Food Science and Quality Management
ISSN 2224-6088 (Paper) ISSN 2225-0557 (Online)
Vol.16, 2013

Viscometric Studies on the Biodegradation of Some Vegetable Oils using Aspergillus Niger

Peter Micheal Dass[*1], Abdulqadir Ibrahim [2], Josiah Nuhu Jabbo [3], Wilson Lamai Danbature[4] and Nuhu Sambo Gin[3]

1. Dept. of Pure and Applied Chemistry, Faculty of Natural and Applied Science, Veritas University of Abuja-Nigeria
2. Dept. of Environmental Management Technology, School of Management Technology, Abubakar Tafawa Balewa, Bauchi-Nigeria
3. Dept. of Science Laboratory Technology, School of Science and Technology, Abubakar Tatari Ali Polytechnic Bauchi-Nigeria
4. Dept. of Chemistry, Faculty of Natural Science, Gombe State University, Gombe-Nigeria

*Email of corresponding author: pmdass66@yahoo.co.uk

Corresponding Author: Dass, P.M; Dept. of Pure and Applied Chemistry, Faculty of Natural and Applied Sciences, VERITAS UNVERSITY OF ABUJA-NIGERIA.

Abstract

The continual utilization of vegetable oils in homes, restaurants, hotels and cosmetic industries require their preservation against microorganisms. Aerobic biodegradation of four different seed oils were carried out using *Aspergillus niger* in order to determined their susceptibility to these common microbes in a temperate climate. The intrinsic viscosities of the inoculated oils, incubated at temperature of 35^{o}C, were determined from the relative viscosities measured at intervals of seven days for six weeks. The viscosities of the un-inoculated oils were determined and used as control. A gradual increase in the viscosities with increase in the time of incubation was observed. This was ascribed to the possible growth of the microbes as they consume the smaller molecules formed during the break down of the oil. A remarkable change in the colour of the oils sample was noticed. This was then attributed to break down of compounds responsible for colours in these oils. The maximum viscosity values were obtained on the twenty eight day of incubation beyond which a decline was observed. This was credited to the formation of secondary metabolites. The Fourier Transform- Infra Red spectrogram and the pH supported the secretion of enzymes and subsequent conversion of oil to secondary metabolites by the *Aspergillus niger*. Absorption peaks at 2923.04-2930.09cm^{-1} and 3359.14-3429.69 cm^{-1} were due to hydroxyl and carboxyl groups in the biodegraded compounds. Edible oils with high free fatty acid value would therefore, biodegrade easily if contaminated by common fungi such as *Aspergillus niger* at temperature of 35^{o}C. The consumption of edible oil without frying commonly practiced in Nigeria may have been the source of diseases such as diarrhea, flu, vomiting, etc. It is recommended that edible Oils such as Palm, Ground nut, Cotton seed oil should be stored at temperature below 30^{o}C and properly covered to prevent any contact with fungi.

Keywords: Oil, Biodegradation, *Aspergillus niger,* Viscosity

1. Introduction

The biodegradation studies on edible oil have continual elicited the interest of scientists especially those in the nutrition and dietetics fields. This is because vegetable oils are largely used domestically i.e. in homes, restaurants, hotels, industries etc, also, due to the ubiquitous nature of microorganisms, they can be found everywhere such as in air, water and soil. Therefore, contact between the oil and any of these sources of microorganisms results in contamination of the oil. The storage of these oils for long period without microbial contamination would therefore be of outmost interest to users. Amongst the microorganisms that are known to contaminate vegetable oils is fungi (Obire, *et. al*; 2008). They are known to cause the deterioration of oil extracted from seeds by changing the free fatty acid content, peroxide, saponification values and total oil content (Kuku, 1979). *Aspergillus* species have been reported to degrade several compounds such as lignin, plasticizers, textiles, dyes, wood, plastics, and metals (Yang, *et. al*; 2011; Gomaa, *et. al*; 2012; Pradeep, et. al; 2012; Premja and Doble, 2005; Jain, 2008). Biodegradation is the processes by which organic substances are broken down by living organisms. In the degradation of vegetables oil the enzyme esterases which catalyzed the cleavage of the ester bond to fatty acid (Broekhuizen, *et al*. 2003) are secreted by the microorganisms. And lipases which are hydrophobic proteins secreted, catalyzed the cleavage of carboxyl, ester bonds in tri-, di- and mono acyglycerols (the major constituents of animals, plants and microbial fats and oils) Saifudin, *et al*. (2006). Glycerol and long chain fatty acids (LCFAs), which serve as the growth substrates for these microbes are produced (Zhengkai, *et.al*; 2005). The composition of fatty acids depends on the plant, the crop, the season, and the growing conditions (Meier, *et. al*; 2007). However, the continuous elucidation of pathways and factors that causes biodegradation in seed oils has tremendously helped in the prevention of deterioration and enhanced storage of oil for utilization

