Journal of Advances in Biology & Biotechnology
8(3): 1-9, 2016; Article no.JABB.27821
ISSN: 2394-1081

SCIENCEDOMAIN *international*
www.sciencedomain.org

Degradation of *Curcubita pepo* Seeds Oil by *Aspergillus niger*

Peter Michael Dass[1*], Umar Usman Modibbo[1], Ayodele Akinterinwa[1] and Munyal Yusuf[1]

[1]Department of Chemistry, School of Pure and Applied Chemistry, Modibbo Adama University of Technology, Yola Adamawa State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author PMD designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors UUM and AA managed the analyses of the study. Author MY managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2016/27821
Editor(s):
(1) James W. Lee, Department of Chemistry and Biochemistry, Old Dominion University, USA.
Reviewers:
(1) Joseph Tanimu, Federal University Wukari, Taraba State, Nigeria.
(2) P. N. Palanisamy, Kongu Engineering College, India.
(3) Aline Elesbao Do Nascimento, Universidade Catolica de Pernambuco, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/15643

Original Research Article

Received 21st June 2016
Accepted 30th July 2016
Published 3rd August 2016

ABSTRACT

Vegetable oil was extracted from *Curcubita pepo* seeds in n-hexane using Soxhlet extractor at 60°C. The physico-chemical parameters of the purified oil was analysed according to American Oil Chemist Society method. The physico-chemical of oil gave the free fatty acids of 0.83 mgKOH/g, Iodine Value of 97.96 I_2 /100 g, viscosity of 4.13 μ, refractive index of 1.46, density of 0.91 g/cm^3 and 1.4 mgKOH/g, 50.34 I_2 /100 g, 3.17 μ, 0.92, 0.602 g/cm^3 for un-degraded and degraded oil respectively. The Fourier Transform Infra Red and Gas Chromatography -Mass Spectroscopy of the oil were determined before and after four weeks of inoculation and incubation with the microorganisms. It was observed that *Aspergillus niger* partly degrade the oil in four weeks of incubation. The decrease in viscosity of oil as the time of incubation increases was observed. The appearance of absorption peaks corresponding to hydroxyl, carbonyl, and carboxylic functional groups in the spectrogram suggested the formation of compounds such as alcohols, carboxylic acids, esters and nitrates as biodegradation metabolites. Mass Spectroscopy provided the mass fragments of the possible components formed during the degradation of the oil to include: heptadecanioc acid, octadecanoic acid and oleic acids. The formation of three fatty acids was

**Corresponding author: E-mail: pmdass66@yahoo.co.uk;*

viewed to be due the oil high stability to this fungal attack thereby alluding to thesubstantial application of the *Curcubita pepo* seeds oil in areas such as soap and detergent as well as usage cooking and or lubricants.

Keywords: Oil; Aspergillus niger; incubation; microorganisms; biodegradation; Curcubita pepo.

1. INTRODUCTION

Recently the Pumpkin plant has witness considerable attention by scientists due to its nutritive and health values [1]. The matured Pumpkin fruit (Plate 1) is a popular delicacy used as ingredient in soup and porridge in most parts of northern Nigeria. It contains many seeds which are imbedded in the mucilaginous fibres at the central cavity of the pod (Plate 2). The seeds are dark brown in colour but it is not unusual to see some that are white (Plate 3). The seeds are mostly thrown away as waste possibly because they can be readily cultivated and therefore present in abundance or adequate attention has not been focused on areas of application [2,3]. However, the medicinal value of the seed oil has now been documented [3]. Treatment of Intestinal disorder due to worms, for vermifuge and as diuretic have been reported [2]. Blood pressure lowering effects and cardiac protection are also some salient features of Pumpkin seed oil [4]. Others are their antioxidation [5], and antidiabetic [6] properties. Their antifungal [7] and antibacterial [8] effects have also been reported. *Aspergillus* species which are highly aerobic fungi found in almost all oxygen-rich environments. They are commonly seen as mold growing on the surface of a substrate. These microorganisms grow on carbon-rich substrates such as monosaccharide's e.g. glucose and polysaccharides e.g. amylase. Evidence of biodegradation which involved the formation of smaller organic compound, toxic or non-toxic has been reported [9]. These compounds could be volatile or non-volatile that may have important physiological effects. Non-volatile products of degradation are important since they remain in the oil during food processing and consumption [10]. This research work is aimed at investigating the degradation of the seed oil using a common fungi that has caused tremendous domestic problems, *Aspergillus niger*.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Materials

Pumpkin Pods *(Cucurbita pepo),* were obtained from Hong of Adamawa State- Nigeria. The seeds were washed thoroughly with water. The clean seeds were dried under laboratory conditions for 3 days. After that outer layer of the seeds were removed manually (Plate 3). The dried seeds were crushed to smaller sizes and pulverized using an iron mortar for extraction of the oil.

