ACTA SCIENTIFIC NUTRITIONAL HEALTH

Volume 3 Issue 6 June 2019

Research Article

Biochemical and Longevity Implications of Consumption of Repeatedly Fried Palm Oil in *Drosophila Melanogaster*

Adeniran OY*

Department of Biochemistry, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria

***Corresponding Author:** Adeniran OY, Department of Biochemistry, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria.

Received: May 03, 2019; **Published:** May 27, 2019

Abstract

Deep fat frying is a food preparation process esteemed by consumers for the pleasurable taste and texture notwithstanding the major harmful health implications it poses. This study was carried out to characterized secondary oxidation products of thermally oxidized palm oil and investigates the biochemical toxicity in *drosophila melanogaster*. Oxidized palm oil generated by intermittent frying of 800g of sweet potato and milled beans in 2000ml of palm oil was used to formulate feed for *D. melanogaster*. Secondary oxidation products of the fried oils were assessed by gas chromatography mass spectrophotometry (GC-MS) technique. Real time-Polymerase Chain Reaction (RT-PCR) expression of selected antioxidant and neurological mRNA genes [Glucose 6 phosphate dehydrogenase (G6PD), Glutathione-s-transferase (GST), Heat shock protein 70A (HSP 70A) and Acetylcholine esterase (AChE)] were evaluated, in addition to Catalase and Superoxide dismutase (SOD) activities. Furthermore, a 39-day survival assay was determined. Our results showed the presence of established toxicants in the fried oils but absent in the fresh oil. There was upregulation in the activities and gene expression of antioxidant enzymes ($P<0.05$). The survival curve analysis revealed that at the end of the 30th day, only 5% of the flies in the test group survived as against 50% remaining in the control group. Our results show the adverse effect of oxidized oil on life span and possible involvement of the nrf2-keap1 pathway in the oxidative toxicity of the secondary oxidation products.

Keywords: Thermally Oxidized Palm Oil; GC-MS, Survival Assay; mRNA Gene Expression; *Drosophila melanogaster*

Introduction

Palm oil (*Elaeis guineensis*), is an edible vegetable oil derived from the mesocarp (reddish pulp) of the fruit of the oil palms with Nigeria as one of the leading producers in the world [1]. Palm oil is reported to have overtaken soybean oil in the world's market [2]. It is a notable source of cooking oil in various countries, especially in Africa and Asia [3] and has continued to enjoy increased patronage in recent times as a result of many nutritional values such as its reportedly being free of trans-fats [4]; richest source of carotenoids which important roles as biological antioxidants and high content of vitamin E (tocotrienols) which possess powerful antioxidant property and are capable of inhibiting cholesterol synthesis [5]. Palm oil is unique in its fatty acid composition as it is the only cooking oil with approximately equal composition of saturated (50%) and unsaturated fatty acids (50%) [1]. In the food industry and culinary practice, the application of heat to edible fats and oil like frying is one of the major methods used for food processing and fried foods have provided culinary delight to people worldwide for centuries. Formation of volatile organic compounds such as aldehyde, ketones, dienes and acids during degradation of edible oil induced by heating has been reported [6]. The oxidative degradation products do not only create unpleasant flavour or reduce the shelf life of cooking oils, they may also cause health challenges [7] due to capacity to cause oxidative stress [8] which has been implicated in the pathogenesis of various degenerative diseases including cancer [9], Alzheimer disease [10], myocardial infarction [11], kidney dysfunction [12], diabetes mellitus [13], and its complications [14].

There is paucity of information on the effects of consumption of repeatedly used palm oil on gene expression in animal models such as *drosophila melanogaster* which has become a better model organism for research especially since the prevalence of "animal rights" which have restricted the use of some other animals as research subjects. This present study was carried out to character-

ized secondary oxidation products of thermally oxidized palm oil and investigates the biochemical toxicity in *D. melanogaster*.

Materials and Methods

The palm oil used was purchased from a local processing company in Ikare, while sweet potatoes and beans were purchased from Ibaka market, Akungba Akoko, Ondo State. The model organism- *Drosophila melanogaster* (Harwich strain) was obtained freely from Dr. A. O. Abolaji of the Department of Biochemistry, University of Ibadan.

Frying process

Exactly 2000 ml each of fresh soya bean oil were used to fry 800g each of sliced potatoes and milled beans at 150ºC for 30mins to simulate the conditions for deep frying normally employed in homes and commercial outlets in Nigeria. The oils were cooled for 5 hours before the frying process was repeated with a fresh batch of samples for five consecutive days. The samples collected were subjected to GC-MS analysis.

