

# STUDIES ON THE PRODUCTION OF METHYL ESTERS FROM FUNGAL DEGRADATION OF SOYBEAN OIL AND MECHANISM OF REACTION

\*<sup>1</sup>B. E. Nyong and <sup>2</sup>M. M. Ekwenchi

<sup>1</sup>Department of Chemical Sciences, Cross River University of Technology, Calabar, Cross River State, Nigeria.

<sup>2</sup>Department of Chemistry, University of Jos, Nigeria

Correspondence: [marknyong@gmail.com](mailto:marknyong@gmail.com)

## ABSTRACT

### Keywords:

Biodiesel;  
Methylesters;  
Soybean oil;  
Fungal degradation;  
Renewable energy;  
Lignocellulose

*The study focused on producing methylesters from fungal degradation of soybean oil using lignocellulose biomass as surface. The methylesters can be used as biodiesel, an alternative energy source. The fungal degradation of Soybean oil by cellulolytic fungus (yeast) using banana leaves as a surface to produce methylester was carried out at optimum operational conditions of concentration and temperature. Bioliquid extraction from the degradation products was carried out for the soybean oil by soxhlet extraction, followed by the separation of the bioliquid into soluble and insoluble components by precipitation. The soluble components of bioliquid was separated into five fractions by column chromatographic method. Urea and thiourea technique was carried out on the first fraction to recover a high yield of the degradation product. GC-MS analysis was then carried out on the urea and thiourea adducts and four methyl esters were confirmed. They were; hexadecanoic acid methylester; 9, 12-octadecadienoic acid methylester; 9-octadecenoic acid methylester and octadecanoic acid methylester. The result of the research shows that it is possible to produce methylesters from soybean oil by fungal degradation using banana leaves as surface. The proposed mechanism was in line with the pathways through which the products were formed.*

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## INTRODUCTION

Soybean oil is a triglyceride extracted from plant. Such oils have been part of human culture for millennia. Although many plant parts may yield oil, in commercial practice,

oil is extracted primarily from seeds.

Soybean, like any other vegetable oil has been identified as having a lot of potentials as alternative diesel engine fuels. This is supported by an interest in a cleaner environment, as well as the increasing cost of mineral deposit

based energy. (Dun and Knothe, 2001).Based on the availability to meet demand for renewable energy, soybean, peanut and sunflower oils have been identified as the most promising fuel sources and when used as fuel, the term "biodiesel is applicable. Biodiesel refers to a vegetable oil or animal fat based diesel fuel consisting of long-chain alkyl (methyl, ethyl or propyl) esters. Biodiesel is typically made by chemically reacting lipids with alcohol to produce fatty acid esters. The oil extracted from soybeans can also be used in margarine, solvent and paint production (Israel, Sunday, Mansong, and Ubong, 2016).

Methyl esters can be produced on a large scale by transesterification process. Transesterification is the reaction of a triglyceride with an alcohol in the presence of a strong acid or base as catalyst to produce a mixture of fatty acids alkylesters and glycerol. The reaction can also be accomplished with the help of enzymes (biocatalysts) particularly lipases (Freedman and Pryde, 1982).

The overall process is a sequence of three consecutive and

reverse reactions in which di and monoglycerides are formed as intermediates. The stoichiometric reaction requires 1 mole of a triglyceride and 3 mole of alcohol. However, an excess of alcohol is used to increase the yield of the alkylesters and to allow its `phase separation from the glycerol formed. Several aspects, including the type of catalyst (alkaline or acid), alcohol/vegetable oil molar ratio, temperature, parity of the reactants (mainly water content) and free fatty acid contents have an influence on the course of the transesterification.

The applicability of transesterification is not restricted to the production of Biodiesel. Several relevant industrial processes use this reaction to produce different types of compounds. An example is the production of PET (polyethylene terephthalate), which involves a step where dimethyl - terephthalate is trans-esterified with ethylene glycol in the presence of zinc acetate as catalyst. Furthermore, a large number of acrylic acid derivatives is produced by transesterification of

methyl acrylate with different alcohols, in the presence of acid catalysts (Drauz, Waldmann and Sauerbrei, 1996).