Plate 1. A matured pumpkin pod

Plate 2. A horizontal matured pumpkin pod with seeds

Plate 3. Matured pumpkin seeds (white and brown coloured)

2.2 Extraction of Oil from Seeds

Oilfromseeds was extracted Usingmethoddecribed by [11]: 300 ml of n-hexane was poured into a round bottom flask. 100 g of the sample was placed in the filter paper thimble and was inserted in the centre of the extractor. The Soxhlet extractor was heated at 60°C. When the solvent was boiling the vapour 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 isolid sample to be extracted. The extract seeped through the pores of the thimble and filled the siphon tube where it flowed back down into the round bottom flask. This was allowed to continue for 4 hours and the condenser was removed at the end of the extraction. The flask containing the liquid mixture was connected to Liebig condenser and heated up to 70°C to evaporate off the n- hexane. The oil obtained was weighed and procedure repeated.

2.3 Isolation of *Aspergillus niger*

Potato Dextrose Agar (PDA) plates were prepared according to manufacturer's specifications. 0.1 g of each of the oil samples were seeded onto a PDA plates each for the isolation of viable fungal cells. These plates were incubated at 36°C for 5 days colonies of the organisms on the PDA plates were purified by repeatedly sub-culturing them onto fresh plates. Each pure culture was then transferred to PDA slants and incubated at 36°C for 5 days for profuse growth of the fungi then stored in refrigeration until needed (Plate 4).

Plate 4. *Aspergillus niger*

2.4 Biodegradation of Pumpkin Seed Oil

The biodegradation of the seed oil was carried out according to the ASTM: D 5864: Standard practice for the evaluation of the action microorganism in oil. Degradation studies were done by dispensing 1.0 g of PDA broth-growncultureoffungalisolateswereaddedtotheoila ndincubatingat (39°C) for 4 weeks in the laboratory of the Department of Microbiology Modibbo-Adama University of Technology Yola, Adamawa State, Nigeria (Figs. 5 and 6) observed the different viscosity flow of the oil samples after degradation by the isolates. After 4 weeks of incubation, all the isolates showed evidence of growth.

2.5 Viscosity Measurement

The viscosity of the vegetable oil was measured using the Ubbelohde viscometer according to ASTM D445-446. The viscosities of the vegetable oil inoculated with *Aspergillus niger* were measured at interval of 7 days whereas, the un-inoculated oil served as controls (t_o). The measurement was done according to Poiseuilles law for capillary tube flow as adopted by [12].

2.6 Fourier Transform- Infra Red (FTIR) Measurement

The FTIR analysis of oils as done at American University of Nigeria using PerkinElmer Spectrum Version 10.03.02. Oil without the *Aspergillus niger (*un-inoculated) was considered as control and the FTIR of oil inoculated with the microbes was measured after the 4th week of incubation. This was considered as reasonable period for biodegradation to have taken place.

2.7 GC-MS Measurement

The GC-MS analysis was done at American University of Nigeria using Model –GC-MS-7890A, Agilent Technologist Inert MSD-597CM. Carrier gas –Helium, ^{63}Ni electron capture detector, low polar HP 5Ms column, Column dimension -30 cm x 0.34 mm, column oven temp-60°C, detector temperature 300°C, injection temperature 250°C, flow rate of carrier gas 1.61 ml/min, pressure of 100.2 kpa, linear velocity of 46.3 cm^{-2} and injection mode- split.

