280 °C at a rate of 10 °C/min and hold for 5 mins.

The sample, 1 μL was injected into injector. All data were obtained by collecting the full-scan mass spectra within the scan range.

The percentage compositions of the constituents were expressed as a percentage by peak area. The identification and characterization of chemical compounds in the samples was based on GC retention time. The mass spectra were computer matched with those of standards available in mass spectrum libraries.

Determination of functional group using FTIR
The FTIR identify the functional group present in the feedstock samples of biodiesel.

It was examined and conducted in Yobe State University chemistry research laboratory using FTIR (Buck Scientific).
Procedure; A drop of liquid of biodiesel sample from *glycine max* is placed or smear onto the ZnSe crystal. The sample was scanned into the machine between 4000 cm^{-}1 to 400 cm^{-}1 in triplicates.

RESULTS AND DISCUSSIONS

RESULTS

Table 1: The physical and chemical properties of oil extracted from *Glycine max* seed.

Parameters	*Glycine max*	ASTM 6751 standard
Oil Yield	17.10±0.56	-
Moisture content	3.33±0.15	-
pH	3.46±0.05	-
Specific Gravity	0.8969±0.0002	0.888
Density	0.9022±0.0001	0.875-0.90
Acid Value	0.84±0.01	0.8 max
Refractive index	1.40±0.02	1.476-1.479

- Not specified; Note: Values are mean ±standard deviation of triplicate determination

DISCUSSIONS

From table 1, the moisture content of *glycine max* is 3.3±0.15 % were in comparable with which reported by Salunke and Desai, (1941) ranged from 3 to 7 %. The oil yield of *glycine max* oil which is 17.1±0.56% is similar to the oil yield reported by Ene-Bong and Carnovale, (1992) observed 18% yields for *glycine max*. it also shows a variation from the report (gunstone,1994)and (langstraat,1976) who reported 18.35 % .The pH oil of *glycine max* 3.41±0.05 were consider to be acidic since it falls from the pH range of 0 – 7 with shows increase acidity. The specific gravity obtain for the oil is 0.8969±0.0002 was in agreement with 0.868 reported by Belewu *et al*., (2010)and Silva *et al*, (2011),and compared with the range specified by ASTM standard of oil fall within the limit 0.888 wt/ml at 25 ^{0}C. The density was 0.9022±0.0001 agree with reports from Silva *et al*, (2011) and also agreed with the specified value reported by ASTM1298 which its limit ranges from 0.875-0.90 gcm^{-3}. The acid value result obtained from this work indicates that the acid value of the edible *glycine max* oils as 0.84±0.01 mgNaOH/g, the values are within the specified ranged reported by Silva *et al*, (2011). This is a remarkable result since the acid value measures the presence of corrosive free fatty acids and oxidation products. The refractive index which is the ratio of the velocity of light in vacuum to the velocity of light in a medium is an indication of the level of saturation of the oil (Oderinde *et al*., 2009). The R.I investigation shows 1.40±0.02 which did not meet the ASTM values limits from 1.476 - 1.479 (ASTM International, 2002). These results could be due to the occurrence of some impurities and other components of the crude oil mixture. The refractive index values were similar to those by (Izuagie *et al*., 2008), for *Cucumeropsis edulis, Colocynthis citrillus and Prunus amygdalus*. The physical and chemical properties of oil extracted from *Glycine max* seed results compared with ASTM standard D6751 are presented in table 1

Table 2: Physicochemical properties result of Methyl ester (biodiesel) produced from *Glycine max seed* compared with ASTM D6751 and EN14214 standard for biodiesel.

|	*Glycine max*	ASTM D6751	EN 14214
pH	6.96±0.01	-	-
Specific Gravity	0.8502±0.0001	0.8544	-
Density	0.8582±0.0002	0.875-0.90	-
Acid Value	0.42±0.02	-	< 0.5
Saponification Value	188.0±1.00	< 200	-
Kinematic value	4.8±0.01	-	3.5-5.0
Iodine value	117.3±0.1	-	Max 120
Flash point	171.0±1.15	-	Min 130
Refractive index	1.44±0.04		

- Not specified; Note: Values are mean ±standard deviation of triplicate determination