Methyl esters produced by transesterification of vegetable oil in the presence of an acid/base as catalyst are extensively used not only as biodiesel but also as intermediates in the manufacture of detergents, emulsifiers, wetting agents, stabilizers, textile treatment and waxes among other applications. Methyl esters are also used in a variety of direct and indirect food additive applications including the dehydration of grapes to produce raisins, synthetic flavouring agents and in metal lubricants for metallic articles intended for food contact use. Methyl esters also find uses in medicine (Shapiro, 1968).

Biodiesel consists of methyl or ethyl esters derived from vegetable oil, animal fat, waste oil, and microalgae oil through the process of "trans-esterification". In the early literature, there are several terminologies for these ester-forming reactions, namely: alcoholysis, acidolysis and ester interchange; but recently, it is more common to use

the term "trans-esterification" to describe the ester reaction, which when carried out with an alcohol in the presence of an acid or base catalyst is known as alcoholysis. Depending on the specific alcohol used, alcoholysis is referred to as methanolysis, ethanolysis, propanolysis, and butanolysis, etc. (Leung *et al.*, 2010). Chemically, biodiesel is a mixture of methyl esters of long chain fatty acids and is formed from vegetable oils, animal fats or waste oils and fats through transesterification in the presence of a catalyst (Ma and Hanna, 1999).

The greatest challenge of this research is designing an efficient and cheaper method of producing methyl esters; a method which can replace the more costly method of transesterification using sodium hydroxide and methanol in the presence of acid/base as catalyst. This research therefore seeks to synthesize methylesters from soybean oil by fungal degradation using lignocellulose from banana leaves as surface. The method employed in this study to produce the methylesters, is expected to replace the high cost of producing the

methylesters by the conventional method of transesterification using alcohol and sodium hydroxide in the presence of acid/base as catalyst.

## **2.0 MATERIALS AND METHOD**

The materials used for the research are Banana leaves, Angel yeast (a fungus), soybean-oil, silica gel, and alumina. The chemicals used were of analytical grade.

### **2.1 Sample collection and preparation**

The Lignocellulose was extracted from banana leaves and the soybean oil purchased from Grand Cereal Plc, Jos. The banana leaves were collected from a group of banana trees planted by Akpabuyo Local Government Council banana plantation in Cross River State. The leaves were fresh and matured at the time of collection. The leaves were first sun-dried for one week and later oven-dried at 37°C for four hours and ground using a pestle and mortar, and then sieved to fine particles.

### **2.2 Experimental procedures**

The experiment was carried out in the following stages:

#### **i. The cleaning of the lignocellulose of banana leaves by fungal degradation and soxhlet extraction:**

About 40g of the processed lignocellulose was weighed and mixed with 250ml of distilled water containing 1.6g of yeast (fungus) in a flat bottom flask. The mixture was kept for 21days for fungal degradation to take place, after which the mixture was soxhlet extracted using absolute methanol. The residual obtained (cleaned lignocelluloses) was dried in a fume cupboard for 48hours and then stored in a stoppered bottle, and was later used as surface for fungal degradation of the soybean oil. The cleaning process of the lignocellulose was repeated by fungal degrading 4g of the cleaned lignocellulose for 21 days using 0.16g of the fungus and 2ml of distilled water followed by soxhlet extraction.

#### **ii. Fungal degradation of soybean oil on the cleaned lignocellulose of banana leaves:**

About 3.6g of the lignocellulose residue obtained from stage 1 above was placed in a flat bottomed flask. About 0.4g of

the Soybean oil was weighed and added to the lignocellulose residue to give 4g of substrate. Accurately 40ml of n-hexane was added to the lignocellulose-oil mixture to help spread the oil evenly on the surface of the lignocellulose residue where the fungus will attack and degrade the oil. The hexane was later evaporated off in a fume cupboard. About 0.16g of fungus was weighed and dissolved in 25ml distilled water and the solution was added to the lignocellulose – oil mixture and the mixture was fungal degraded for 21 days. Followed by soxhlet extraction using absolute methanol to obtain the bioliquid.