3. RESULTS AND DISCUSSION

3.1 Biodegradation of Pumpkin Seed Oil

Physico- chemical parameters of undegraded (A1) and degraded (A2) oil extracted from *Cucurbita pepo* seed oil is given in Table 1. The extracted oil has a yellow colour with pleasant odour. The colour often the oil changed to green

yellow by four weeks of inoculation with *Aspergillus niger* resulting into a bad smelling product. This aromatic compounds in the virgin oil may have been broken down by the microorganisms thereby forming compounds such as ammonia. The Iodine value of 97.9266 I_2 /100 g oil similar to 84.4 I_2 /100 g for groundnut seed oil but lower than some non -edible seed oil such as *Lagenaria siceraria* (Bottlegourd) seed oil, 126.90 I_2 /100 g [13]. This high Iodine value indicates a high degree of unsaturation in the oil. The Iodine value of degraded oil is 50.3401 I_2 /100 g was lower indicating a reduction in the amount of the degree of unsaturation. This was viewed to either be the conversion of double bonds into single bonds products or the consumption of possible unsaturated compounds initially present in the oil. The acid value of 0.82665 mgKOH/g of the undegraded oil is lower than many vegetable seeds oils reported [13]. There is slight decrease in Acid value 0.71130 mgKOH/g of the degraded oil. The low acid value indicates stability against oxidation possibly at low temperature by these microorganisms. The specific gravity is 0.8188 for undegraded oil while 0.6702 is obtained for the degraded oil. The decrease in specific gravity could due to the reduction in some smaller organic compounds as the microbes utilized them as sources of energy for growth.

3.2 Viscosity Measurement

Fig. 1 is a plot of the viscosity against incubation time in days. The viscosity of the inoculated oil with the microbes shows gradual decrease as the incubation time increases. The decrease could be due to the reduction in the chain length of the macromolecule by the breakdown of the triglycerides into fatty acids and glycerol. [13] however, observed increase in viscosity of *Adansonia digitata* seed oil as the incubation time increases using similar microorganisms. They opined that it could be due to the growth of the microorganisms as they utilized the organic molecules produced in the process of the breaking down of the oil. Although, the *Aspergillus niger* acted on the *Curcubita pepo* seed oil the decrease in viscosity could be seen to be minimal indicating a rather resistant oil to the microbial activity. Even though an increase in acid number of the degraded oil is obtained the few fatty acids produced may have hindered the growth of the microbes. The pH 6.75 of the oil measured before inoculation may have served as a favourable medium for the growth of the microbes [13]. This could be seen to only slightly increase for the period of incubation (Fig. 2).

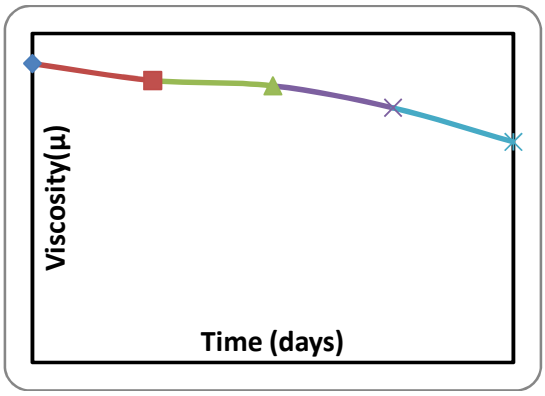

Fig. 1. A plot of viscosity against time (days) for pumpkin oil

The favourable pH of *Aspergillus niger* activity has been shown to be between 6-7.5 9 [14,15]. However, the decrease in the pH as incubation progresses may be due to the formation of saturated carboxylic acids and the depletion of the unsaturated long chain molecules.

Table 1. Physico- chemical parameters of undegraded (A1) and degraded (A2) oil extracted from *Cucurbita pepo* seed oil

Parameters	Properties	
	Undegraded oil (A1)	Degraded oil (A2)
Amount of oil (ml)	150	-
Color	Yellow	Green yellow
Odour	Pleasant	Unpleasant
Refractive index	1.4600	0.91500
Iodine value (I_2/100 g)	97.9666	50.3401
Specific gravity	0.8188	0.6702
Acid value (mg KOH/g)	0.82665	0.71130
pH value	6.75	5.58
Density (g/ cm^1)	0.911	0.911

