Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.3, No.5, 2013

EXTRACTION and BIODEGRADATION OF BAOBAB (*Adansonia digitata*) SEEDS OIL BY FUNGI (*Aspergillus niger*)

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Abstract

Baobab (*Adansonia digitata*) seed oil was extracted using the soxhlet extractor in n-hexane at 60^{o}C and some of its physical parameters determined. The oil was biodegraded using a Fungi *(Aspergillus niger)* between 25-30^{o}C for 49 days. The pH and viscosity of the oil were measured as incubation period progressed. The result revealed a gradual decrease in pH but an increase in viscosity of the oil as the incubation time increases. It was opined that the decrease in pH could be due to the secretion of enzymes, and the subsequent breaking down of the substrates into smaller acidic molecules whereas, the increase in Viscosity could have been due to the assimilation of these molecules by the microorganisms which may have led to their growth and or increase in the population and the formation of long chain metabolites. The Fourier Transform Infrared determined showed shifts in the absorption bands of the functional groups such as carbonyl, hydroxyl, carboxylic and amide of the biodegraded oil. The GC - MS analysis showed the esterification of unsaturated carboxylic acid to 9-octadecanoic acid and the formation of 2-hydroxy-1, 3-propanediyl ester by the *Aspergillus niger*. Therefore, it was concluded that biodegradation of *Adansonia digitata* has taken place in 35 days.

Keywords: Biodegradation**,** Seed oil, *Aspergillus niger*, Viscosity, Baobab (*Adansonia digitata*)

1. Introduction

The Baobab (*Adansonia digitata*) tree has been subjected to intensive scientific studies considering its agricultural, industrial and medical importance. Virtually, all parts of the plant have or were being studied. The seed has been reported to be eaten raw or roasted (Ezeagu, 2005), having a nutty flavor and this can be a substitute for Coffee and Ground nut seeds.

Fungi infection of oily seeds has been reported to deteriorate oils extracted from seeds (Adekunle and Ume, 1996). Properties such as the free fatty acid content peroxide and saponification values, as well as, oil quantity are known to be reduced by microbial infections (Al-Nasrawi, 2012). Such microorganisms use the oil as sources of carbon and energy for metabolism (Adekunle and Adebambo, 2007). The effect of oil on microbial population depends upon the chemical composition of the oil, the nature of the species and sometimes the population of microorganisms.

The degradative end product of any oil is distinct depending on the degradation pathways. The ability of *Aspergillus* species on crude oil, diesel, spent engine oil, kerosene and many oil extract have been reported (Adebambo, 2007). Although, microorganisms used in biodegradation process are ordinarily required to be isolated from related sources however, isolation could also be done from unrelated environment but with the same result (Ojo, 2005).

The study is aimed at investigating and documenting the capability of *Aspergillus niger* isolates to degrade Baobab (*Adansonia digitata*) seed oil extract.

2. Materials and Methods

Baobab (*Adansonia digitata*) seeds were collected from Kaltungo of Gombe State, Nigeria (Plate 1). Seeds were soaked in water with detergent and washed thoroughly in overflowing tap water (Plate 2). The clean seeds were dried in laboratory at a room temperature of 30^{o}C for 48 hrs (Plate 3). The dried seeds were crushed in a crucible to smaller sizes and pulverized using a grinding machine for easy extraction of the oils.

The oil was extracted according to method reported by Kyari (2008). 300ml of n-hexane was poured into a round bottom flask; 100g of the sample was placed in the filter paper thimble and was inserted in the centre of the extractor. The soxhlet was heated at 60-65℃. When the solvent was boiling, the vapor rose through the vertical tube into the condenser at the top. The liquid condensate dripped into the filter paper thimble in the centre which contains the solid sample to be extracted. The extract seeped through the pores of the thimble and filled the siphon tube, flowing back down into the round bottom flask. This was allowed to continue for 3-4 hours. At the end of the extraction the condenser was removed. The flask containing the resulting mixture was connected to Liebig condenser and heated up to 65-70℃ during which the n-hexane evaporated off and was recovered in a conical flask; the oil extracted weight was taken. This procedure was repeated all through the work to obtain the required quantity of the oil for further analysis.