Group of oil	Treatment
CPO	Control (Unheated) palm oil
SPO	Thermally oxidized palm oil used to fry sweet potato chips
BPO	Thermally oxidized palm oil used to fry bean cake
HPO	Heated palm oil

Table 1: Grouping of oil based on treatment.

Chromatographic and mass spectrophotometry determination of secondary oil oxidation products

A Hewlett Packard HP 6890 Series gas chromatograph coupled with a Hewlett Packard 5973 mass spectroscopy detector (GC/MS) system was used. A HP"5 MS capillary column (30 m length, 0.25 mm i.d., 0.25 µm film thickness) was used for the GC system. The temperature program was set up from 50°C to 250°C at 4°C/min, both the injector and detector temperatures were 280°C and Helium was used as carrier gas. The injection volume was 2µL. Ionization energy EI of 70 eV was used for mass spectroscopy detector, with a source temperature of 150°C, scan range 50-300 amu and scan rate $1s^{-1}$. The mass spectra were compared with the NIST/EPA/NIH Mass Spectral Library 2.0 [15].

Feed formulation

A modified semi synthetic diets (Sigma Aldrich diet for flies) containing the fresh palm oil as well as the thermoxidized oils were formulated as presented in table while the flies were grouped based on the diets as shown in table 2.

Ingredient	Amount in diet (g/kg)	
	Fresh oil diet	Fried oil diet
Corn Meal	276.29	276.29
Yeast, Brewers	50.29	50.29
Glucose	166.05	166.05
Sucrose	82.88	82.88
Soybean Fiber	500.54	500.54
p-Hydroxybenzoic Acid, Methyl Ester	2.72	2.72
Oil	11.24	11.24
Agar	10.00	10.00

Table 2: Composition of feed diet fed to drosophila.

Group of animals	Diets
GC (CONTROL)	Commercial diet
TPPO	Feed + thermally oxidized palm oil from potato chips
TBPO	Feed + thermally oxidized palm oil from bean cake
FPO	Feed + fresh palm oil

Table 3: Drosophila grouping based on diet.

Animal treatment

The flies were allowed to mate in vials monitored under a regulated temperature until the eggs metamorphosed into young adult fruit flies under a natural photoperiod of about 12 hours light and 12 hours dark daily for the period of administration of the feed composition.

Flies were collected and separated into experimental groups (based on experimental diets) in seven vials of 20 flies each (in three replicates) and the flies were allowed to eat freely.

The flies were transferred every 3 to 5 days in order to prevent feed contamination and overpopulation due to emergence of new flies.

Longevity assay

Drosophila melanogaster (both gender) of 1 to 3 days old were divided into seven vials containing 20 flies each. A 39-days longevity assay was carried out, in order to determine the effect of toxicity of the oxidized oil on *Drosophila melanogaster's* life span.

The longevity assay consists of three replicates of the test and control groups in vials containing 20 flies each. The formulated diet was change once a week. The longevity assay was determined by recording the number of live and dead flies daily. At the end of the 39-days, the data was analyzed and plotted as number of death [16].

Enzyme assay

- **Preparation of sample for biochemical assays**: 20 flies (of both genders) were placed on the formulated diets for 6hrs, 24hrs and 2 weeks. At the end of the treatment period, flies were anaesthetized on ice, weighed, and homogenized in 0.1 M phosphate buffer, pH 7.0 (ratio of 1 mg:10 mL), and centrifuged at 4000 g for 10 min at 4^0C in a Biofuge 13 (Baxter Scientific Products, Germany). The supernatant was separated from the pellet into labeled Eppendorf tubes, and used for the determination of the activities of superoxide dismutase (SOD) and catalase (CAT).
- **SOD Activity Determination:** An aliquot of 20 samples and of 25.1M phosphate buffer pH 7.8 was transferred to separate wells of a clear flat-bottom 96-well plate and the reaction started by the addition of 30µl of freshly prepared 0.3nM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 25µl of phosphate buffer 30µl of substrate (adrenaline) and 20µl of water. The increase in absorbance at 480nm was monitored every 30seconds for 150seconds.
- **Catalase activity determination:** 1ml of the supernatant was mixed with 49ml distilled water to give a 1 in 50 dilution of the tissue homogenate. The assay mixture contained 4ml of H_2O_2 (800µmoles) solution and 5ml of phosphate buffer and mixed with the reaction mixture by a gentle swirling motion. The reaction was run at room temperature. 1 ml portion of the reaction mixture was withdrawn and blown into 2ml dichromate/acetic acid reagent at 60seconds intervals. The H_2O_2 contents of the withdrawn sample were determined by taking the absorbance at 570nm.