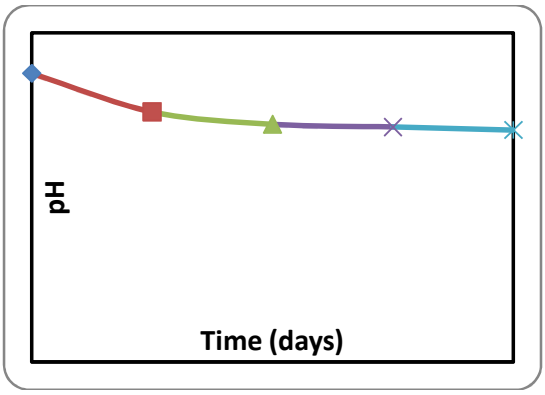

Fig. 2. A plot of pH against time of incubation (days) of pumpkin seed oil

Also, the accumulation of hydroxylic molecules could plasticized remnant long chains molecules thereby increasing fluidity of the mixture. It is observed that the curve of Fig. 8 fattens as the incubation time increases. As the secretion of enzymes by *Aspergillus niger* continues the medium became more acidic which could have stopped further microbial growth action. However, vegetable seeds oil such as palm oil have been shown to have less inhibitory effect on the growth of *Aspergillus niger* [16,17].

3.3 Fourier Transform- Infra Red (FTIR) Measurement

The result of FTIR shows appearance of absorption peak at 2853.07 cm^{-1}, disappearance at 1159.90 cm^{-1} and reduction in the height of some absorption peaks (Table 2 and Fig. 3). The FTIR spectroscopy of the oxidation of Pumpkin oil showed bands at 1755- 16550 cm^{-1} suggested ester C = O stretching which indicates possible formation of aldehydes or ketones whereas absorption at 2853.07 corresponded to C-O stretching possibly of carboxylic acids. At 2922.31 cm^{-1} the absorption corresponds to O-H stretching of hydroxyl compounds. The use of hydrogenated seed oil in food has never been satisfactory since partially hydrogenated oils and their trans- fats have been viewed to increase the risk of blood clothing inside blood vessels [18], however, the consumption of polyunsaturated and monounsaturated fatty acids could be responsible for the improvement of the lipidic profile in relation to the saturated fatty acids. They are viewed to have no negative effects because they provoke an increase in the LDL cholesterol oxidation and reduction of the HDL cholesterol levels [19].

Table 2. Result of the FTIR of un-inoculated (A_1) and inoculated (A_2) pumpkin seed oil

Absorption band (cm^{-1})	Peak(cm^{-1})	Peak(cm^{-1})
	A_1	A_2
50-59	_____	2853.07
60-64	2922.30	2922.31, 1744.58
65-69	2853.10	1160.24
70-74	1159.90	_____
75-79	1457.24, 1116.37	1464.44,1782.56,721.99
80-84	1238.87, 86271.64	1378.02
85-89	1377.83,	1542.11, 668.04
90-94	1541.97	----------

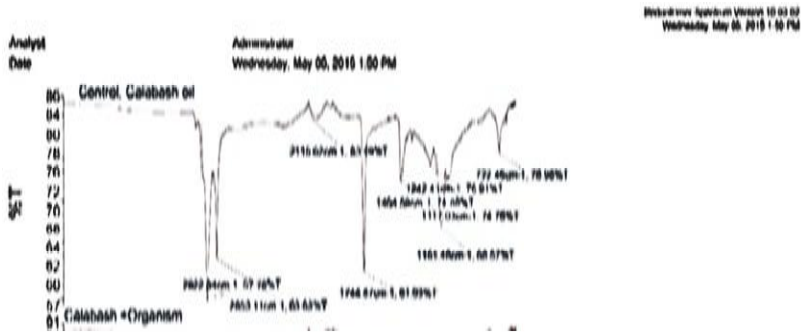

Fig. 3. FTIR of both un-inoculated and inoculated pumpkin seed oil with *Aspergillus niger*

3.4 GC-MS Measurement

Figs. 4 and 5 give the GC spectrogram of both the un-inoculated and inoculated Pumpkin seed oil with *Aspergillus niger*. Peaks corresponding to C=O and O-H were absent in the un-inoculated Pumpkin seed oil whereas, these peaks where seen in the biodegraded seed oil (Table 3). The appearance of functional groups of C=O and O-H in the biodegraded oil may be inferred to be due to the formation of acidic and alcoholic metabolites by the microorganisms as they acted up on the oil. In addition, were height of the carboxylic acid groups could be considered to be proportional to the amount of the group of these compounds produced by the microorganisms. Triglycerides of the oil may have been hydrolysed to their alcohol and carboxylic acid components by the enzymes secreted by the microbes, suggesting the occurrence of biodegradation of the oil. Interestingly, the MS fragmentation of the biodegradation uponmetabolites gave credence to the formation of fatty acids: heptadecanioc,octadecanoic and oleic acids (Figs. 6, 7 and 8).