**iii. Separation of the insoluble components of the bioliquid from soybean oil by precipitation:**

About 1.0g of the concentrated bioliquid from the fermented slurry was dissolved in a mixture of 1cm<sup>3</sup> methanol and 40cm<sup>3</sup> n-hexane in a 250cm<sup>3</sup> beaker and kept in a refrigerator for 24 hours. At the end of 24 hours, a separating funnel was used to separate the insoluble

component from the soluble component.

**iv.** The soluble component of the bioliquid from the lignocellulose and soybean oil were separated into five fractions by column chromatographic technique.

**v.** For further analysis of the degradation products, the first fraction obtained from the soluble component of bioliquid from soybean oil by column chromatographic technique was subjected to urea and thiourea adduction technique in order to recover a high yield of the degradation products. The urea and thiourea adducts were then subjected to GC-MS analysis.

### **3.0 RESULTS AND DISCUSSION**

#### **3.1 Soxhlet extraction result of products from fungal degradation of soybean oil**

The Soxhlet extraction results from fungal degradation of soybean oil are presented in Table 1. The result shows the amount of bioliquid generated from soybean oil

degradation. The result also shows the amount of soluble and insoluble component of the bioliquid.

**Table 1: Soxhlet Extraction of Products from Fungal Degradation of Banana Leaves and Soybean Oil**

<b>Parameter (g)</b>	<b>Soybean oil (g)</b>
Amount of Bioliquid produced	3.48
Amount of Soluble Component of Bioliquid Produced	2.75
Amount of Insoluble Component of Bioliquid Produced	0.73

Results from Table 1 shows the amount of bioliquid produced from the fungal degradation of soybean oil. The result also shows the amount soluble and insoluble component of the bioliquid.

**3.2 Column chromatographic separation products of the soluble components of bioliquid from soybean oil.**

The column chromatographic separation results of the soluble components of bioliquid from soybean oil are presented in Table 2.

The result shows the five fractions obtained from the soluble components of bioliquid from soybean oil. The result also shows that fraction 1, (n-hexane separated), had the highest amount of degradation product followed by fractions 2, 3, 4 and 5 in decreasing order. The high amount of fraction 1 indicates that most of the degradation products was present in the n-hexane separated.

**Table 2: Column Chromatographic Separation Products of the Soluble Component of Bioliquid from soybean oil**

<b>Separation products</b>	<b>Weight (g)</b>
Fraction 1	0.1566
Fraction 2	0.0619
Fraction 3	0.0586
Fraction 4	0.0462
Fraction 5	0.0255

**Key:**

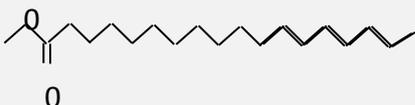
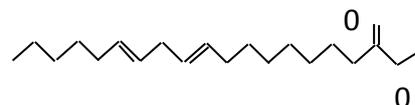
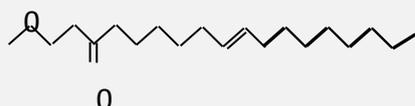
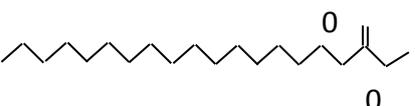
- Fraction 1 = n-hexane separated fraction
- Fraction 2 = 5% benzene/hexane separated fraction
- Fraction 3 = 15% benzene/hexane separated fraction
- Fraction 4 = 20% benzene, 20% diethyl ether, 60% methanol fraction
- Fraction 5 = methanol separated fraction

### 3.3 Product of Analysis of Soybean Oil to Esters

The results of the GC.MS analysis of the urea adduct (degradation products of soybean oil on banana leave surface) are presented in table 3. The GC-MS analysis confirmed the presence of four methyl esters which appeared as

four peaks on the chromatogram (figure 2) namely peaks 5, 6, 7 and 8 representing hexadecanoic acid, methylester, 9, 12-octadecadienoic acid, methyl ester, 9- octadecenoic acid, methylester and octadecanoic acid methylester.