both domestically and industrially. The use of several spectroscopic techniques in monitoring biodegradation has also advanced the cause of study (Meier, *et. al*; 2007). Others have monitored the extent of biodegradation of vegetable oils through the measurement of the concentration of primary, secondary oxidation products, the amount of oxygen consumed and peroxide value (Vlactos, *et al*. 2012). The viscosity of the degrading oil has also been used to monitor biodegradation. This is because viscosity is a property of fluids and slurries which indicates their resistance to flow. The viscosity increases with increasing molecular weight and decrease with increase unsaturation and temperature (Azian, *et. al*., 2001). The aim of this research was to use viscometric measurement to monitor the biodegradation of some seed oils such as Baobab (*Adansonia digitata*), Palm oil, Cotton oil, Ground nut oil and their results compared.

2. Materials and Methods

Baobab seeds (*Adansonia digitata)*, were obtained from Kaltungo of Gombe State, Nigeria and transferred in Polyethene bags to the Gombe state University Laboratory and oil extracted in n-hexane using a Soxhlet extractor at 60^{o}C. Palm oil, Cotton seed oil and Ground nut oil were purchased from the market and purified by the removal of moisture using a Rotary evaporator in the laboratory.

2.1 Isolation of Fungi

Colony of microbes was dissolved in 100ml of sterile distilled H_2O. It was seal in flask with a tape and was incubated at 30^{o}C for 48 hours. A few drops of the culture were added in to 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) the plate was seal and incubated at 30^{o}C for 6 days. Without inversion, the colony of the visible organism was picked and purified by sub-culturing into freshly prepared ager plates using the streak plate technique. The pure fungi isolates was transferred into PDA slant as stock cultures.

2.2 Viscosity Measurement

The viscosity of each of the vegetable oil was measured using the Ubbelohde viscometer according to ASTM D445-446. The Ubbelohde viscometer is filled with the liquid using a pyreusball until the liquid fills the capillary viscometer. Then the Ubbelohde is placed vertically in a stand inside a water bath at 35^{o} C temperature. The liquid is then allowed to flow through the capillary tubes and into the spheres. The time taken for the liquid to fill the spheres is measured (t). The viscosities of the vegetable oils inoculated with *Aspergillus niger* were measured at interval of 7 days whereas, the un-inoculated oils served as controls (t_o). The measurement was done according to Poiseuilles law for capillary tube flow;

$Q=V/t= \Delta P\Pi r^4 /8\mu l$ (1)

Where t is the time required for a given volume v of liquid with density ρ and viscosity μ to flow through the capillary tube of length l and radius r by means of pressure gradient ΔP is;

$V/t= \Pi r^4 \rho gl/8\mu l$ (2)

or

$\mu= \Pi r^4 \rho gl/8V$ (3)

The intrinsic viscosity μ is then calculated as,

$\mu_{rel} = \mu/ \mu_o$ (4)

$\mu_{rel} = t/t_o$ (5)

where, t and t_o are the flow times for inoculated and un-inoculated oil and μ_{rel} is the relative viscosity

$\mu'_{rel} = \mu=t/t_o$ (6)

the intrinsic viscosity does not have the units of absolute viscosity, it is an intrinsic measure of the properties of macromolecules.

2.3 Fourier Transform- Infra Red (FTIR) Measurement

The FT-IR analysis of oils was done at NARICT, Zaria-Nigeria using the Shimadzu model 8400S. Oils without the *Aspergillus niger* were considered as controls whereas, the FT-IR of oils inoculated with the microbes was measured after the 4th week of incubation. This was considered as reasonable period for biodegradation to have taken place.