Procedure for gene expression

Briefly, the anesthetized animals were placed in trizol reagent and homogenized. Chloroform was added for phase separation, allowing collection of the aqueous phase containing RNA. Centrifugation (Abbott 10117429-3) was done at 3000 rpm for 15 min. and the aqueous phase was carefully transferred to a fresh tube where equal volume of 100% iso-amyl propanol was added to precipitate the RNA.

The RNA was converted to cDNA, by adding sequentially: Buffer, nuclease free water, the primers, dNTPs and then transcriptase to the RNA solution in the eppendorf tube. The solution was then loaded into the thermocycler (PCR machine Labnet International Inc.) to run at 42°C for one hour in order to amplify the cDNA.

Agarose gel electrophoresis (VWR Scientific Products model 300) was used to allow the genes expressed themselves along the column of the well on the gel.

The gel was removed from the electrophoretic tank and then placed on the photophoresis machine (Fotodyne Incorporated) for visualization and therefore analyzed using the DNA ladder in the first lane as a guide [17].

Target gene	FORWARD 5'-3'	REVERSE 5'-3'
GapDH	GTCTCACCCCATTCTAC-CGC	AGTTTGGCTACTCCAAC-CGC
Ache	CGCGGACTA-ATAGCCCCAAG	TCATCTTCGTCTTT-GCCCCG
GST	CGTGGAGGTAAACACTC-GGG	GTTTTGCCGTATTTG-GACACC
G6PD	GCTATGCCCGTTCCATGCT	GCGTCCGTCATATCT-GCCG
HSP70A	AATTGAGACCGCTGGAG-GTG	GACAGATCGAAGGT-GCCCAA

Table 4: List of PCR Primer for Drosophila Target Genes

Result

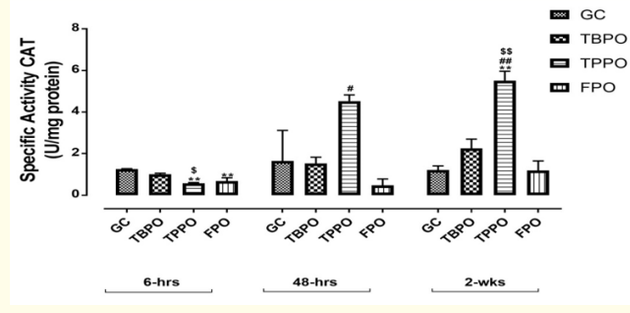


Figure 1: Changes in catalase activity in D. melanogaster at 6hrs, 48hrs and 2 weeks of feeding.

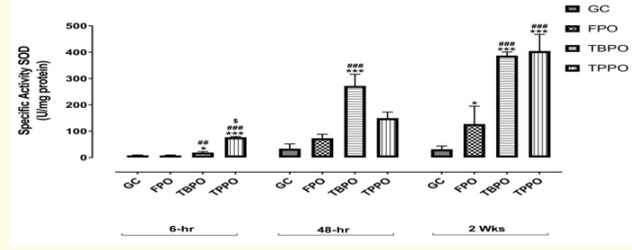


Figure 2: Changes in SOD activity in D melanogaster at 6hrs, 48hrs and 2 weeks of feeding.