From table 2, the Specific gravity of *Glycine max* methyl ester 0.8502±0.0001 is within the specified standard value of ASTM 1298 which limits 0.8544 wt/ml. The density result for the biodiesel of *Glycine max* is 0.8582±0.0002 was in agreement with the specified value of 0.83 to 0.86 g cm $^{-3}$ for biodiesel, and also in agreement with 0.868 reported by (Silva *et al*,2011), (Belewu *et al*, 2010). The density values of the biodiesels obtained is 0.8582±0.0002 which correspond to what report by Silva *et al*, (2011) and the feed stocks meet the density value specified by the ASTM D6751 ranged from 0.82–0.90 g cm^{-3}. Acid value result obtain for *Glycine max* methyl ester is 0.42±0.02 is in agreement with report from Silva *et al*, (2011) and compared with the specified standard values of ASTM D6751 limits less than < 0.8 and EN14214 limits less than < 0.5.The saponification value results obtain from the methyl ester of *Glycine max is* 188±1.00 mg KOH/g. The S.V of the biodiesel is comparable with the result stated for quality oil. S. values is been reported to be inversely related to the average molecular weight of the fatty acids in the oil fractions. Oil portions with saponification values of 200 mg KOH/g and above, had been stated to have low molecular weight fatty acids (Abayeh *et al*., 1998). Refractive Index values of the obtained from *Glycine max* biodiesel range is 1.44±0.04 were in agreement with those reported by Domínguez, (1996).Ullah et al., (2013) also reported that pure biodiesel possesses an RI in the range of 1.45.The kinematic viscosity at 40 ºC of biodiesel result from *Glycine max* is 4.8±0.01 and is in comparable with the standard EN ISO 1304 ranged from 3.5-5.0 mm^2/s, which falls within the limit of biodiesel. But lower compared to 9.60 reported by Belewu *et al*, (2010). The Iodine value From the result of methyl ester obtain is 117.3 ±0.1 gI /100g compared with the standard EN1411 is agreed which has the maximum value of 120 gI/100g.The Flash point of biodiesel result from *Glycine max* is 171±1.15 ºC which are higher than the minimum requirement of 130 ºC specified by ASTM D93 of biodiesel and in agreement with Silva *et al*,. Flash point helps to observe the safe handling and storage of fuel. The more the flash point the safer the fuel and vice versa.

Table 3 The Fatty acid result of *Glycine max* based on relative amount.

Fatty acid	**Chemical name**	**Structure**	***Relative amount***
Palmitic acid	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	15.4
Oleic Acid	9-Octadecenoic acid, methyl ester Z	$C_{18}H_{34}O_2$	28.9
Linoleic	9-12 octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	54.5
Arachic	Eicosanoic acid	$C_{20}H_{40}O_2$	1.2
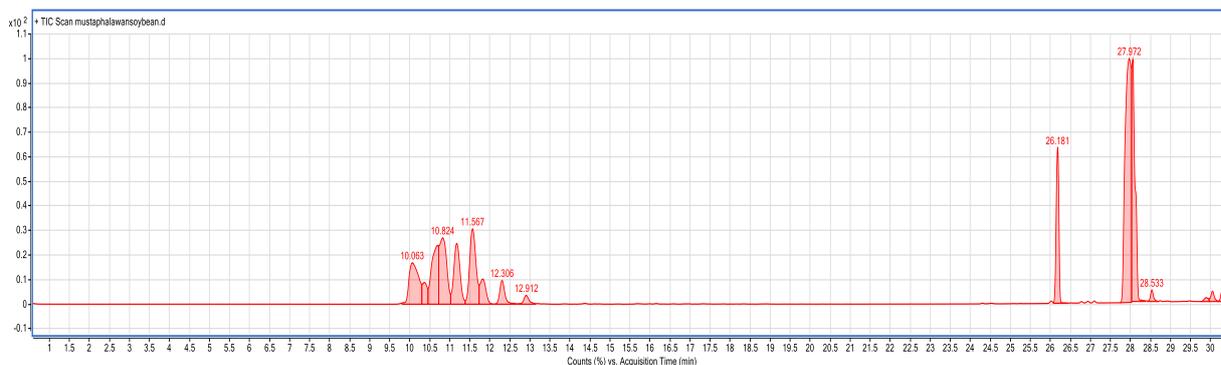
Fig. 1: GCMS spectra on *Glycine max* biodiese