3. Results and Discussions

The result of viscosities of four different oils not inoculated with *Aspergillus niger* is shown in Table I. Palm oil has the highest value of 191.84 while Baobab seed oil the lowest (41.75). The viscosities of the oils that were inoculated with the microbes gradually increased with incubation time (Table II). This increase could be due to the growth of the microorganisms (Plate 1) as the substrate (oil) is broken down into smaller molecules which are subsequently utilized as source of food and energy. It could also be due the formation of long chain macromolecules as free radical intermediates terminated. The highest viscosities values were recorded on the

28th day of inoculation for all samples. This may be because the microorganisms are most active on the substrates due to favourable pH. Furthermore, the formation of long chain products may quickly entangled giving a kind of branched products which resist flow this could be temporal since as the microorganisms accumulate more bonds would be broken down and products consumed. This could be the reason gradual decline in viscosities after the 35th day of incubation was obtained. The accumulation of small molecules may have resulted in the plasticitization of the remnant long chains thereby increasing fluidity. Also, the continuous secretion of enzymes due to the growth of *Aspergillus niger* may have caused the medium to more acidic which in turn stopped their action on the substrate. It has been reported that these microbes are destroyed at high acidic condition (Table 5).

Table 1: Determination of the Viscosities of un-inoculated oils (Control) sample

Time (days)	Viscosity(**μ**)			
	A1	B1	C1	D1
0	41.75	191.84	60.64	66.87
7	41.73	191.84	60.58	66.79
14	41.70	191.84	60.56	66.78
21	41.74	191.83	60.62	66.82
28	41.76	191.84	60.60	66.86
35	41.75	191.84	60.64	66.85
42	41.74	191.84	60.63	66.86

Key: A_1 (Baobab seed oil), B_1 (Palm oil), C_1 (Cotton seed oil) and D_1 (Groundnut oil).

Table 2: Determination of the Viscosities of inoculated oils sample

Time (days)	Viscosity (μ)			
	A2	B2	C2	D2
0	41.75	191.84	60.64	66.87
7	49.02	229.91	62.08	69.90
14	55.35	269.97	64.61.	72.73
21	59.41	272.27	67.54	75.93
28	70.08	275.37	69.84	79.53
35	67.25	269.86	68.92	76.64
42	64.42	238.91	65.74	73.92

Key: A_2 (Baobab seed oil), B_2 (Palm oil), C_2 (Cotton seed oil) and D_2 (Groundnut oil).

The favourable pH level of activity of *Aspergillus niger* has been shown to be between 6 to 7.5 (Obire, *et. al*., 2008; Yang, *et al*. 2011). Table V revealed a gradual increase in acidity of the medium as the time of incubation increases. Beyond the fifth week of incubation the medium becomes more acidic and this could have hindered the continuous activities of the microorganisms. Vegetable seeds oil such as Palm oil have been shown to have less inhibitory effect on the growth of *Aspergillus niger* (Ekwenye and Ijeomah, 2005; Tagoe, *et. al.,* 2012). Therefore, the formation of free radical intermediates suggested in the mechanism by Joseph (1977) may have reduced significantly. The initial high values of viscosities (Fig. 1) of biodegraded Palm oil (B) compared to Baobab (A), Cotton seed oil (C) and Ground nut (D) may be due the high proportion of unsaturated fatty acids, such as the mono-unsaturated oleic acid and the polyunsaturated linoleic acid (Dunn, 2005), (Gertz *et al*., 2000). Low molecular weight triacylglycerols are less viscous as high molecular weight triacylglycerols (Azian, *et al*., 2001). So when hydrogenated it increases the resistance to rancidity (oxidation) or change its physical characteristics of the oil. As the degree of saturation increases however, the oil's viscosity and melting point increase (Rabelo, *et. al*., (2000) due to intermolecular attractions of the long chain molecules. The decrease in viscosities of the oils beyond the 5th weeks of incubation suggests the formation of low molecular weight compounds possibly due cleavage of the glycosidic bonds (depolymerization). This could be the products that are detected by the FTIR analyzer.