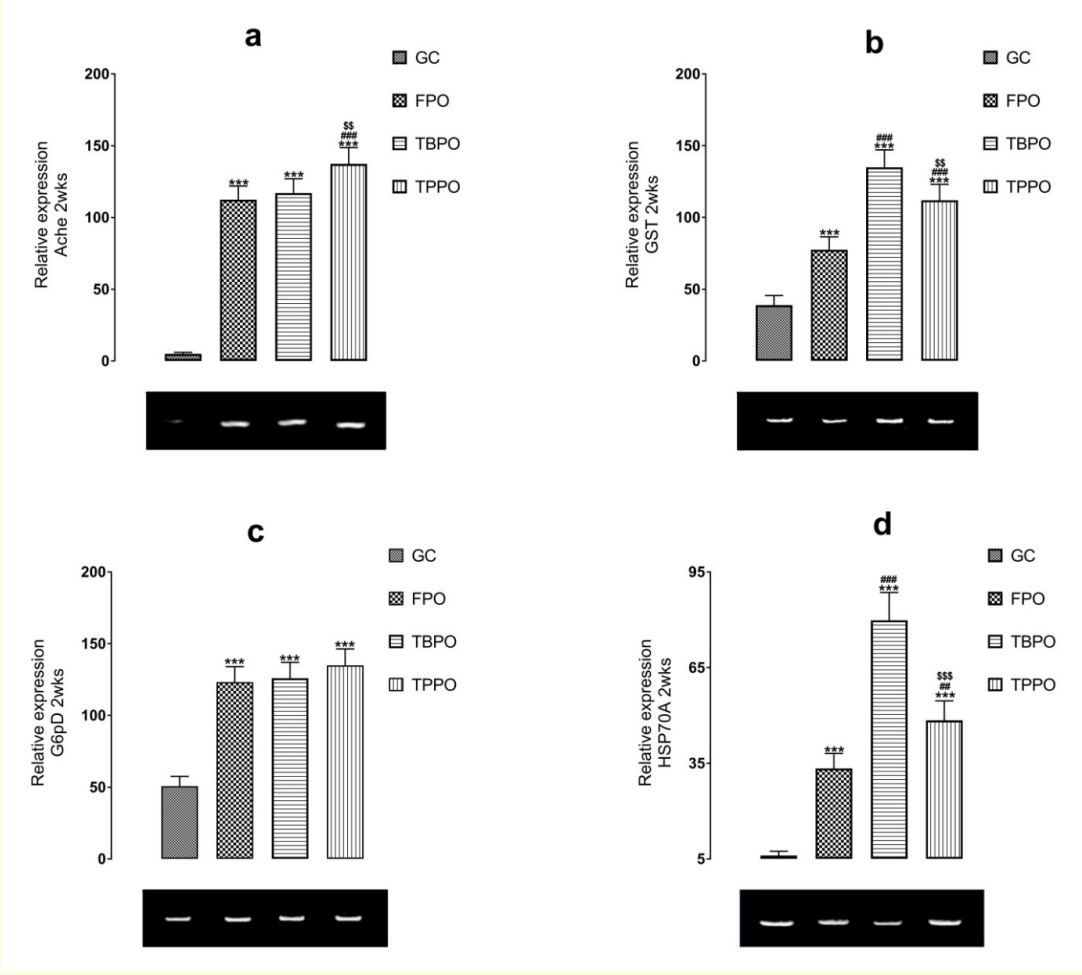
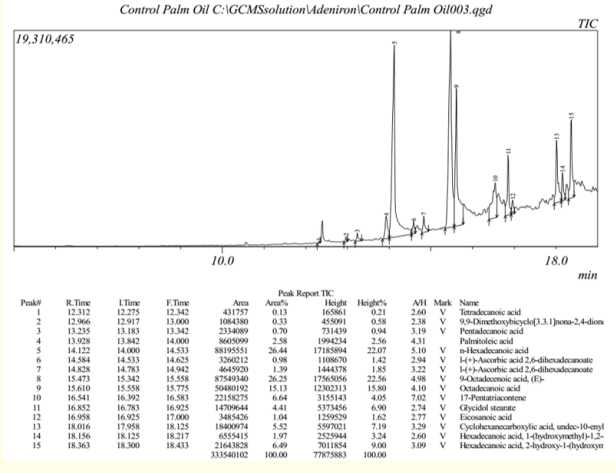


Figure 3: Expression of (a) AChE, (b) GST, (c) G6PD and (d) HSP 70A gene expressions in D. melanogaster placed on diets containing used and unused palm oil.

Statistical analysis

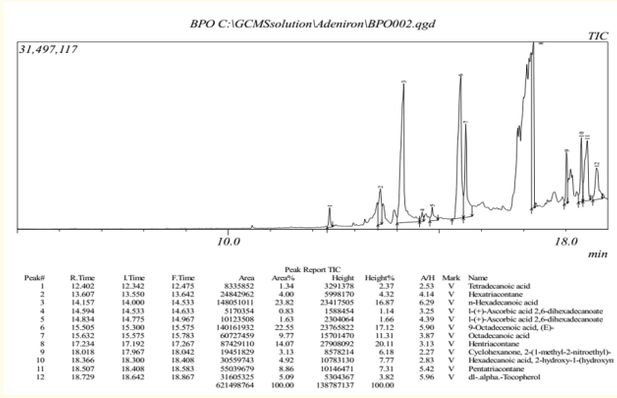
The obtained data were expressed as mean ± standard error of mean (SEM), analysis of variance (ANOVA), followed by Tukey's multiple comparisons test, was used to detect any significant differences among means. Statistical analysis was performed using GraphPad prism 7.04 software (GraphPad Software, San Diego, CA, USA).

Relative expression of each protein within groups; *p < 0.05 vs. GC group, **p < 0.01 vs. GC group, ***p < 0.001 vs. GC group; #p < 0.05 vs. FPO group, ##p < 0.01 vs. FPO group, ###p < 0.001 vs. FPO group; $p < 0.05 vs. TBPO group, $$p < 0.01 vs. TBPO group, $$p < 0.001 vs. TBPO group.



Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	12.312	12.275	12.342	431757	0.13	165861	0.21	2.60	V	Tetradecanoic acid
2	12.966	12.917	13.000	1084380	0.33	455091	0.58	2.38	V	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dion
3	13.235	13.183	13.342	2334089	0.70	731439	0.94	3.19	V	Pentadecanoic acid
4	13.928	13.842	14.000	8605099	2.58	1994234	2.56	4.31		Palmitoleic acid
5	14.122	14.000	14.533	88195551	26.44	17185894	22.07	5.10	V	n-Hexadecanoic acid
6	14.584	14.533	14.625	3260212	0.98	1108670	1.42	2.94	V	1-(+)-Ascorbic acid 2,6-dihexadecanoate
7	14.828	14.783	14.942	4645920	1.39	1444378	1.85	3.22	V	1-(+)-Ascorbic acid 2,6-dihexadecanoate
8	15.473	15.342	15.558	87549340	26.25	17565056	22.56	4.98	V	9-Octadecenoic acid, (E)-
9	15.610	15.558	15.775	50480192	15.13	12302313	15.80	4.10	V	Octadecanoic acid
10	16.541	16.392	16.583	22158275	6.64	3155143	4.05	7.02	V	17-Pentatriacontene
11	16.852	16.783	16.925	14709644	4.41	5373456	6.90	2.74	V	Glycidol stearate
12	16.958	16.925	17.000	3485426	1.04	1259529	1.62	2.77	V	Eicosanoic acid
13	18.016	17.958	18.125	18400974	5.52	5597021	7.19	3.29	V	Cyclohexaneearboxylic acid, undec-10-enyl
14	18.156	18.125	18.217	6555415	1.97	2525944	3.24	2.60	V	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-
15	18.363	18.300	18.433	21643828	6.49	7011854	9.00	3.09	V	Hexadecanoic acid, 2-hydroxy-1-(hydroxym
				333540102	100.00	77875883	100.00			

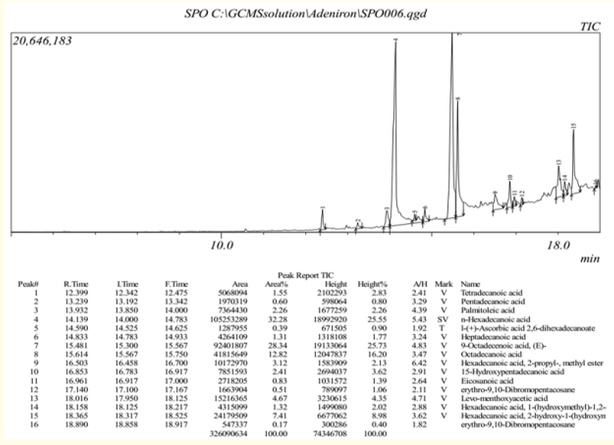
Figure 4a: GC-MS chromatogram and components identified in unheated palm oil (CPO).



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	12.402	12.342	12.475	8335852	1.34	3291378	2.37	2.53	V	Tetradecanoic acid
2	13.607	13.550	13.642	24842962	4.00	5998170	4.32	4.14	V	Hexatriacontane
3	14.157	14.000	14.533	148051011	23.82	23417505	16.87	6.29	V	n-Hexadecanoic acid
4	14.594	14.533	14.633	5170354	0.83	1588454	1.14	3.25	V	l-(+)-Ascorbic acid 2,6-dihexadecanoate
5	14.834	14.775	14.967	10123508	1.63	2304064	1.66	4.39	V	l-(+)-Ascorbic acid 2,6-dihexadecanoate
6	15.505	15.300	15.575	140161932	22.55	23765822	17.12	5.90	V	9-Octadecenoic acid, (E)-
7	15.632	15.575	15.783	60727459	9.77	15701470	11.31	3.87	V	Octadecanoic acid
8	17.234	17.192	17.267	87429110	14.07	27908092	20.11	3.13	V	Hentriacontane
9	18.018	17.967	18.042	19451829	3.13	8578214	6.18	2.27	V	Cyclohexanone, 2-(1-methyl-2-nitroethyl)-
10	18.366	18.300	18.408	30559743	4.92	10783130	7.77	2.83	V	Hexadecanoic acid, 2-hydroxy-1-(hydroxym
11	18.507	18.408	18.583	55039679	8.86	10146471	7.31	5.42	V	Pentatriacontane
12	18.729	18.642	18.867	31605325	5.09	5304367	3.82	5.96	V	dl-.alpha.-Tocopherol
				621498764	100.00	138787137	100.00			

