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Degradation of Groundnut Oil Using Yeast on Organic Surface

[1]**Fai F. Yirankinyuki,** [2]**Buhari Magaji**[*]**,** [3]**Abdurrahaman Yusuf Muazu**

Department of Chemistry,
Gombe State University, Nigeria

E-mail: magaji.buharii@gmail.com

Abstract

The present research present degradation of ground nut oil with Saccharomyces cerevisiaeon organic surface produced from banana leaves. The products of degradation were fractionated into aqueous and organic phases. Gas chromatography mass spectroscopy (GC-MS) analysis was carried out on the aqueous and organic phases of yeast degraded groundnut oil to confirm biodegradability. The fractions were found to contain twenty-six (26) new compounds. All these new compounds generated by Saccharomyces cerevisiae degradation are found to be useful compounds in different industries. Yeast degradation of groundnut oil could therefore be a substitute for source and technology for producing these compounds in future.

Keywords: Biodegradability, Gas chromatography-mass spectroscopy, Organic surface, *Saccharomyces cerevisiae,* Yeast degradation

INTRODUCTION

Groundnut (*Arachis hypogaea L.*) also known as peanut is a legume crop grown mainly for its edible seeds. Groundnut is classified as both a grain legume and oil crop due to its high oil content. China is the largest producer as well as consumer of groundnut in the world with 166.24 × 10^5tonnes, India (68.57 ×10^5 tonnes), Nigeria (30.28 ×10^5 tonnes) and United States of America (25.78 ×10^5tonnes) as reported by Nautiyal, 2002; Madhusudhana, 2013 and Ajeigbe *et al*., 2014. In West Africa, Nigeria is the largest groundnut producing country, accounting for 51% of the region's groundnut production. The country contributes 10% of total global production and 39% of Africaproduction (Nautiyal, 2002; Nautiyal *et al*., 2002; Madhusudhana, 2013; Ajeigbe *et al*., 2014; Okello *et al*., 2013).Between 1956 and 1967, Nigeria's most valuable single export crop was groundnut(Ajeigbe *et al*., 2014).Groundnut is major source of edible oil as well as livelihoods for small-scale farmers in Northern Nigeria. It generates employment for the rural dwellers because it is a capital-intensive crop. Groundnut products like oil and cake accounted for a significant percentage of total Nigerian export earnings. And also, local demand from oil expressing industries and confectioners make groundnut a cash crop of significance to the economy of Nigeria.

Groundnut oil is an organic substance that can undergo biodegradation. Organic materials can be degraded aerobically or anaerobically. Biodegradation can be referred to as chemical breakdown or transformation of a substance caused by organisms or their enzymes

*Author for Correspondence

(Emmanuel *et al*., 2009).Yeasts have a wide range of applications mainly in food industries including wine making, brewing, distilled spirit production, and baking and in biomass production (Zymanczyk-Duda *et al*., 2017).More recently, yeast has also been used in the biofuel industry and for the production of heterologous compounds. Many yeast strains can function under both anaerobic as well as aerobic conditions of environment, switching their metabolism types easily (Otterstedt *et al*., 2004).Moreover, yeasts as fungi have been exploited by mankind for thousands of years for food and fermentation processes. Traditionally the yeast has been used for the production of alcoholic beverages, biomass and glycerol. *Saccharomyces cerevisiae* has been described as mankind's most domesticated organism and still widely exploited yeast species in industries today (Zymanczyk-Duda *et al*., 2017). The number of yeast species described so far is about 1500 and only about a dozen is used at industrial scale (Barnett and Barnett, 2011).Some 70-80 species have been shown to possess potential value for biotechnology (Kurtzman *et al*., 2011). According to modest estimate (Hawksworth, 2004), known yeast species represent roughly 5% of the total number that may inhabitant Earth surface. Modern applications of yeasts have been greatly expanded beyond classical applications. Yeasts, especially *S. cerevisiae* and other non-*saccharomyces* yeasts today are increasingly used for the heterologous production of enzymes and pharmaceutical proteins (Çelik and Çalık 2012). Yeasts have important roles in environmental applications such as bioremediation and removal of heavy metals from waste waters (Tondee, 2008). Yeasts are also used in agriculture as biocontrol agents (Buzzini and Margesin, 2014). Several chemicals can be produced using yeast as a biocatalyst (Kapoor and Gupta, 2012). To the best of our knowledge, little or no attention has been given to degradation of groundnut oil with *Saccharomyces cerevisiae*. Thus, the focus of this research is to study the degradation process of groundnut oil with yeast and find out if the products of fungal degraded groundnut oil could contain useful chemicals for various industries.