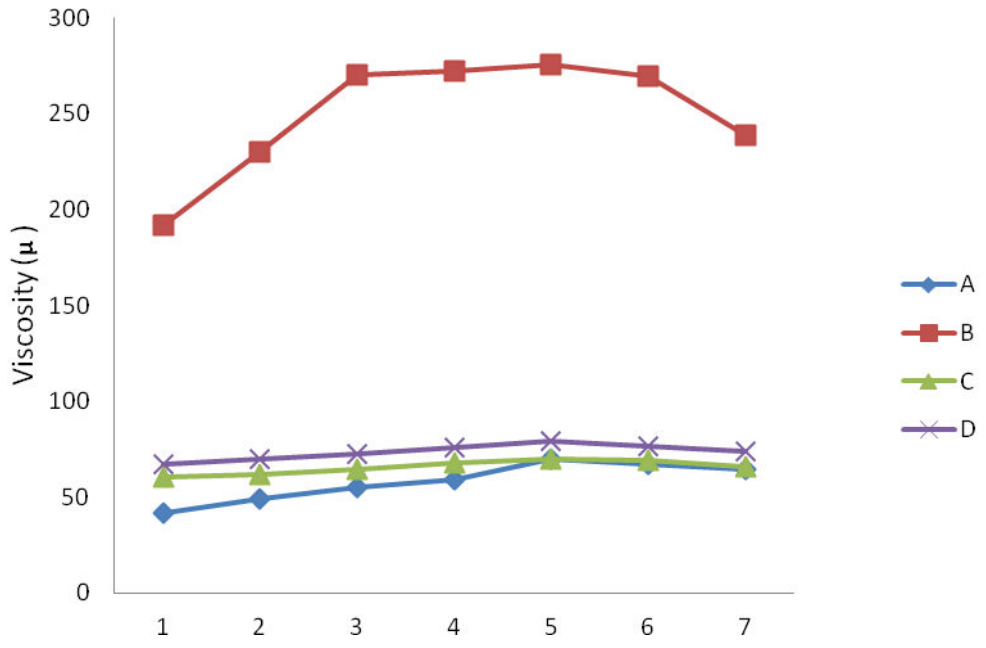

Fig. 1: Graph of Viscosity against Time (days) of A_1 (Baobab seed oil), B_1(Palm oil), C_1 (Cotton seed oil) and D_1 (Groundnut oil)

The use of hydrogenated oils in foods has never been completely satisfactory since partially hydrogenated oils and their trans- fats have been viewed to increase the risk of blood clotting inside blood vessels (Atinafu and Bedemo, 2011).

3.1 FTIR Analysis of Biodegraded oils

The FTIR analysis of biodegradation of oils given in figure IV revealed the disappearance of absorption peaks in degraded Palm oil (B2) and the introduction of an absorption peak in degraded Ground nut oil (D2) after the 5th week of incubation. This supports the assertion that biodegradation of the four different oils may have been completed on the 5th weeks irrespective of the composition of the oil. Carboxylic group absorption band appeared in Baobab seed oil and Palm oil unlike the hydroxyl group which is seen in all the oils. These may suggest the formation of different primary or secondary metabolites during the biodegradation of the oils. This could be due to the difference in composition the triglycerides but unique to every seed oil (Bharathi,*et. al*., 2012). Since triglyceride molecule is bound to three fatty acids molecules, the formations of other metabolic products without carboxyl or hydroxyl functional groups are possible. Groups such as ketone, aldehyde, esters, etc. have been reported also as possible degradation products (Vlactos, *et al*, 2012) from vegetable oils.

Table 3: Result of the FTIR of Un-inoculated oils

No. of peaks	Peaks (cm^{-1})			
	A1	B1	C1	D1
1	423.39	450.39	444.61	445.57
2	715.61	717.54	722.37	714.65
3	939.36	1166.97	1166.97	1166.01
4	1173.72	1449.55	1357.93	1449.55
5	1442.8	1735.99	1451.48	1742.74
6	1729.24	2927.08	1742.74	2352.27
7	2670.53	3464.27	2929.97	2928.04
8	2929.00		3472.96	

Key: A_1 (Baobab seed oil), B_1 (Palm oil), C_1 (Cotton seed oil) and D_1 (Groundnut oil).