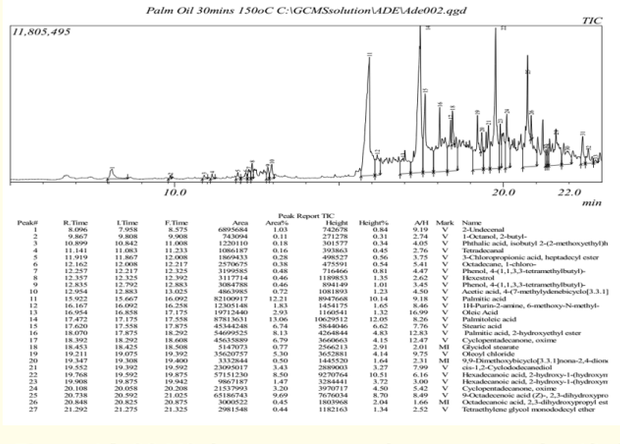
Figure 4b: GC-MS chromatogram and components identified in thermally oxidized palm oil used to fry bean cake (BPO)



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	12.399	12.342	12.475	5968094	1.55	2102293	2.83	2.41	V	Tetradecanoic acid
2	13.239	13.192	13.342	1970319	0.60	598064	0.80	3.29	V	Pentadecanoic acid
3	13.932	13.850	14.000	7364430	2.26	1677259	2.26	4.39	V	Palmitoleic acid
4	14.139	14.000	14.783	105253289	32.28	18992920	25.55	5.43	SV	n-Hexadecanoic acid
5	14.590	14.525	14.625	1287955	0.39	671505	0.90	1.92	T	l-(+)-Ascorbic acid 2,6-dihexadecanoate
6	14.833	14.783	14.933	4264109	1.31	1318108	1.77	3.24	V	Heptadecanoic acid
7	15.481	15.300	15.567	92401807	28.34	19133064	25.73	4.83	V	9-Octadecenoic acid, (E)-
8	15.614	15.567	15.750	41815649	12.82	12047837	16.20	3.47	V	Octadecanoic acid
9	16.503	16.458	16.700	10172970	3.12	1583909	2.13	6.42	V	Hexadecanoic acid, 2-propyl-, methyl ester
10	16.853	16.783	16.917	7851593	2.41	2694037	3.62	2.91	V	15-Hydroxypentadecanoic acid
11	16.961	16.917	17.000	2718205	0.83	1031572	1.39	2.64	V	Eicosanoic acid
12	17.140	17.100	17.167	1663904	0.51	790097	1.06	2.11	V	erythro-9,10-Dibromopentacosane
13	18.016	17.950	18.125	15216365	4.67	3230615	4.35	4.71	V	Levo-menthoxyacetic acid
14	18.158	18.125	18.217	4315099	1.32	1490080	2.02	2.88	V	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-
15	18.365	18.317	18.525	24179509	7.41	6677062	8.98	3.62	V	Hexadecanoic acid, 2-hydroxy-1-(hydroxym
16	18.890	18.858	18.917	547337	0.17	300286	0.40	1.82		erythro-9,10-Dibromopentacosane
				326090634	100.00	74346708	100.00			

Figure 4c: GC-MS chromatogram and components identified in thermally oxidized palm oil used to fry sweet potato (SPO).