2.1 Isolation of Fungi

Colony of microbes from bread (substrate) was dissolved in 100 ml of sterile distilled H_2O and sealed in flask with a tape then incubated at 30℃ for 48 hours. A few drops of the culture were added unto a plate containing solidified PDA by the Pour Plate Technique. Drops of lactic acid were added to inhibit the growth of bacteria (Bonnet et. al; 2002) and the sealed plate incubated at 30℃ for 6 days. Without inversion, the colony of the visible organisms were picked and purified by sub-culturing into freshly prepared agar plates using the Streak Plate Technique. The pure fungi isolates was transferred into PDA slant as stock cultures and stored in a refrigerator.

2.2 Biodegradation Test

The biodegradation of Baobab seed oil was carried out according to the ASTM: D 5864: Standard practice for the evaluation of the action microorganism in oil.

2.3 Viscosity Measurement

The viscosity of the oil was measured using the Ubehlob Viscometer at 30^{O}C. Measurement of the viscosity of the oil with microbes was taken at interval of 7 days for six weeks whereas, the viscosity of oils without the microbes was taken once to serve as the control.

2.4 Fourier Transform-Infra Red (FTIR) Measurement

The FTIR analysis of oils was done at the National Research Institute of Chemical Technology (NARICT), Zaria-Nigeria laboratory using the Shimadzu model 8400S FTIR. The FTIR of the biodegraded oil was measured at interval of two weeks of incubation to ascertain when biodegradation began and possibly ended. The FTIR of oil without microbes was also taken which serve as the control.

2.5 GC-MS Analysis

The GC-MS analysis was done at NARICT, Zaria-Nigeria laboratory using GC-MS QP2010 PLUS Shimadzu, Japan. The Column Oven Temperature 70.0^{o}C Hold Time 2.0 min, Injector Temperature 250.0^{o}C, Pressure 116kPa, Total Flow 40.8mL/min, Linear Velocity 49.2cm/sec, Column Flow 1.80mL/min, Purge Flow 3.0 Ml/min and Carrier Gas was off.

3. Results and Discussions

Some physico - chemical parameters of Baobab seeds oil extract were determined (Table 1). The percentage oils yield of the *Adansonia digitata* seeds are in the range of 22- 25 cm^3/100g. This could be considered as a reasonable yield, though, higher percentage oil yield have been reported for several vegetable seeds oil such as Cotton, Rapeseed (Lobos-Moysa, et.al; (2009), Ground nut, Sesame (*Sesamum indicum* L.) (Nzikuo, et. al. 2010), etc, whereas, soybean contains 20% oil (Narine and Kong, 2005). The lower percent oil yield could be

due to the source (Kaltungo) of the seed since the storage and recovery method has not be shown to significantly affects the oil content (Thobunluepop, 2009; 1984).

The free fatty acid value is 4.22%, which is lower than that obtained by Bahruddin (2008) suggesting low level of free fatty acid in the oil. This could be responsible for the non degradation of components shown by peaks 1, 3 and 6 in the GC-MS analysis result. The microbes were noticed to have acted on the unsaturated fatty acids to form 9-Octadecanoic acid and 2-hydroxy-1, 3-propanediyl ester.

Table 1: Some physical and chemical parameters of Baobab (*Adansonia digitata*) seed oil

Parameter	Crude Oil Extract
Total Weight of Powder Seed (g)	1100
Total Weight of the Oil Extract (ml)	255.64
Oil Content (cm^3/100g)	22-24
Colour and Odour	Yellow and Pleasant Odour
Density	0.88
Viscosity (g/cm^3)	41.75
FFA (%)	4.22
Temperature (^oC)	35-40

3.1 Biodegradation of the seeds oil

The biodegradation process of the oil was monitored by the measurement of viscosity. A gradual increase in the viscosity of the oil was observed as the incubation time increases (Fig. 1) in the first four weeks.