MATERIALS AND METHODS

Sample collection and preparation

The organic surface (lignocellulose) was extracted from banana leaves. Groundnut oil was collected from Gombe town (i.e. bought from groundnut millers at British Cotton Ginnery Area (BCGA) industrial/commercial layout), Gombe. About 100g of fresh and matured banana leaves were collected from banana plants growing in the staff quarters of Gombe State University. The leaves were sun-dried for one week and later oven-dried at 40 °C for 4 hours and grounded using a pestle and mortar, sieved to fine particles using a sieve of 250 microns. A 40g of grounded dried banana leaves were weighed and extracted with n-hexane in a soxhlet extractor for 72 hours, and dried in an oven.

Experimental procedures

In other to know if *S. cerevisiae* will digest the organic surface, about 4 g of the processed organic surface and 0.016 g of the yeast (*S. cerevisiae*) were weighed and mixed with 25 ml of distilled water in a 250 ml flat bottom flask and stoppered loosely. The mixture (which serves as a control) was kept for 21 days for fungal degradation to take place. The product of the degraded organic surface by yeast was analyzed with a GC-MS so as to be able to differentiate the degradation products of the oil and any possible degradation products of the organic surface.

In a 100 ml flask containing 25 ml of distilled water, 0.4 g of the groundnut oil and 0.016 g of the *S. cerevisiae* were added and mixed thoroughly. The mixture was transferred to a to 250 ml flask containing 3.6 g of the organic surface and allowed to digest for 21 days. More so,

25 ml of water and 30 ml of hexane was added to the digested solution, stirred, filtered and vacuum pump. The hexane layer and the water layer were separated and each of the extracts was transferred to a weighed beaker and dried to a constant weight. The hexane fraction was dried in a fume cupboard while the water fraction was dried in a water bath. GC-MS analysis was carried out to identify the composition of the digestion products.

Gas chromatography-mass spectrometry (GC-MS)

The GC-MS analysis was done at the American University of Nigeria (AUN), Adamawa. The GC-MS model used was GC-MS-7890A, Agilent Technologist Inert MSD-597CM with the following conditions: Column agilent-1 fused silica capillary column (30 m x 250 µm x 0.25 µm, composed of 5% Phenyl Methyl Silox). For GC-MS detection, an electron ionization system with ionization energy of 74eV was used. Helium gas was used as the carrier gas at constant flow rate of 3.8379ml/min and an injection volume of 1 µl was employed with splitless injection mode, injector temperature 270 ◦C and ion source temperature 250 ◦C. The oven temperature was programmed initially at 80 ◦C for 0 minute, decreased to 10 ◦C for 1 minute then increased to 300 ◦C for 5 minutes. The flow control mode was at an average velocity of 72.418 cm/sec, pressure 32.475 psi, the column flow was 3.8379ml/minute the purge flow was 1 ml/minute. The total flow was 54.838/minute. Mass spectra were taken at 74eV; a scan of 27 minutes and fragment from 50 to 550.

RESULTS AND DISCUSSION

The GC-MS analysis of the groundnut oil showed the presence of twenty-four (24) compounds as shown in Figure 1. The compounds are presented in Table 1 with their retention time (RT), molecular formula (MF), molecular weight (MW), and percentage composition.