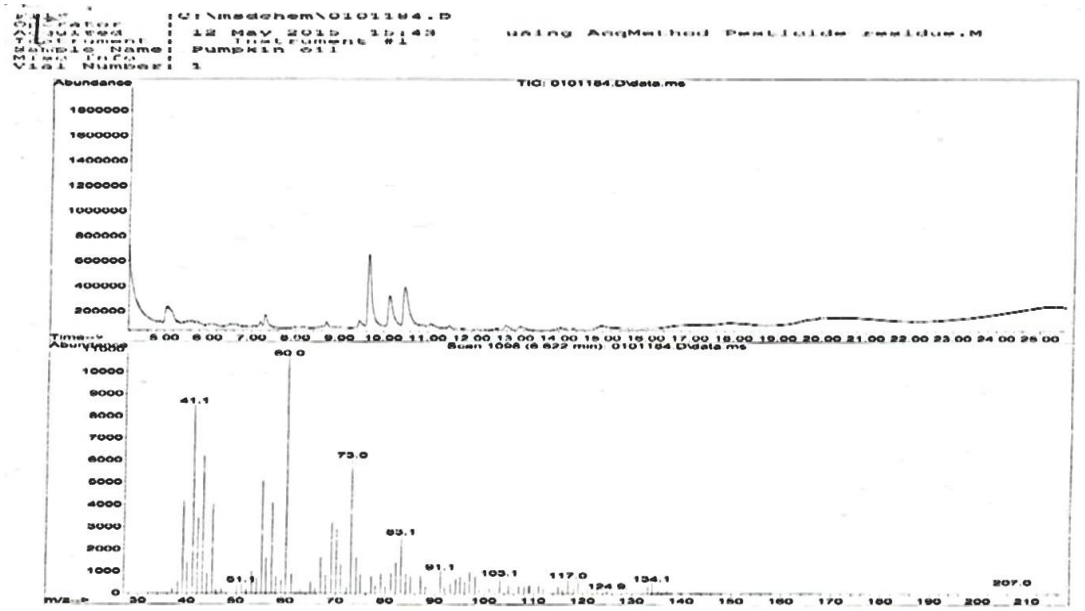

Fig. 4. GC –MS absorption peaks of the un-inoculated pumpkin seed oil (control)

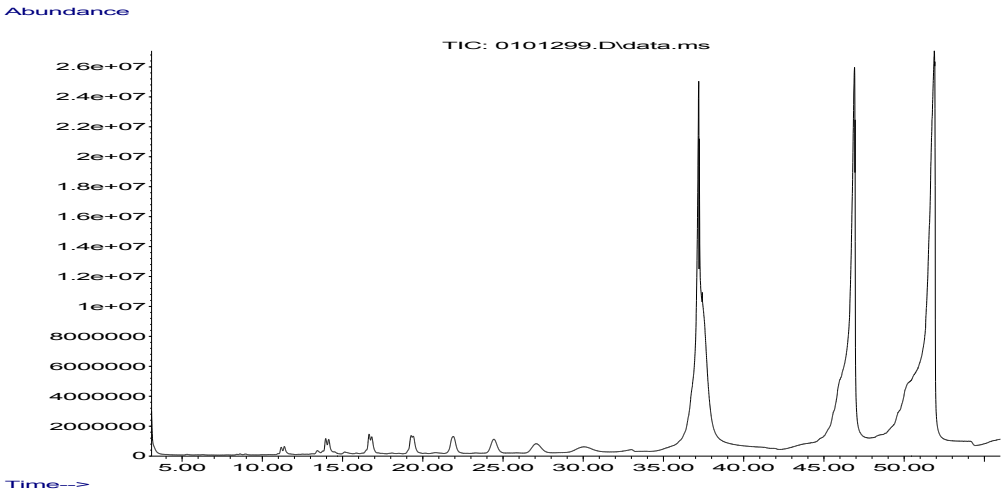

Fig. 5. GC absorption peaks of the degraded pumpkin seed oil after four weeks of incubation with *Aspergillus niger*