Plate 1: The Baobab Pulps

Plate 2: The Horizontal Section of Baobab Pulps

Plate 3: The Dry Baobab Seeds

This could be as a result of the growth of the microorganisms as they act on the substrate (oil) possibly by the secretion of enzymes to facilitate the breaking down process (Aluyor, et. al; 2009). Biodegradation is expected to have been initiated by the enzymatic hydrolysis of triglycerides to glycerol and long chain fatty acids (LCFAs), which serve as the growth substrates for *Aspergillus niger* (Zhengkai, et.al; 2005) Some of the metabolites released during the breaking down of the oil are acidic. This may explain the gradual decrease in pH (Table 2) of the oil as incubation time increases. Furthermore, some physical properties of the oil such as colour (yellow to whitish), moisture, regeneration, were observed to change to more viscous substance. The growth of *Aspergillus niger* on oil has been observed to decrease the colour intensity of oil mill waste, possibly due to the degradation of some phenolic compounds and adsorption of the polyphenols and tannins on the fungal mycelium (Hamdi, et. al; 1990).

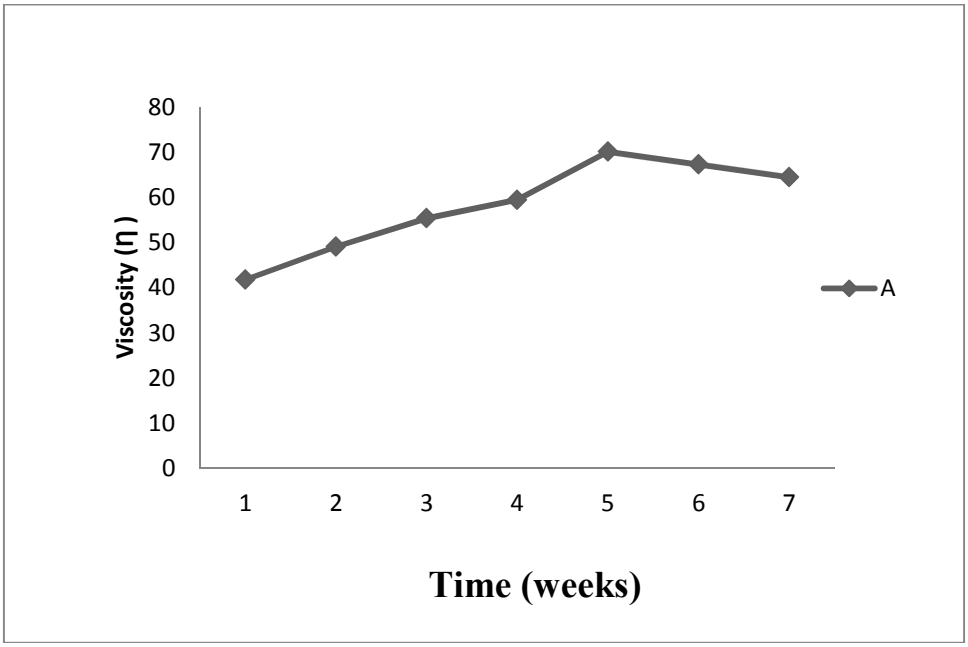


Fig 1: A Plot of Viscosity of Baobab Seed Oil (A) against

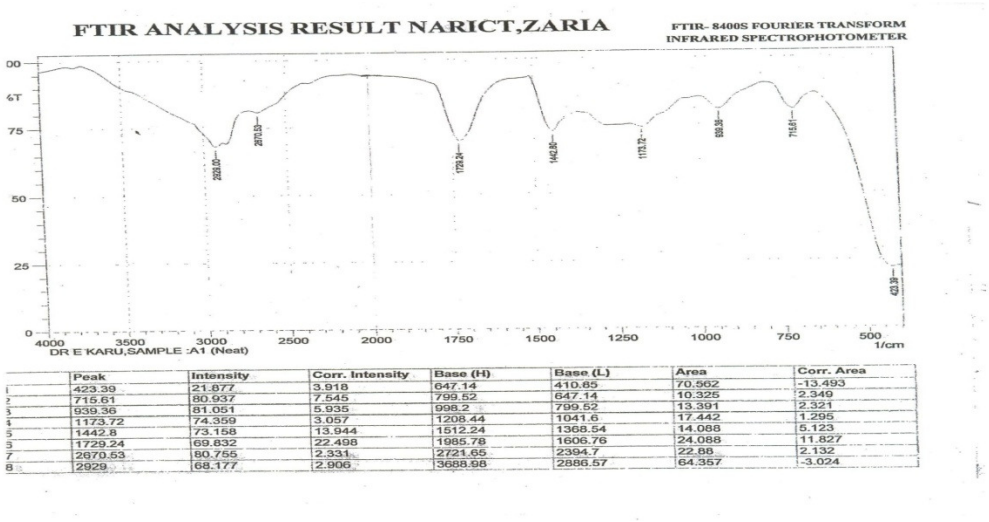
Time of Incubation.

Important metabolic intermediates of vegetable oil include among others acetic and oleic acids (Aluyor et. al; 2009) and biodegrade in hours or few weeks of incubation. The *Aspergillus* sp has been reported to degrade *osinolate sinigrin* in liquid culture associated with the expression of intracellular myrosinase (Sakorn et al. 1999, 2002). The maximum curve obtained in week 5 (fig.1) could be due to the combined effects of the growth of the

microorganisms, the productions of secondary metabolites and recombination of radical intermediates ((Joseph, 1977) to form long chain products at a favourably pH >6 (slightly acidic). However, the slight decrease in viscosity beyond the 5th week of incubation may be due to the termination of reactive intermediates by the acid which could prevent any formation of long chain products leading to a highly viscous liquid.

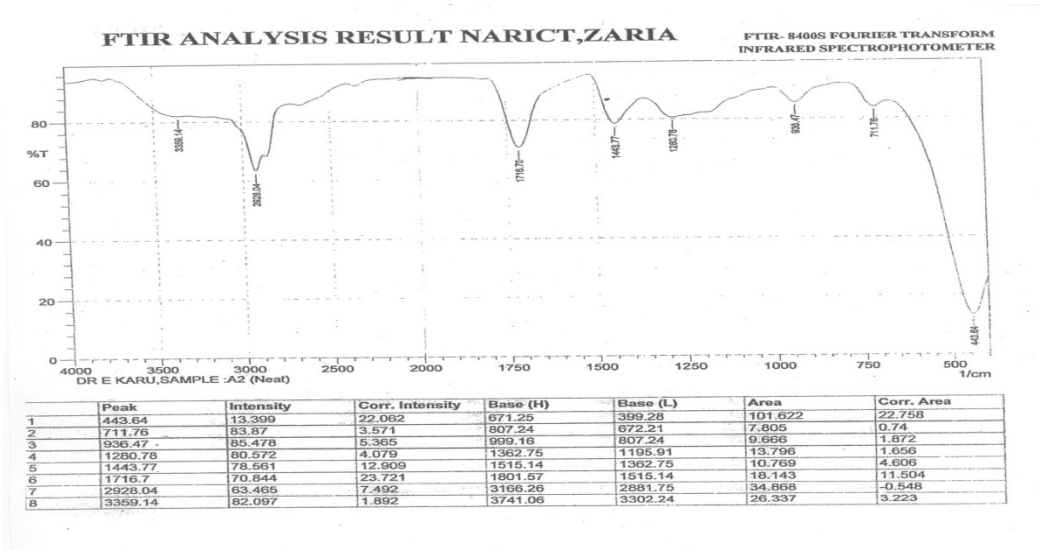
Table 2: Determination of the pH of oil inoculated with Fungi (*Aspergillus niger*)