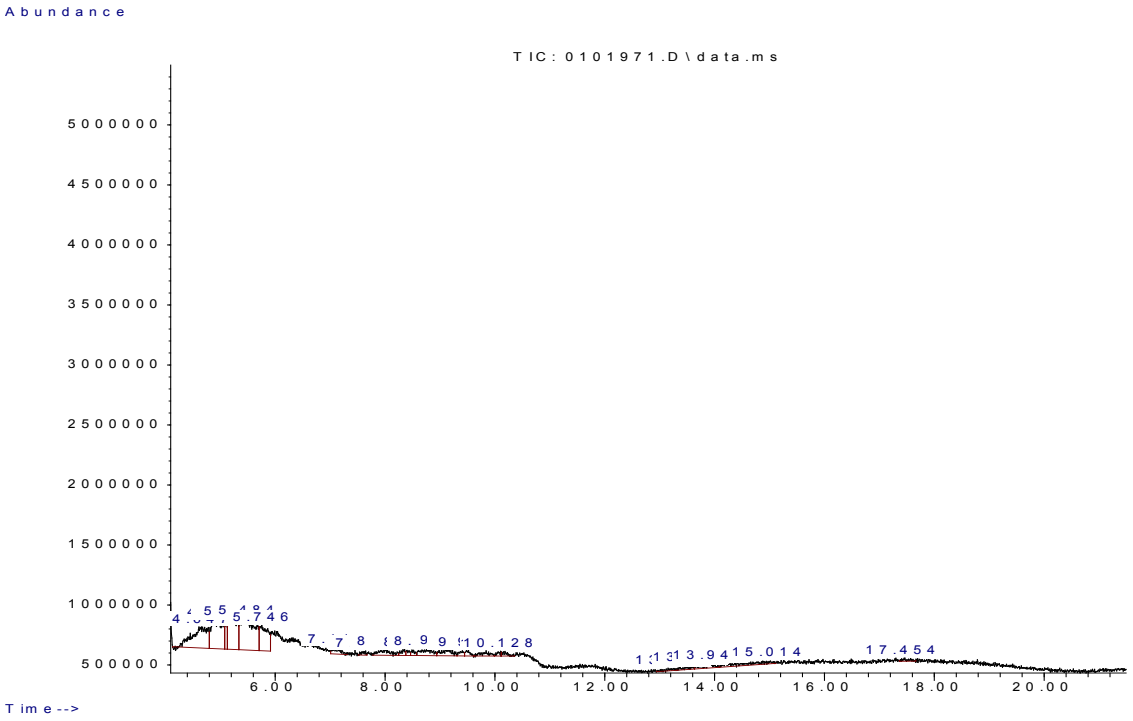

Figure 1: Detection of compounds in groundnut oil using GC-MS.

Table 1: Characteristics of the detected compounds in groundnut oil obtained from GC-MS analysis.

Peak#	RT	Area%	Name of compound	MF	MW
1	4.6	14.2	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.5
2	4.9	13.9	Cyclopentadecanone, 2-hydroxy-	$C_{15}H_{28}O_2$	240.4
3	5.1	2.6	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.5
4	5.2	11.6	n-Propyl 11-octadecenoate	$C_{21}H_{40}O_2$	324.5
5	5.5	20.2	Oleic Acid	$C_{18}H_{34}O_2$	282.5
6	5.7	9.4	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282.5
7	7.1	2.0	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.5
8	7.6	0.7	Oleic Acid	$C_{18}H_{34}O_2$	282.5
9	8.0	2.9	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5
10	8.3	1.0	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5
11	8.4	0.7	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.5
12	8.5	0.8	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5
13	8.7	3.3	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.5
14	9.2	2.9	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.5
15	9.4	1.0	Oleic Acid	$C_{18}H_{34}O_2$	282.5
16	9.5	1.1	6-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	282.5
17	9.8	1.6	Oleic Acid	$C_{18}H_{34}O_2$	282.5
18	10.1	0.7	Oleic Acid	$C_{18}H_{34}O_2$	282.5
19	10.1	0.9	Oleic Acid	$C_{18}H_{34}O_2$	282.5
20	13.3	0.7	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5
21	13.6	1.1	cis-10-Nonadecenoic acid	$C_{18}H_{34}O_2$	282.5
22	13.9	1.8	9-Tricosene, (Z)-	$C_{23}H_{46}$	322.6
23	15.1	3.9	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5
24	17.5	0.7	Oleic Acid	$C_{18}H_{34}O_2$	282.5

*RT = Retention time, MF = Molecular formulae and MW = molecular weight

The GC-MS analysis of the aqueous fraction of the degraded groundnut oil revealed the presence of nineteen (19) compounds as shown in Figure 2. The compounds with their retention time (RT), molecular formula (MF), molecular weight (MW), and percentage composition are presented in Table 2.