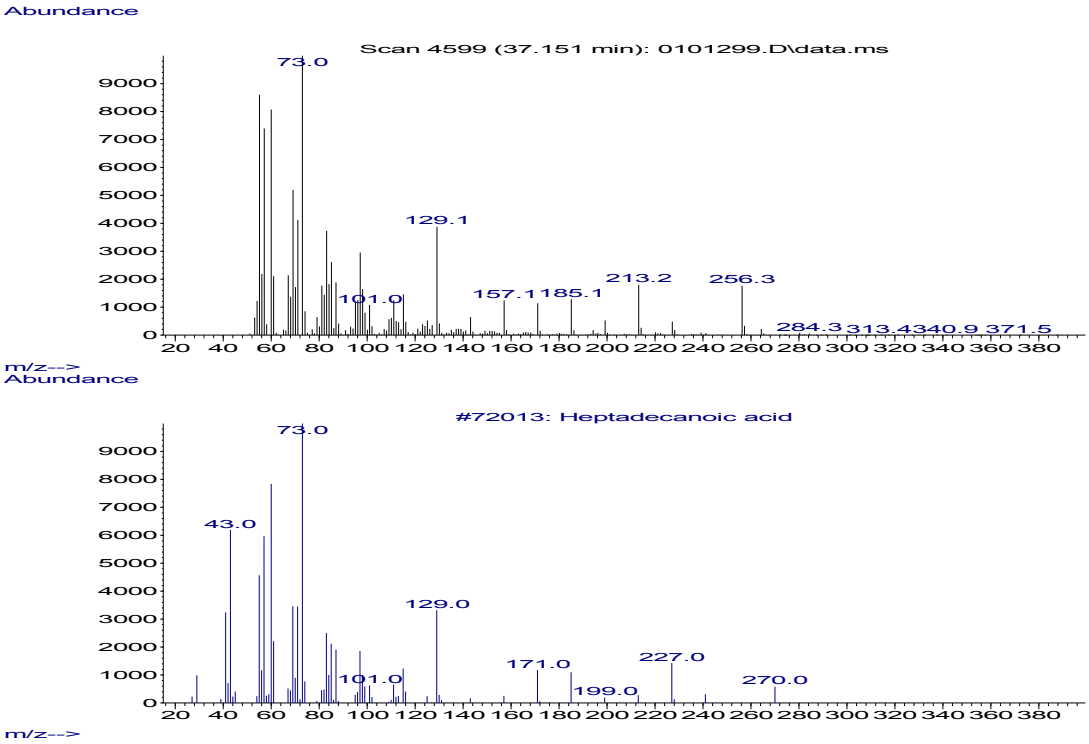

Fig. 6. MS fragmentation of heptadecanioc acid pumpkin seed oil after four weeks of incubation with *Aspergillus niger*

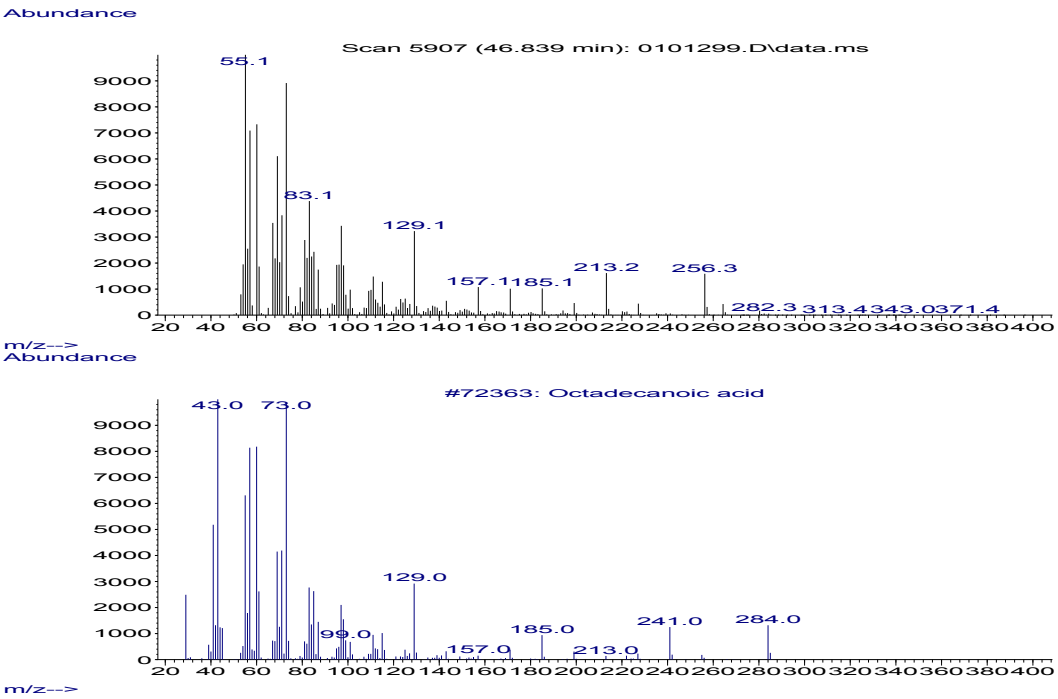

Fig. 7. MS fragmentation of octadecanoic acid pumpkin seed oil after four weeks of incubation with *Aspergillus niger*