**Table 3: Product of Analysis of Soybean Oil to Esters**

RETENTION TIME (MIN)	MOLECULAR WEIGHT	MOLECULAR FOMULAR	STRUCTURE	NAME
21.342	270	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>		Hexadecanoic acid Methyl ester
23.435	294	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>		9, 12- Octadecadienoic acid, Methyl ester
23.475	292	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>		9- Octadecenoic acid, Methyl ester
23.708	298	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>		Octadecanoic acid. Methyl ester

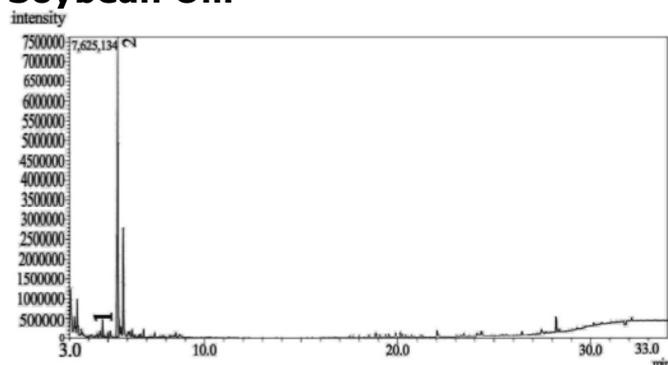
### 3.4 Chromatogram of banana leaves surface used for fungal degradation of soybean oil

The chromatogram of banana leaves surface used for fungal degradation of soybean oil is presented in figure 1. The absence of peaks on the chromatogram indicates that the

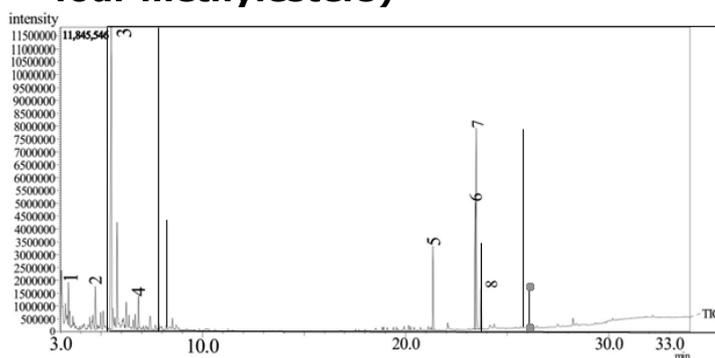
surface was thoroughly cleaned, that is, the oil of the lignocellulose from the Banana leaves and other degradable products were removed during degradation to ensure a cleaned surface. The essence of cleaning the surface of the banana leaves before

using it for degradation of the soybean oil was to ensure that the degradation products from the banana leaves does not interfere with that from the soybean oil. This guarantees that the degradation products from the soybean oil will be solely from the soybean oil and not, contaminated with the degradation products from banana leaves surface.

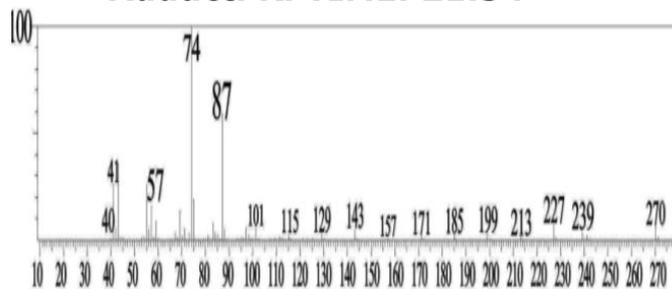
**Figure 1: Chromatogram of the Cleaned Banana Leaves Surface Used for Fungal Degradation of Soybean Oil.**