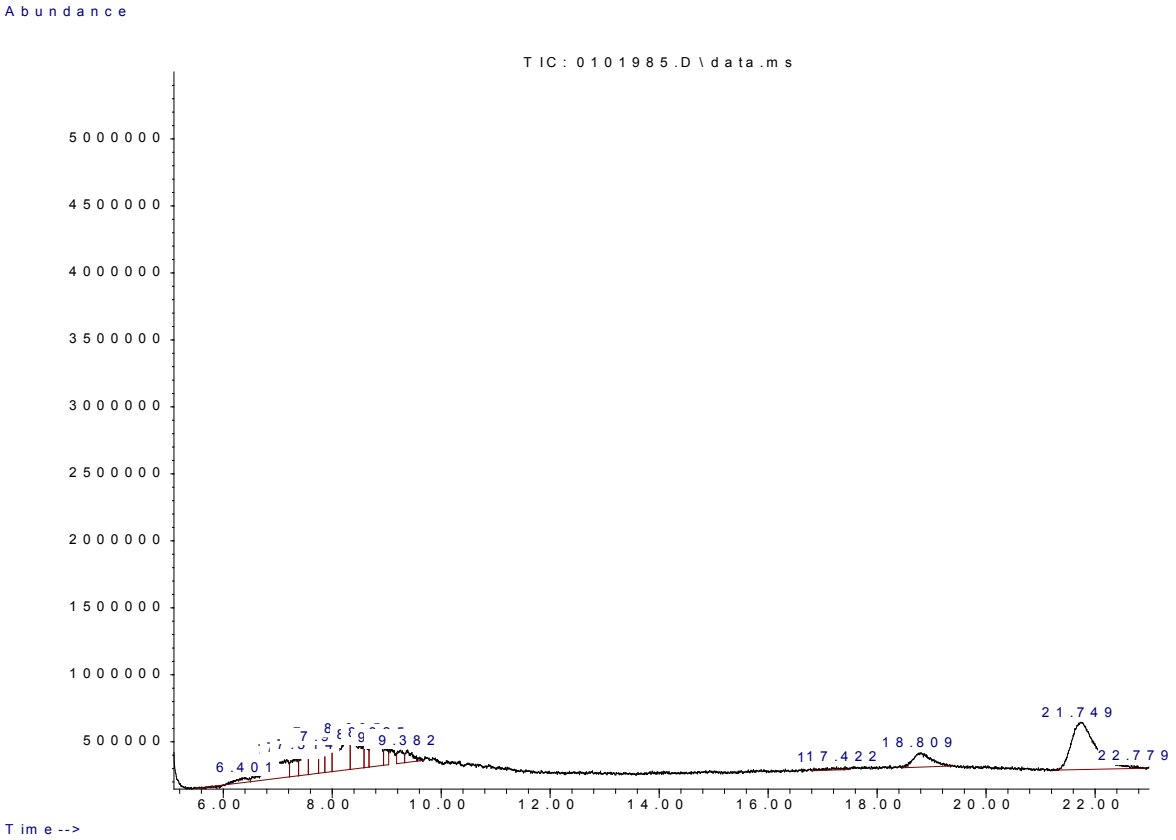

Figure 2: Detection of compounds in aqueous fraction of degraded groundnut oil using GC-MS.

Table 2: Characteristics of the detected compounds in aqueous fraction of the degraded groundnut oil obtained from GC-MS analysis.

Peak#	RT	Area%	Name of compound	MF	MW
1	6.4	1.2	N,N,2,7-Tetramethyl-2,7-octadien-1-amine	$C_{12}H_{23}N$	181.3
2	7.2	8.4	cis-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.5
3	7.3	3.2	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.5
4	7.5	3.9	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.5
5	7.7	5.5	13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.5
6	7.8	3.7	7-Hexadecenoic acid, methyl ester,(Z)-	$C_{17}H_{32}O_2$	268.4
7	7.9	3.9	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{32}O_2$	296.5
8	8.3	10.0	cis-9-Hexadecenal	$C_{16}H_{30}O$	238.4
9	8.4	7.5	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296.5
10	8.6	2.2	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.5
11	8.8	6.6	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.5
12	8.9	1.8	cis-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.5
13	9.3	1.9	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296.5
14	9.4	2.3	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296.5
15	17.3	0.8	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	356.5
16	17.4	0.1	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.5
17	18.8	6.8	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256.4
18	21.8	29.9	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.5
19	22.8	0.2	i-Propyl 9-octadecenoate	$C_{21}H_{40}O_2$	324.5

*RT = Retention time, MF = Molecular formulae and MW = molecular weight

The GC-MS analysis of organic fraction of the degraded groundnut oil revealed the presence of twenty-three (23) compounds as shown in Figure 3 and, the compounds are listed with their retention time (RT), molecular formula (MF), molecular weight (MW), and percentage composition in Table 3.