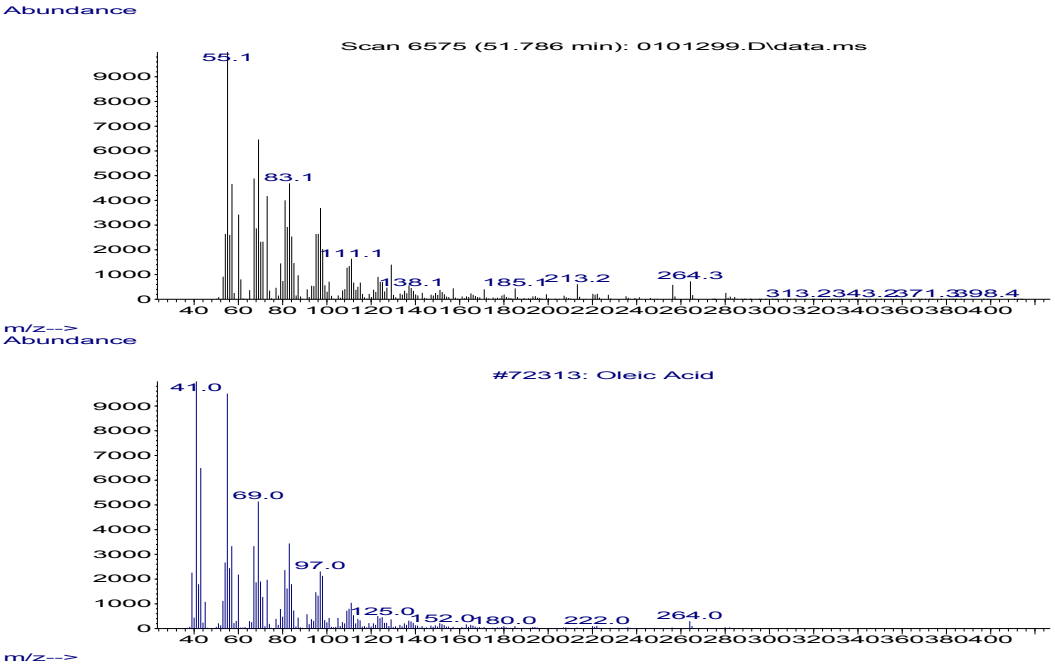

Fig. 8. MS fragmentation of oleic acid pumpkin seed oil after four weeks of incubation with *Aspergillus niger*

Table 3. GC Peaks and corresponding functional group of un- inoculated and inoculated pumpkin seed oil

S/No	Peak and functional group for un-inoculated oil (cm^{-1})	Peak and functional group for biodegraded oil (cm^{-1})	Respective functional group for biodegraded oil
1	41.1 C—H	43.1 C= O	Esters
2	51.1 C — O	51.9 C—O	Esters
3	_____	73.0 C = O	Esters
4	73.0 C—H	83.1 C ≡ N	Nitrates
5	83.1 C≡C	97.1 C≡ C	Esters
6	91.1 C— O	107.1 O – H	Carboxylic acids
7	103.1 C — H	117.0 C≡ N	Nitrates
8	117.0 C — H	133.0 C≡ N	Nitrates
9	124.4 C— H	149.1 O – H	Carboxylic acids
10	134.1 C — H	171.1 O – H	Carboxylic acids
11	207.0 C — H	185.1 O – H	Carboxylic acids
12———	————	199.0 O – H	Carboxylic acids
13———	————	213.1 O – H	Carboxylic acids