Determination of fatty Acid Composition

From table 3 and figure 1 shows the result and Spectra of *Glycine max* biodiesel which has high proportion of linoleic acid 54.5 % compare with considerable amount oleic, palmitic and Arachic acid very negligible. The capability of biodiesel to come across ASTM D 6751 standard is dependent on the fatty acid composition as presented in table 3.
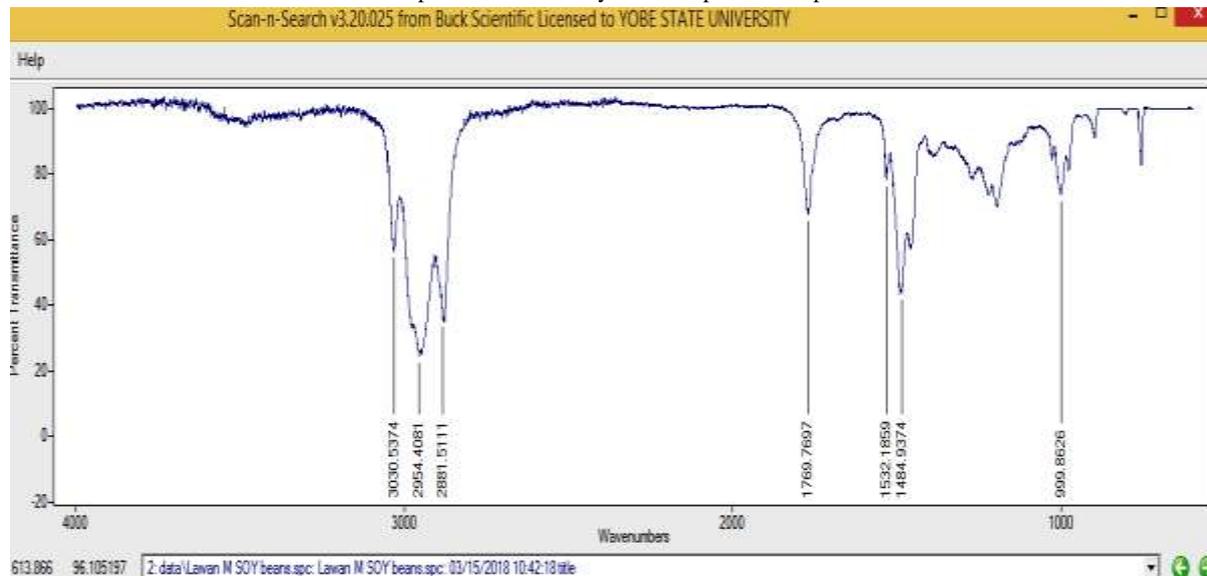

Fig. 2: I.R Spectra of *Glycine max* biodiesel

Determination of Functional Groups using FTIR

From figure 2, In the region from 1800-1700 cm^{-1}, often 1769 cm^{-1} it can be observed peaks that can be attributed to the stretching of C=O, which represent esters functionality, and thus are common in FAME and absent in petro diesel. The main spectrum region that allows for chemical discrimination between *glycine max* oil and its respective FAME is in the range 1500-900 cm^{-1}, known as "fingerprint" region. The peak at 1484 cm^{-1} corresponds to the asymmetric stretching of -CH$_3$ present in the biodiesel spectrum (Soares *et al*., 2008).

CONCLUSION

The *Glycine max* oil content is classified as economically viable for biodiesel production. From the experimental result it could be concluded that, the percentage oil content of *Glycine max* seed was found to be 17.10 %. As such a satisfactory result can be gotten by solvent extraction process by laboratory Soxhlet apparatus. *Glycine max* seed oil and methyl ester (biodiesel) product produced in this research work were examined for pH, moisture content, specific gravity, iodine value, Kinematic Viscosity at 40℃, refractive index, acid value, saponification value, flash point, Fatty acid methyl ester (FAME) composition and identification of their functional groups respectively. Since all the results of the seed oil and the biodiesel product were conformed to the standard specified by USA (ASTM D6751) and European organization (EN 14214) and have met the specified standard recommendation, It can be confirmed that the biodiesel production from *Glycine max* seed oil is suitable for use in conventional diesel engines while the production and effective usage of biodiesel will help to reduce the cost of protecting the atmosphere from the hazards in using fossil diesel and hence will boost the economy of the country.

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