Incubation Time (days)	pH of oil inoculated with Fungi
0	7.04
7	6.45
14	6.34
21	6.09
28	5.92
35	5.89
42	5.91



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	423.39	21.877	3.918	647.14	410.65	70.562	-13.493
2	715.61	80.937	7.545	799.52	647.14	10.325	2.349
3	939.36	81.051	5.935	998.2	799.52	13.391	2.321
4	1173.72	74.359	3.057	1208.44	1041.6	17.442	1.295
5	1442.8	73.158	13.944	1512.24	1368.54	14.088	5.123
6	1729.24	69.832	22.498	1985.78	1606.76	24.088	11.827
7	2670.53	80.755	2.331	2721.65	2394.7	22.88	2.132
8	2929	68.177	2.906	3688.98	2886.57	64.357	-3.024

Fig. 2: FT-IR of un-inoculated Baobab Seed Oil

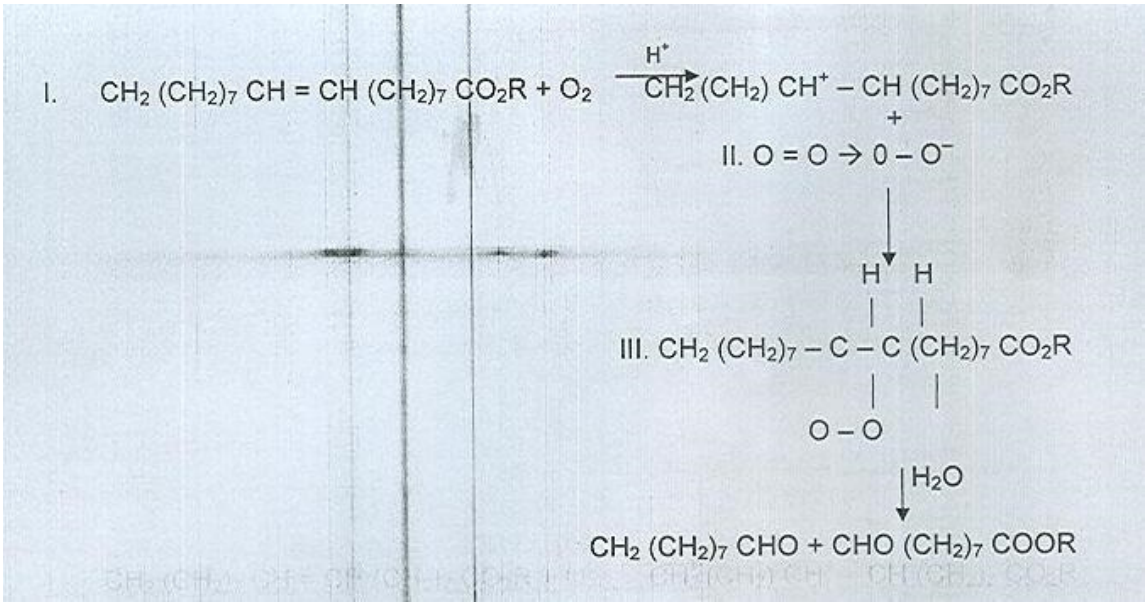


	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	443.64	13.399	22.062	671.25	399.28	101.622	22.758
2	711.76	83.87	3.571	807.24	672.21	7.805	0.74
3	936.47	85.478	5.365	999.16	807.24	9.666	1.872
4	1280.78	80.572	4.079	1362.75	1195.91	13.796	1.656
5	1443.77	78.561	12.909	1515.14	1362.75	10.769	4.606
6	1716.7	70.844	23.721	1801.57	1515.14	18.143	11.504
7	2928.04	63.465	7.492	3166.26	2881.75	34.868	-0.548
8	3359.14	82.097	1.892	3741.06	3302.24	26.337	3.223