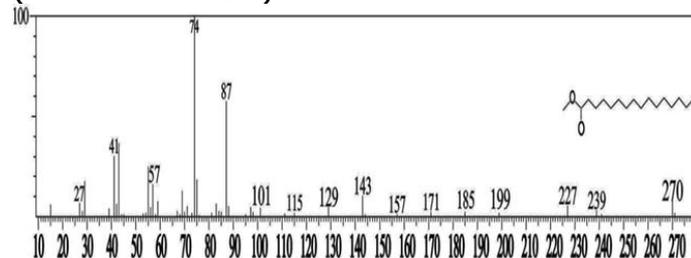
**Figure 2: Chromatogram of product from urea adduct (the four methylesters)**



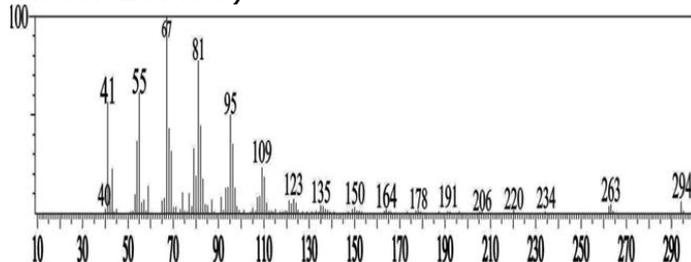
**Fig. 3a: Mass Spectra of Unknown Compound from Urea Adduct. R. TIME: 21.34**



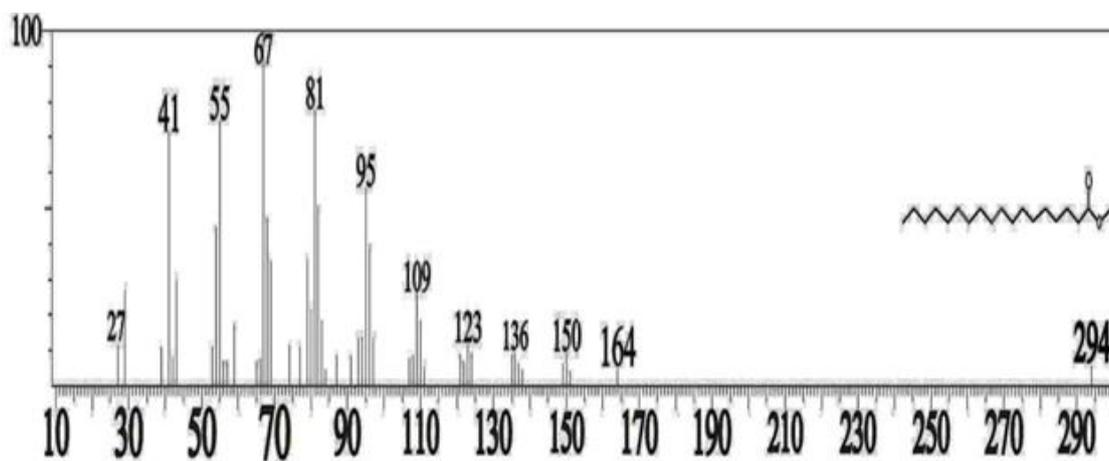
**Fig. 3b: MASS SPECTRA OF KNOWN COMPOUND FROM NIST LIBRARY (STANDARD)-Mass Spectra of Hexadecanoic Acid, Methylester (R.TIME: 21.34)**



**Fig. 4a: MASS SPECTRA OF UNKNOWN COMPOUND FROM UREA ADDUCT- Mass Spectra of Peak 6 from Chromatogram of Urea Adduct. (R. Time: 23.435)**

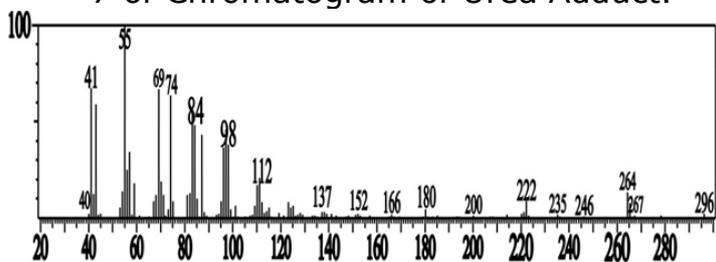


**Fig. 4b: MASS SPECTRA OF KNOWN COMPOUND FROM NIST LIBRARY (STANDARD) (R. Time: 23.435)- Mass Spectra of 9,12-Octadecadienoic Acid, Methylester**