Table 4: Result of the FTIR of inoculated (Biodegraded) oils

No. of Peaks	Peaks (cm^{-1})			
	A2	B2	C2	D2
1	443.64	452.32	458.11	445.57
2	711.76	1442.80	717.54	716.58
3	936.47	1719.60	1168.90	1166.97
4	1260.78	2930.93	1358.90	1363.72
5	1443.77	3425.69	1448.59	1449.55
6	1716.70		1741.78	1740.81
7	2928.04		2346.48	2348.41
8	3359.14		2929.00	2930.93

Key: A_2 (Baobab seed oil), B_2 (Palm oil), C_2 (Cotton seed oil) and D_2 (Groundnut oil)

Table 5: Result of the pH Values of inoculated Vegetable Oils

Time of Incubation (days)	pH			
	A2	B2	C2	D2
0	7.04	7.03	7.06	7.09
7	6.45	6.53	6.47	6.59
14	6.34	6.39	6.40	6.45
21	6.09	6.22	6.33	6.09
28	5.92	5.89	5.86	5.79
35	5.89	5.85	5.88	5.91
42	5.91	5.90	5.89	5.89

Key: A_2 (Baobab seed oil), B_2 (Palm oil), C_2 (Cotton seed oil) and D_2 (Groundnut oil)

Plate 1: Photograph of the biodegraded oils (from left to right) Groundnut D, Baobab seed A, Palm oil B, and Cotton seed C.

Conclusion

Biodegradation of some vegetable oils was determined by the measurement of viscosity and spectroscopic methods. The oil showed a gradual increase in viscosity due to the growth of microbes. The oil is broken down and used as source of food and energy. Also, the viscosity increase may be due the formation of long chain compounds which temporally entangled. The growth of microbes may have resulted un-bonding of long chain molecules and lubrication of chains therefore, a decrease in viscosity. The presence of high molecular weight polyunsaturated molecules and low free fatty acids in oil such as Palm oil is obtained to be responsible for a high value of viscosities. Oils such as Baobab seed oil with higher free fatty acids showed lower viscosities and therefore, degrade easily. The FTIR spectrogram revealed the formation biodegradable products. Edible oils with high free fatty acid value would therefore, biodegrade easily if contaminated by common fungi such as

Aspergillus niger at temperature of 35^{o}C. The consumption of edible oil without frying commonly practiced in Nigeria may have been the source of diseases such as diarrhea, flu, vomiting, etc. It is recommended that edible Oils such as Palm, Ground nut, Cotton seed oil should be stored at temperature below 30^{o}C and properly covered to prevent any contact with fungi.

References

Atinafu, D.G and Bedemo, B (2011), Estimation of Total fatty Acid and Cholesterol Content in some Commercial Edible Oils in Ethopia Bahir DAR. *Journal of Cereals and Oil Seeds* 2(**6**): 71-76.

Azian, M.N; Mustafa- Kammal, A.A; Panau, F and Ten, W.K (2001), Viscosity Estimation of Triacylglycerols and of some Vegetable Oil, Based on their Triacylglycerol Composition. *Journal of American Oil Chemists' Society*. 78(**10**):1001-1005.

Bennett, J.W (2010), "An overview of the Genus *Aspergillus*." *Aspergillus* Molecular Biology and Genomic. Caister Academic Press 53 **(1)** pp 978-9044 Availablethrough*http://www.open-access biology.com/Aspergillus/Aspergillus1.pdf* (accessed 19th December, 2011).

Broekhuizen P. Theodori D. Le Blanch K. Ullmer (2003), Lubrication in Inland and Coastal Water Activities. A.A. Balkema Publishers, Tokyo, p 34-36.

Bharathi, P; Elavarasi, N and Mohanasundaram, S (2012), Studies on Rate of Biodegradation of Vegetable (Coconut) oil by using *Pseudomonas aeruginosa*. *International Journal of Environmental Biology* 2(**1**):12-1

Dunn R.O (2005), Effect of Antioxidant on the Oxidative Stability of Methyl Soyate (Biodiesel). *Fuel Processing Technology* 86: 1071-1085.