4. CONCLUSION

Pumpkin seeds which are widely thrown away as waste in Nigeria has been found to contain reasonable quantity of oil. The iodine value suggest that the oil is high in unsaturation. However, the fatty acids detected, the reduction in viscosity and the formation of by-products by FTIR, GC and MS revealed that *Aspergillus niger,* a common fungi degrades the oil when exposed for a period of four weeks. The sweet smelling odour, low unsaturation and other physicochemical properties of *Cucurbita pepo* seed oil favour its application as lubricants and ingredients in food industry. However, adequate care should be taken during the extraction and utilization of the oil to avoid contamination by this microorganisms.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Abd-El-Aziz AB, El- Kalek A. Antimicrobial proteins and oil seeds from pumpkin (*Cucrbita pepo)*. Natural Science. 2011;9:105-119.
2. Caili FS Huan, Quanlong L. A review on pharmacological activity and utilization technologies of pumpkin plant. Foodhuman Nutrition. 2006;61:73-80.
3. Cltonoko UG, Rufai AB. Antibacterial activity of *Cucurbita pepo* (pumpkin) against *Staphiylococcus aureus* and *Salmonella typzi.* Bayera. Journal of Pure Applied Science. 2011;1:145-147.
4. Dass PM, Abduladir A, Josiah NJ, Wilison DL, Gin NS. Viscometric studies on the biodegradation of some vegetable oils using *Aspergillus niger*. Food Science and Quality Management. 2013;16:1-8.
5. Dass PM, Wilson LD, Karu E, Abuduladir I, Abunakarji B. Extraction and biodegradation of Boabob (*Adansonia digitata*) seed oil using fungi *Aspergillus niger.* Journal of Natural Science Research. 2013b;3(5):128-136.
6. Dhiman AK, Sharma KD, Atlri S. Functional constituents and processing of pumpkin: A review. Journal of Food Science and Technology. 2009;46:411-417.
7. Dolatabadian A, Sanavy SAMM, Chashmi NA. The effects of foliar application of ascorbic acid (vitamin C) on antioxidant enzymes activities, lipid peroxidation and proline accumulation of canola (*Erassica nopus* L.) under conditions of salt stress. Journal of Agriculnn'e and Crop Sciences. 2008;194:206-213.
8. Eman MH, El-Kinawy OS. Food toxicology and contaminants. Journal of American Science. 2011;7(9):615-622.
9. Pericin D, Krimmer V, Trivic S, Radulovic L. The distribution of phenolic acids in pumpkins hull-less seed, skin oil cake meal, dehulledhernel and hull. Journal of Food Chemistry. 2009;113:450-456.
10. Tagoe SMA, Dickinson MJ, Apetorgbor MM. Factors influencing quality of palm oil produced at the cottage industry level in Ghana. International Food Research Journal. 2012;19(1):271-278.
11. Wang H, Ng T. Isolation of *cucurmoschin*, a novel antifungal peptide abundant in arginine, gluatamate and glycine residues from black pumpkin seeds. Journal of Food Chemistry. 2003;24:969-972.
12. Kyari MZ. Extraction and characterization of seed oil. International Agrophysics. 2008;22:139-142.
13. Dass PM, Ku bmarawa D, Akinterinwa A, Mohammed B. Spectroscopic elucidation of the biodeterioration potentials of *Aslergillus niger* on *Lagenaria siceraria* seed oil. American Chemical Science Journal; 2016.
14. Obire O, Anyanwa EC, Okigbo RN. Saprophytic and crude oil degrading fungi from cow dung and poultry dropping as bioremediation agents. Journal of Agriculture Technology. 2008;4(2):81-89.
15. Yang YS, Zhou JT, Lu H, Yuan YL, Zhao LH. Isolation and characterization of fungus. *Aspergillus niger* sp strain F-3 capable of degrading almaliliginin. Biodegradation. 2011;22:1017-1027.
16. Ekwenye UN, Ijeomah CA. Antimicrobial effects of palm kernel oil and palm oil. KMITL Science Journal. 2005;5(2):502-505.
17. Atinafu DG, Bedemo B. Estimation oftotal fatty acid and cholesterol content in some commercial edible oils in Ethopia Bahir DAR. Journal of Cereals and Oil Seeds. 2011;2(6):71-76.
18. Zambiazi RC, Przybylski R, Zambiazi MW, Mendonca CB. Fatty acid composition of vegetable oils and fats. B. CEPPA, Curitiba. 2007;25(1):111-120.
19. Gohari A, Farhoosh R, Khodaparasat MHH. Chemical composition and physico-chemical properties of Pumpkin seeds (*Cucurbita pepo* subsp. pepo var.styriaka) grown in Iran. Journal of Agriculture Science and Technology. 2011;13:1053-1063.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/15643