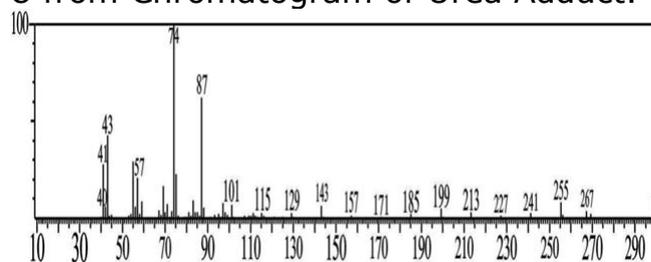


**Fig. 5a:** MASS SPECTRA OF UNKNOWN COMPOUND FROM UREA ADDUCT (R. TIME: 23.475) - Mass Spectra of Peak 7 of Chromatogram of Urea Adduct.

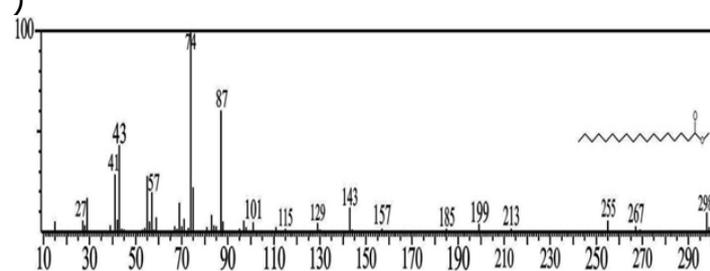
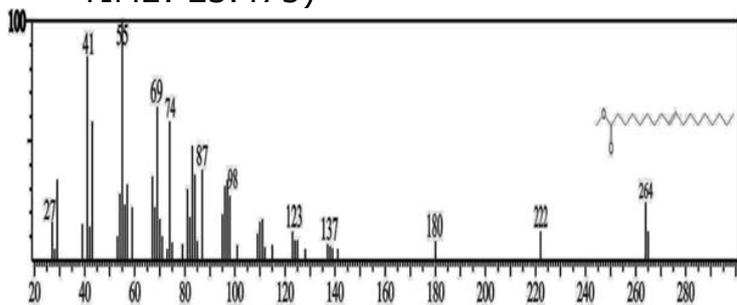
**Fig. 6a:** MASS SPECTRA OF UNKNOWN COMPOUND FROM UREA ADDUCT (R. TIME: 23.708) - Mass Spectra of Peak 8 from Chromatogram of Urea Adduct.



**Fig 5b:** MASS SPECTRA OF KNOWN COMPOUND FROM NIST LIBRARY (STANDARD)-Mass Spectra of 9-Octadecenoic Acid, Methyl ester (R. TIME: 23.475)



**Fig. 6b:** MASS SPECTRA OF KNOWN COMPOUND FROM NIST LIBRARY (STANDARD) - Mass Spectra of Octadecanoic Acid, Methyl ester (R. TIME: 23.708 )



### 3.5 Proposed mechanism for the fungal degradation of the soybean oil on banana leaves surface.

There are three possibilities for the mechanism: -

- (i) By Alkane formation pathway
- (ii) By Acid formation pathway
- (iii) By Methyl ester formation pathway

to release the alkyl radical and liberate the carbon dioxide (decarboxylation).