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	8.096	7.958	8.375	6895684	1.03	742678	0.84	9.19	V	2-Undecenal
2	9.867	9.808	9.908	743094	0.11	271278	0.31	2.74	V	1-Octanol, 2-butyl-
3	10.899	10.842	11.008	1220110	0.18	301577	0.34	4.05	V	Phthalic acid, isobutyl 2-(2-methoxyethyl)h
4	11.141	11.083	11.233	1086187	0.16	393863	0.45	2.76	V	Tetradecanal
5	11.919	11.867	12.008	1869433	0.28	498527	0.56	3.75	V	3-Chloropropionic acid, heptadecyl ester
6	12.162	12.008	12.217	2570675	0.38	475591	0.54	5.41	V	Octadecane, 1-chloro-
7	12.257	12.217	12.325	3199585	0.48	716466	0.81	4.47	V	Phenol, 4-(1,1,3,3-tetramethylbutyl)-
8	12.357	12.325	12.392	3117714	0.46	1189853	1.35	2.62	V	Hexestrol
9	12.835	12.792	12.883	3084735	0.46	894149	1.01	3.45	V	Phenol, 4-(1,1,3,3-tetramethylbutyl)-
10	12.954	12.883	13.025	4863985	0.72	1081893	1.23	4.50	V	Acetic acid, 4-(7-methyldenebicyclo[3.3.1]
11	15.922	15.667	16.092	82100917	12.21	8947668	10.14	9.18	V	Palmitic acid
12	16.167	16.092	16.258	12305148	1.83	1454175	1.65	8.46	V	1H-Purin-2-amine, 6-methoxy-N-methyl-
13	16.954	16.858	17.175	19712440	2.93	1160541	1.32	16.99	V	Oleic Acid
14	17.472	17.175	17.558	87813631	13.06	10629512	12.05	8.26	V	Palmitoleic acid
15	17.620	17.558	17.875	45344248	6.74	5844046	6.62	7.76	V	Stearic acid
16	18.070	17.875	18.292	54699525	8.13	4264844	4.83	12.83	V	Palmitic acid, 2-hydroxyethyl ester
17	18.392	18.292	18.608	45635889	6.79	3660663	4.15	12.47	V	Cyclopentadecanone, oxime
18	18.453	18.425	18.508	5147073	0.77	2566213	2.91	2.01	MI	Glycidol stearate
19	19.211	19.075	19.392	35620757	5.30	3652881	4.14	9.75	V	Oleoyl chloride
20	19.347	19.308	19.400	3332844	0.50	1445520	1.64	2.31	MI	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dion
21	19.552	19.392	19.592	23095017	3.43	2889603	3.27	7.99	V	cis-1,2-Cyclododecanediol
22	19.768	19.592	19.875	57151230	8.50	9270764	10.51	6.16	V	Hexadecanoic acid, 2-hydroxy-1-(hydroxyn
23	19.908	19.875	19.942	9867187	1.47	3284441	3.72	3.00	V	Hexadecanoic acid, 2-hydroxy-1-(hydroxyn
24	20.108	20.058	20.208	21537993	3.20	3970717	4.50	5.42	V	Cyclopentadecanone, oxime
25	20.738	20.592	21.025	65186743	9.69	7676034	8.70	8.49	V	9-Octadecenoic acid (Z)-, 2,3-dihydroxypro
26	20.848	20.825	20.875	3000522	0.45	1803968	2.04	1.66	MI	Octadecanoic acid, 2,3-dihydroxypropyl est
27	21.292	21.275	21.325	2981548	0.44	1182163	1.34	2.52	V	Tetraethylene glycol monododecyl ether

Figure 4d: GC-MS chromatogram and components identified in heated palm oil at 150ºC for 30 mins (HPO).

Figure 5: 39-day survival curve analysis of fruit flies exposed to thermally oxidized palm oil diets.

Discussion

The GC-MS analysis of fresh, heated oils at 150oC for 30mins and fried oils obtained from beans cake and sweet potato at 150oC for 30mins are presented in Figure 4a-d.

- **Heated Palm oil**: During heating, reactions such as oxidation, hydrolysis, polymerization and isomerization occurs leading to the generation of some fatty acid derivative compounds not present in fresh palm oil such as Cyclopentadecanone, oxime, Oleoyl chloride, Cis-1,2-cyclododecanediol, Hexadecanoic acid, 2-hydroxyl-(hydroxymethyl) ethyl ester and lot more. Moreover, there is significant reduction in the level of saturated palmitic acid and monounsaturated oleic acid. The polyunsaturated linoleic acid and linolenic acid present in the fresh oil has been degraded to a large extent due to the heating condition. Some of the saturated fatty acid such as palmitic acid has probably undergone primary oxidation to produce fatty acid derivative such as Hexadecanoic acid, 2-hydroxyl-(hydroxymethyl) ethyl ester and Palmitic acid, 2-hydroxyethyl ester. While monounsaturated oleic acid produce 9-Octadecenoic acid (Z)-2,3-dihydroxypropyl ester. The level of palmitoleic acid increased in the heated palm oil as compared to the fresh oil, this might result from the oxidation of palmitic acid present predominantly in fresh palm oil. Furthermore, some cyclic compounds were generated in the heated oil such as Cyclopentadecanone, oxime, Cis-1,2-cyclododecanediol and [1,1-bicyclopropyl]-2-octanoic acid, 2-hexyl-methyl ester. These compounds may result from the reaction within or between triacylglycerol radicals generated during oxidation. Choe and Min [18] stated that the formation of cyclic compounds in frying oil depends on the degree of unsaturation and the frying temperature while Tompkins and Perkins [19] reported that the formation of cyclic mono-

mers and polymers increases as the amount of linolenic acid increased. Hence, it can be deduced that most of the cyclic compounds generated in the heated oil are from oleic, linoleic and linolenic acid present in the fresh palm oil.