Fig.3: FT-IR of inoculated Baobab Seed Oil on the 5th week of incubation

3.2 Fourier Transform-infrared Spectroscopy of Baobab Seeds Oil

The result of the Fourier transform – infrared (FT-IR) of the Baobab seeds oil is presented in fig. 2 and 3. Carboxylic, hydroxide, amide and aldehyde groups shifts were observed in the biodegraded oil as compared to the un-inoculated oil incubated for six weeks at temperature of 30°C. The use of FT-IR in the elucidation of adulteration of vegetable oils has been reported by Vlachos, et.al; (2006). They showed band shift observed at 3009 cm^{-1} assigned to the C-H stretching vibration of the *cis*-double bond, which allows the determination of extra virgin olive oil adulteration. Changes in the 3050–2800 cm^{-1} and 1745 cm^{-1} spectral region appear after heating at elevated temperatures and aid the oxidation process. The appearance and /or disappearance of certain absorption peak suggest that the microbes may have catalyzed the oxidative degradation of the oils (Joseph, 1977) and could be represented by the mechanism below:



3.3 GC-MS Analysis

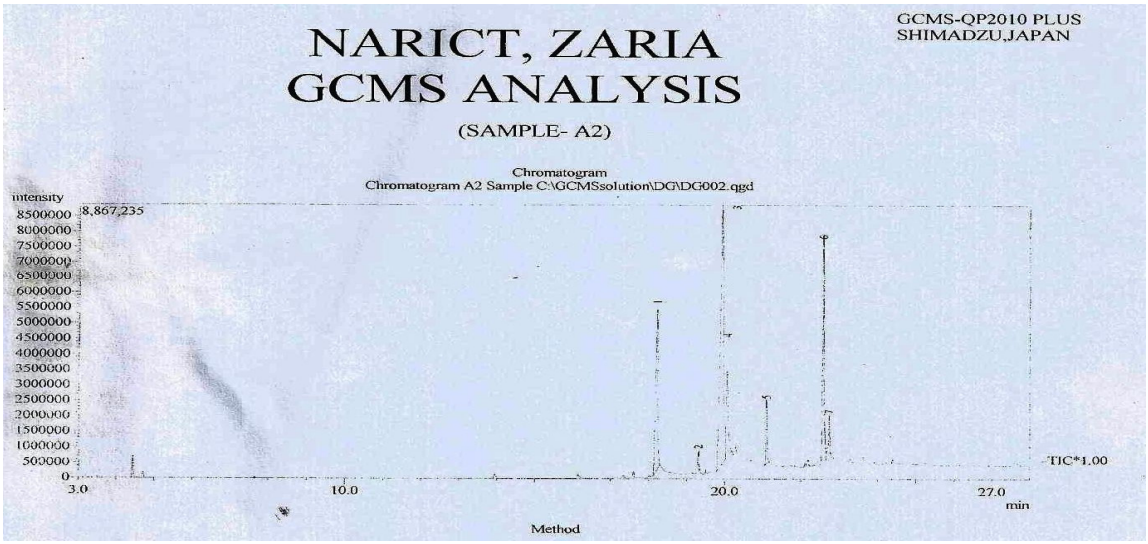


Fig. 4: GC Analysis of biodegraded Baobab seed oil (A2) inoculated with *Aspergillus niger* and incubation for 5 weeks.

The GC-MS analysis of the Baobab seed oil inoculated with *Aspergillus niger* is shown in figures 4 and 5. Seven components were detected at the end of the 5th week of incubation and a reduction in quantity was observed for components 2 and 5 in figure 4 whereas, an increase in quantity of components 3 and 6 compared with figure 5 (A1). This could be as a result of the microorganisms acting on the substrate which must have broken down some of these components into smaller molecules that are easily consumed. The appearance of the seventh component (Fig. 4) could therefore be a product of the biodegradation. This component may be a long chain macromolecule which possibly may have led to the increase in viscosity of the oil due to chains entanglement. Arguably highly branched macromolecules (Fig.5) must have been formed during the termination stage involving free radicals. This also suggests that other metabolic products from the action of theses microbes must have been consumed putting their growth into consideration.