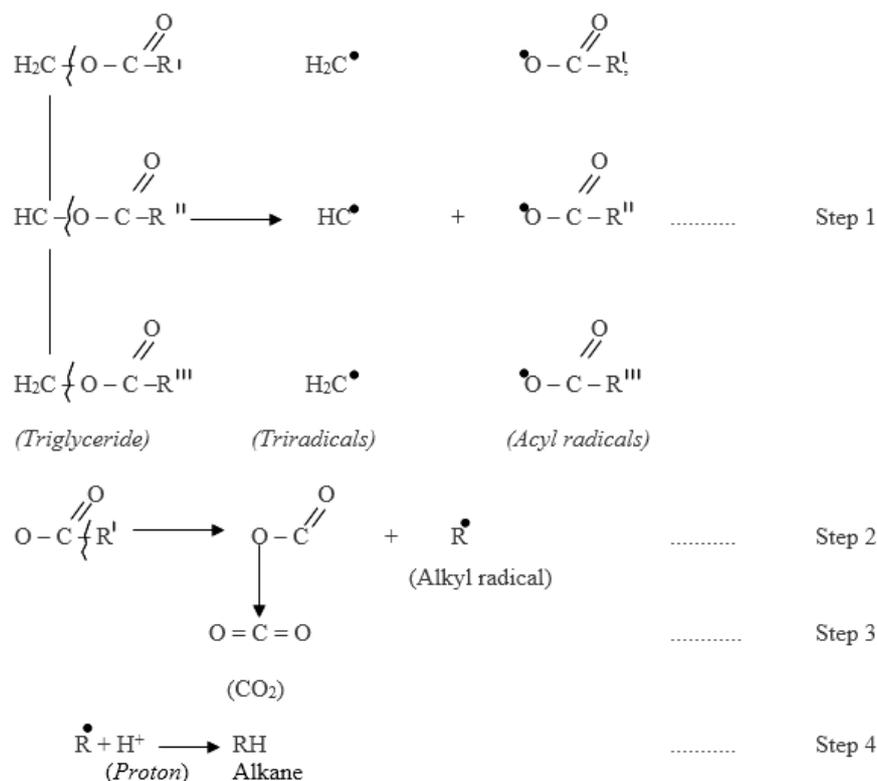
The alkyl radical was expected to pick proton from the system to form

#### i. Alkane Formation pathway

Based on the structure of the oil (Triglyceride), it is anticipated that the enzyme of the fungus would degrade the oil and effect cleavage at the (C-O) bond thereby release the triradicals and Acyl radicals as reactive intermediates. The Acyl radical finally undergoes cleavage at the (C-R) bond

alkanes, but since the result of the GC-MS analysis did not confirm the presence of alkane, the alkane formation route was discarded.

#### Scheme 1: Mechanism of Alkane Formation Pathway



## ii. Acid Formation Pathway

It was envisaged that there is probably a competition between the protons and methyl radicals in the system for combination with the acyl radicals to form the products. Whichever species dominated depended on the concentration in the system, that is, the species present in higher concentration would dominate and combine with the Acyl radicals to form the products.

If the proton were in higher concentration, then the following reaction would have taken place and an acid would have formed.

## Scheme 2: Mechanism of Acid Formation Pathway

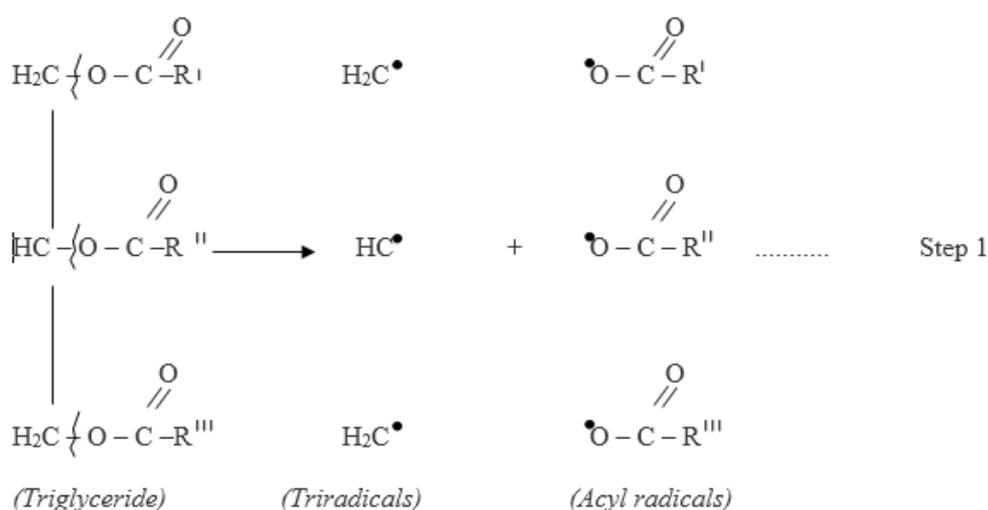


But since the GC-MS analysis did not confirm the presence of acid, the acid formation route of the mechanism was discarded.