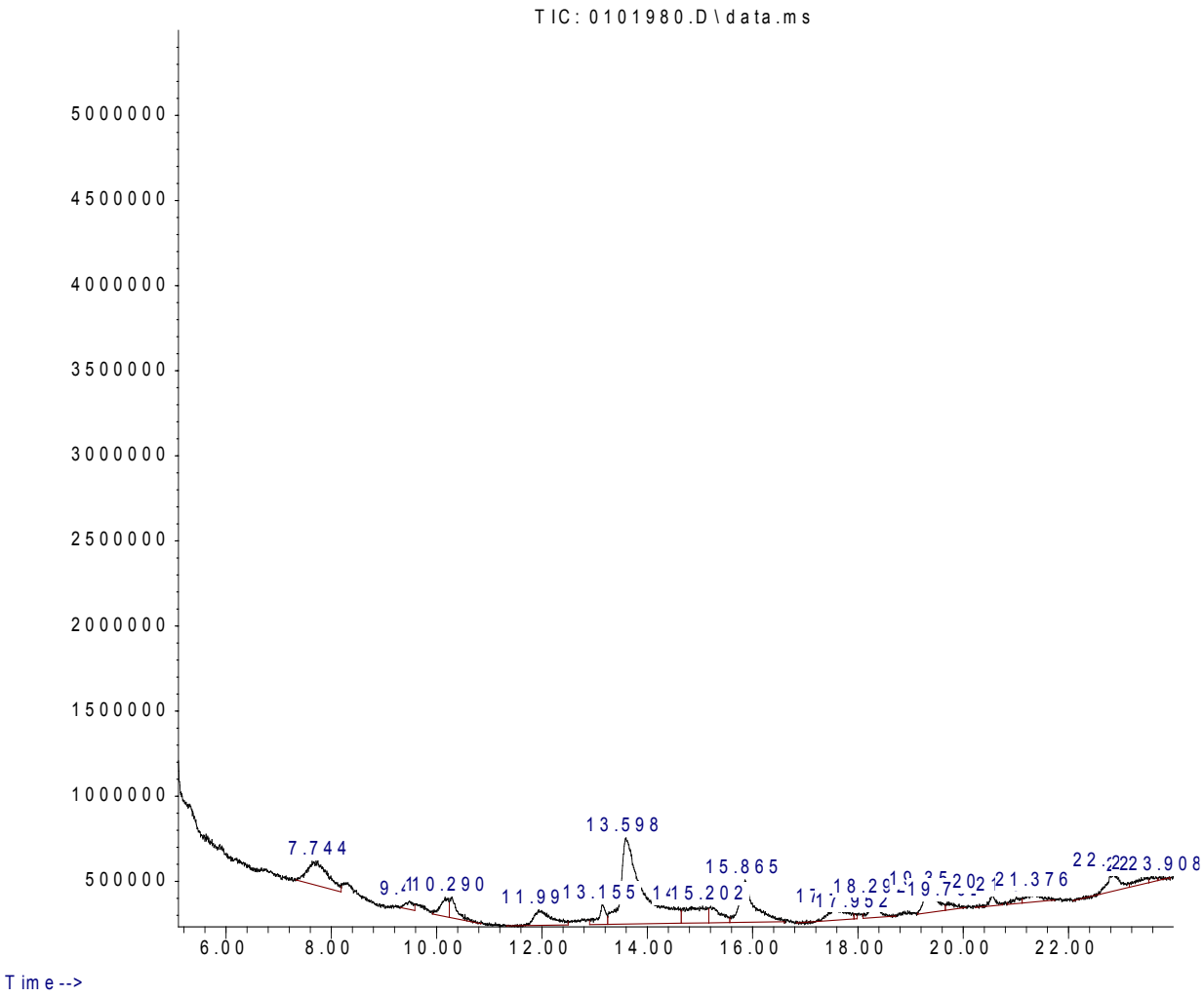

Figure 3: Detection of compounds in organic fraction of degraded groundnut oil using GC-MS.

Table 3: Characteristics of the detected compounds in organic fraction of the degraded groundnut oil obtained from GC-MS analysis.