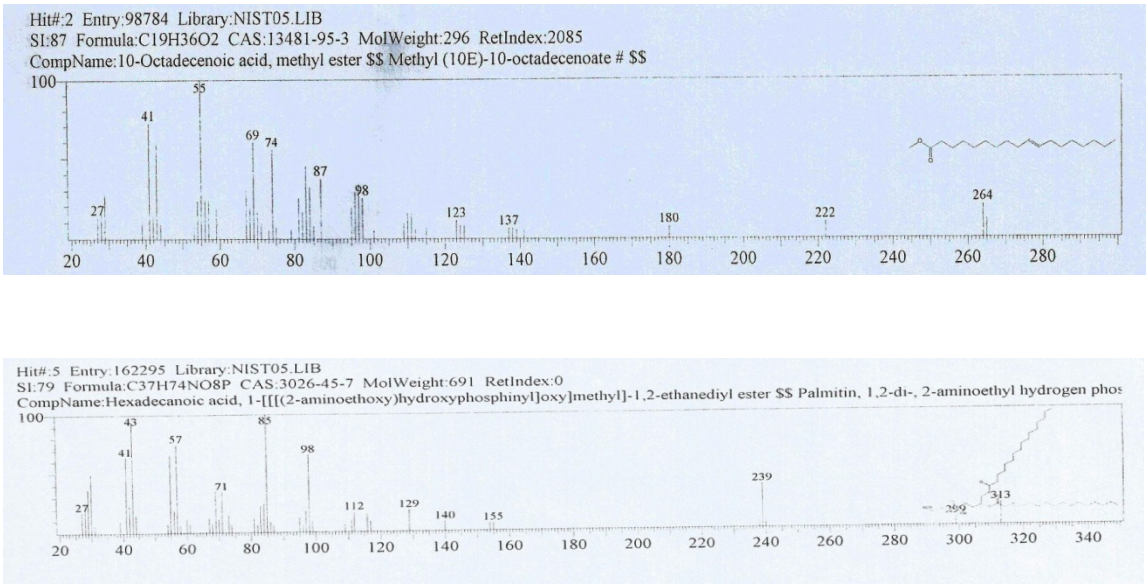


Fig. 5: MS Analysis of metabolites in the Biodegraded Baobab Seed oil (A2)