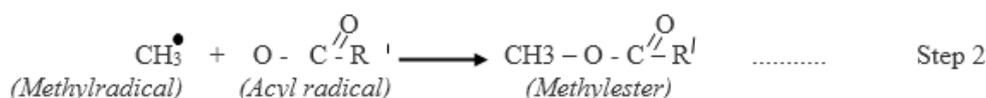
## iii. Methyl ester Formation Pathway

Since the result of the GCMS analysis confirmed the presence of methyl esters, it implied that the concentration of methyl radicals was higher than that of the protons in the system. The methyl radicals combined with the Acyl radicals to form the methyl esters.

## Scheme 3: Mechanism of Methyl ester Formation Pathway



The Acyl radicals picked up methyl radicals ( $^\bullet\text{CH}_3$ ) which were present in high concentration in the system to form the methylesters



**Note:** The slow step of the mechanism of the reaction is step one. In this step, energy is needed to break the (C-O) bond of the triglyceride and this is slow. The slow step is also the rate determining step. Other steps in the mechanism are fast.

## CONCLUSION

The limited reserve of fossil fuels and problems of environmental pollution has prompted many researchers to look for alternative fuels which can be produced from renewable feedstock. This research was therefore designed to generate methylesters from soybean oil by fungal degradation. The methylesters can be used as biodiesel, an alternative energy source.

The fungal degradation of soybean oil on banana leaves as surface was successfully carried out at optimum operational conditions of concentration and temperature. The percentage of bio liquid generated from 0.4g of soybean oil by fungal degradation was evaluated to be 50.33%. The fungal degradation of soybean oil on banana leaves surface was successfully carried out. On the

basis of the aim and objectives of the research, the goal was met, that is, methyl esters were produced and GC-MS analysis results clearly identified the methyl esters. The path ways through which the enzyme of the fungus degraded the oil to produce the methyl esters were confirmed by the proposed mechanisms.

The method employed in this study to produce the methyl esters, which is fungal degradation of soybean oil on banana leaves as surface, can replace the high cost of producing methylesters by transesterification using alcohol and sodium hydroxide in the presence of acid/base as catalyst.

## REFERENCES

- Amamatu, D.T. (1995). Physico-chemical studies of biogas production, Pp 4-15.
- Dunn, R. O. and Knothe. G. (2001). Alternative diesel fuels from vegetable oils and animal fats. *Journal of Oleo Science*, 50,415-426.
- Dangoggo, S. M. and Fernando, C.E. (1986). A simple biogas plant with additional gas storage system. *Nigerian Journal of solar energy*, 5 (2), 133-141.

Drauz, K., Waldmann, H., and Saverbrei, B. (1996). Applied Homogeneous catalysis with organometallic compounds. P. 769.

Freedman, B. and Pyrd, E. H. (1982). Vegetable oil fuels – proceedings of the international conference on plant and vegetable oils as fuels, 31(5), 466-487.

Israel, A. U., Sunday, E. A., Mansong, S. and Ubong, U. V. (2016). Agro-based Raw Materials and the Chemical Industries in Nigeria. *Journal of Industrial Technology*, 1(2): 1-15.

Leung, D.Y.C., Wu, X. and Leung, M.K.H. (2010). A review on biodiesel production using catalysed transesterification, *Applied Energy*, 87, 1083-1095.

Ma, F. and Hanna, M. (1999). Biodiesel production: a review. *Bioresource Technology*, 70;1-15.

Shapiro, S. H. (1968). Fatty acids and their industrial applications, 15 (3): 77-154.