Peak#	RT	Area%	Name of compound	MF	MW
1	7.8	8.7	9-Oxatetracyclo[5.3.1.0(2,6).0(8,10)]undec-3-ene	$C_{10}H_{12}O$	148.2
2	9.5	1.1	2-Octyne	C_8H_{14}	110.2
3	10.2	2.7	Butylated Hydroxytoluene	$C_{15}H_{24}O$	220.4
4	10.3	2.7	Butylated Hydroxytoluene	$C_{15}H_{24}O$	220.4
5	11.9	4.7	Methoxyacetic acid, tetradecyl ester	$C_{17}H_{34}O_3$	286.4
6	13.2	2.6	Dithioerythritol, O,O',S,S'-tetrakis(trimethylsilyl)-	$C_{16}H_{42}O_2S_2Si_4$	443
7	13.6	34.1	Cyclohexane, 1-ethyl-2-methyl-, cis-	C_9H_{18}	126.2
8	14.9	6.2	cis-10-Heptadecenoic acid	$C_{17}H_{32}O_2$	268.4
9	15.2	3.3	3-Eicosene, (E)-	$C_{20}H_{40}$	280.5
10	15.9	10.9	1-Tridecene	$C_{13}H_{26}$	182.4
11	17.6	3.5	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280.5
12	17.9	0.2	E-15-Heptadecenal	$C_{17}H_{32}O$	252.4
13	18.3	2.8	Mandelic acid, 2TBDMS derivative	$C_{20}H_{36}O_3Si_2$	380.7
14	19.4	4.9	Tetracontane, 3,5,24-trimethyl-	$C_{43}H_{88}$	605.2
15	19.7	1.1	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	234.1
16	20.6	1.3	Heptasiloxane, hexadecamethyl-	$C_{16}H_{48}O_6Si_7$	533.1
17	21.0	0.8	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282.5
18	21.4	2.2	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.5
19	22.9	4.1	1-Decanol, 2-octyl-	$C_{20}H_{42}O$	298.6
20	23.5	1.3	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.5
21	23.6	0.5	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.5
22	23.7	0.2	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5
23	23.9	0.1	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.5

*RT = Retention time, MF = Molecular formulae and MW = molecular weight

The GC-MS analysis of the fungal degraded groundnut oil showed the presence of twenty-six (26) new compounds which include; N,N,2,7-Tetramethyl-2,7-octadien-1-amine, cis-13-Octadecenoic acid, methyl ester, 11- Octadecenoic acid, methyl ester, 13- Octadecenoic acid, methyl ester, 7- Hexadecenoic acid, methyl ester, (Z)-, 9- Octadecenoic acid, methyl ester, cis- 9- Hexadenal, 9- Octadecenoic acid (Z)-, 2,3- dihydroxypropyl ester, Pentadecanoic acid, methyl ester, i-Propyl 9-Octadecenoate, 9-Oxatetracyclo[5.3.1.0(2,6).0(8,10)]undec-3-ene, 2-Octyne, Butylated Hydroxytoluene, Methoxyacetic acid, tetradecyl ester, Dithioerythritol, O,O',S,S'-tetrakis (trimethylsilyl)-, Cyclohexane, 1-ethyl-2-methyl-, cis-, cis-10-Heptadecenoic acid, 3- Eicosene, (E)-, 1- Tridecene, 2- Methyl-Z,Z-3,13- Octadecadienol, E-15- Heptadecenal, Mandelic acid, 2TBDS derivative, Tetracontane, 3,5,24-trimethyl-, 2-Piperidinone, N-[4-bromo-n-butyl]-, Heptasiloxane, hexadecamethyl-, and 1-decanol, 2- octyl-.

Some of the identified compounds found in the aqueous and organic fraction of the degraded groundnut oil have shown some biological activities.9-Octadecenoic acid (Z)-, methyl ester has been reported to be anticarcinogenic and antioxidant. Heptacosane also has antioxidant activity. Hexadecanoic acid**,** methyl ester was reported to have hypocholesterolemic, antifungal, antioxidant, potent antimicrobial, nematicide, pesticide, anti-androgenic, flavour, haemolytic, 5-Alpha reductase inhibitory activities (Ogukwe *et al*., 2016).Several authors have shown that natural aromatic compounds possess important biological activities, such as antitumor, antihepatotoxic, antioxidant, anti-inflammatory, estrogenic and antibacterial activities (Ogunlesi *et al*., 2010). Therefore, these compounds with these activities could be obtained from digestion of groundnut oil instead of getting

them from petrochemical industries using petroleum which is not renewable as a raw material.

CONCLUSION

In the present study, groundnut oil was degraded by using *S. cerevisiae* in aqueous and organic phases. Twenty-six (26) new compounds were identified from both the aqueous and organic fractions of the degraded groundnut oil by GC-MS. This study has shown that the degraded groundnut oil in both fractions is rich in bioactive compounds which could have high potential industrial and biological activities. Therefore, fungal degraded groundnut oil could be a good alternative source for many industrial chemicals that are currently source from petroleum.

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