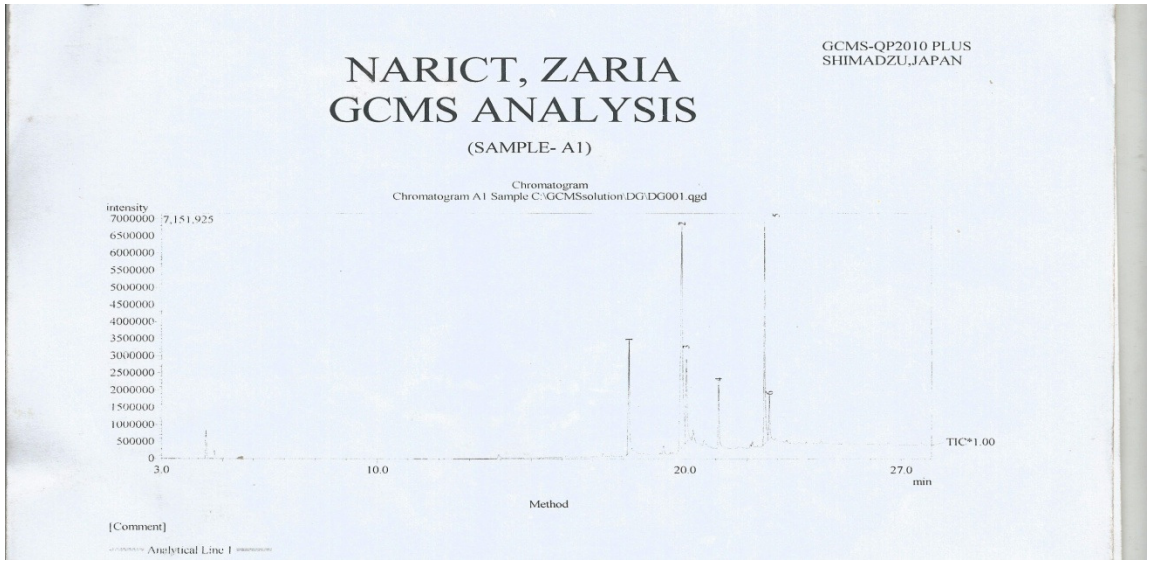
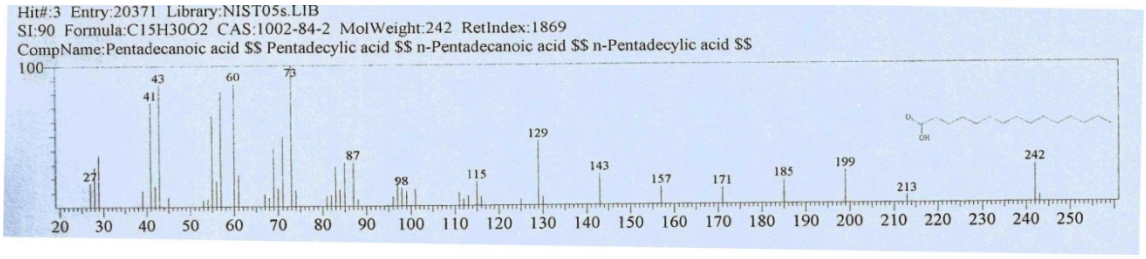


Fig. 6: GC Analysis of un-inoculated Baobab seed oil (A1)



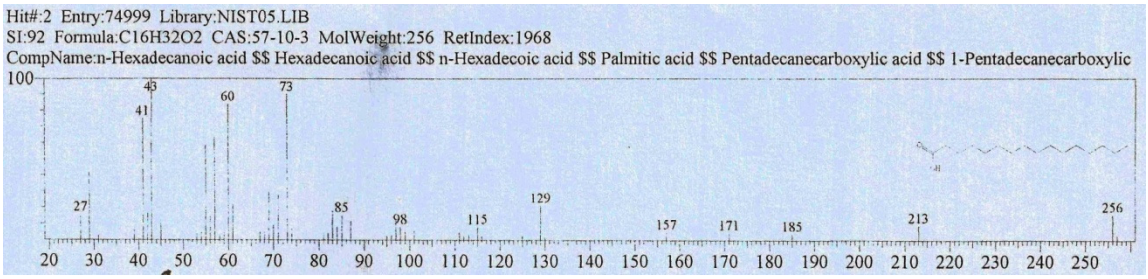


Fig. 7: MS Analysis of Components of the Baobab seed oil (A1)

4. Conclusion

Oil extracted from Baobab seeds in n-hexane yielded a reasonable quantity of about 25% though, not as high as that obtained in other seed oils like Groundnut, Soybean and Jatropha seeds. *Aspergillus niger* were used to degrade the Baobab seed oil at temperature between 25-30^{o}C in 35 days of incubation. Biodegradation metabolites were noticed to have effect on the pH of the medium. Also, physical properties such as colour, liquid state and moisture content were found to change as degradation proceeded. The Fourier transform-infrared measurement showed the appearance of a shift in the absorption peaks corresponding to carboxylic, hydroxyl, amide and aldehyde functional groups of the inoculated oil. The GC-MS analysis of the oil revealed the formation of some metabolites such as 9-octadecanoic acid and 2-hydroxy-1, 3-propanediyl ester. The production of the metabolites by these microbes could have been through a complex mechanism however, the pathway given by Joseph (2007) was adopted.

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Acknowledgement
Authours are profoundly grateful to Gombe State University for the financial support and NARICT Zaria-Nigeria for the laboratory analysis.

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