Ekwenye, U.N and Ijeomah, C.A (2005), Antimicrobial Effects of Palm Kernel Oil and Palm Oil. *KMITL Science Journal* 5(**2**): 502-505.

Gertz C. Klostermann S. Kochhar SP. (2000), Testing and Comparing Oxiative Stability of Vegetable Oils and Fats at Frying Temperature. *Europia Journal of Lipid Science Technology* 102 (**8**): 543-541.

Gomaa, O.M; El Kareem, H.A and Fatahy, R (2012), Assessment of the Efficacy of *Aspergillus* sp. EL-2 in Textile Waste Water Treatment. *Biodegradation* 23:243-251

Jain, P.C. (2008), Microbial Degradation of Grains, oil Seeds, Textiles, Wood, Corrosion of Metals and Bioleaching of Mineral Ores**.** *Applied Microbiology*: 1-37

Joseph, A.F (1977), Measuring Flavour Deterioration of Fats**,** Oils and Foods. General Food Cooperation Technical Centre, New York. P 1-7.

Larsen, H (2007), Behaviour of Polymer Muds under High Pressure – High Temperature Conditions. A Master Thesis Presented at IPT, NTNU pp 10-30

Li, Z; Wrenn, B.A and Venosa, A.D (2005), Aerobic Biodegradation of Vegetable Oil and its Metabolic Intermediates in oil-enriched Freshwater Sediments. *Biodegradation* 16:341-352.

Meier, M.A.R; Metzger, J.O and Schubert, U.S (2007), Plant Oil Renewable Resources as Green Alternatives in Polymer Science. *Critical Review* 36:1788-1802

Obire, O; Anyanwu, E.C and Okigbo, R.N (2008), Saprophytic and Crude Oil Degrading Fungi from Cow Dung and Poultry Dropping as Bioremediation Agents. *Journal of Agriculture Technology* 4(**2**):81-89.

Pradeep, S; Fasela, P; Sarath-Josh, M.K; Balachandran, S; Sudha-Devi, R and Benjamin, S (2012), Fungal Biodegradation of Phthalate Plasticizer in situ. *Biodegradation*

Rabelo, J; Batista, E; Cavaleri, F.W and Meirelles, A.J.A (2000), Viscosity Prediction for fatty systems. *Journal of American oil Chemists Society* 77(**12**):1255-1258

Rodenbush, C.M; Hsieh, F.H and Viswannath, D.S (1999), Density and Viscosity of Vegetable Oils. *Journal of American Oil Chemists Society* 76(**12**):1415-1417

Saifudin N, Chua KH (2006), Biodegradation of Lipid-rich Waste Water by Combination of Microwave Irradiation and Lipase Immobilized on Chitosan. *Biotechnology* 5 (**3**): 315-323.

Stanciu, I (2012), A New Viscosity-shear Rate Relationship for Rapeseed oil. *Journal of Science and Arts* 3(**2**):313-316

Tagoe, S.M.A; Dickson, M.J and Alexander, M.M (2012), Factors Influencing Quality of Palm Oil Produced at the Cottage Industry Level in Ghana. *International Food Research Journal* 19(**1**):271-278

Thota, P; Bhogavalli, P.K; Vallem, P.R and Sreerangam,V (2012), Screening and Identification of Potential Fungal Strains for the Production of Extracellular Lipase from Soil. *Plant Sciences Feed* 2(**5**):79-84

Vlachos, N; Skopelitis, Y; Psaroudaki, M; Konstantinidou, V; Chatzilazarou, A and Tegou, E (2006), Applications of Fourier Transform-infrared Spectroscopy to Edible Oils. *Analytica Chimica Acta* 573–574:

Yang, Y.S; Zhou, J.T; Lu, H; Yuan, Y.L and Zhao, L.H (2011), Isolation and Characterization of Fungus *Aspergillus* sp. Strain F-3 Capable of Degrading Alkali Liginin. *Biodegradation* 22:1017-1027.

Aknowledgement
The authors are grateful to Mr. Umar Galadima for the microbiological analysis conducted. The contribution of a senior laboratory technologist, Mr. Shauibu Gombe is also appreciated. We are indebted to Gombe State University and NACRICT Zaria-Kaduna State for their financial support and spectroscopic analysis done respectively.