- **Fried Bean cake oil**: n-Hexatriacontanoic acid, a saturated fatty acid with 36-carbon long carboxylic acid have the predominant percentage in the bean cake fried palm oil. This might have occurred largely due to polymerization reaction taking place during the frying process which might result due to hydrolysis of some triacylglyerol due to the presence of the moisture in the bean cake. The formation of trans fatty acid was also noted in the bean cake fried palm oil. The formation of trans fatty acids is as a result of isomerization reaction that occurred during the frying process. The 9-octadecenoic acid, (E) generated during the frying process is trans unsaturated fatty acids, an isomer of oleic acid that is present in the fresh palm oil sample. It was also noted that the level of palmitic acid, oleic acid, steric acid, linoleic acid present in the fresh palm oil greatly reduced due to frying condition and the fried product.
- **Fried Sweet potato oil:** n-Hexadecanoic acid (also known as palmitic acid) was the predominant fatty acid present in the oil sample after use. This indicated that the level of degradation of oil sample was minimal due to the fried product as compared with bean cake. However, the level of monounsaturated oleic acid reduced slightly due to oxidation reaction during the frying process. The formation of trans fatty acid was also noted namely 9-Octadecenoic, (E). This occurred due to isomerization reaction during frying. Some primary oxidation products were generated during the frying process such as Hexadecenoic,2-hydroxy-1-(hydroxymethy) ethylester, and 15-hydroxypentadecenoic acid.

Acetylcholinesterase (AchE) is an important cholinesterase enzyme present in the neuromuscular junctions and cholinergic synapses in the CNS. It terminates the signal transmission by hydrolyzing acetylcholine (ACh), a neurotransmitter that conducts nerve impulses across neuromuscular junctions in the nervous system of vertebrates as well as insects [20]. Therefore AChE is considered as a key enzyme in detecting the neurotoxicity. Akinyemi., et al. [21] reported that exposure of *D. melanogaster* to oxidative stress lead to up-regulation of AChE genes. Pertubation in the expression or activity of AChE could lead to decrease in cellular metabolism, disturb metabolic and nervous activity and lead to ionic refluxes and differential membrane permeability in addition to increase lipid peroxidation. The result obtained in this study is in line with previous studies as there was overexpression of this gene in the test groups especially TPPO and TBPO compared to the control group as at 2 weeks of feeding.

Heat shock proteins (HSPs) have been associated with a number of cytoprotective functions, including the protection of stable proteins [22], chaperoning and folding of nascent polypeptides [23], and degradation of aggregated proteins [24]. As a general rule the more severe the stress, the greater the expression of HSPs in the cells. At 2weeks, an obvious significant increase in the expression of HSP 70 was observed in the test groups compared to the control, especially TBPO.

The high expression of g-6-p-d may be due to thermal oxidation as a result of re-using these heated oils for frying which may generate more free radicals that are harmful to tissues [25]. Over expression of glucose-6-phosphate dehydrogenase in *drosophila melanogaster* and consequent increase in the ability to synthesize NADPH would enhance resistance to oxidative stress and extend the lifespan of the flies.

Figure 3b revealed that Glutathione s-transferase is highly expressed in the TBPO and TPPO compared with FPO after 2 weeks of feeding. Mazari [26] reported that overexpression of *Drosophila melanogaster* GSTE7 protects female flies from initial acute toxic effects of phenethyl isothiocyanate (PEITC). The significant linear correlation among the antioxidative genes investigated in this study suggest that these genes are co-regulated by a common factor, probably by an antioxidant response element (ARE) regulatory region in the promoter region [27] in response to oxidative stress induced by the secondary oxidative products produced during the frying process.

Figure 2a and 2b, clearly shows that there is a significant increase in the sod and catalase activities in the test groups compared with the control. However, the increase in the TBPO is higher compared with other groups. SOD thus functions to protect cells and mitochondria from free radical damage due to superoxide [28]. Observing the activities of catalase in the group of *Drosophila melanogaster* at two weeks, it is clearly seen that there is significant increase in the TBPO and TPPO groups compared with the control. Ageing is a slow process characterized by progression at cellular, tissue and organ level during the fag end of an organism's life. It results in gradual functional decline and decrease the adaptability of an organism [29]. It is known that free radicals play an important role in ageing and age-associated diseases [29]. This is because accumulation of free radicals from thermally oxidized oil diet tends to overwhelm the body antioxidant defense systems leading to damage to biomolecules. When the unrepaired damage accumulates, it put at risk the homeostasis of the organism, consequently provoking ageing and death. This study revealed that consumption of

TBPO diet (Figure 5) led to high death rate of the flies (less than 5%), and this trend increases significantly as the study proceeds, this might possibly be due to the fact that overheated fats are toxic, if fed to the animals.

Conclusion

It is therefore concluded that repeated frying of palm oil at elevated temperature generates toxic secondary oxidation products which has potentials to induce oxidative stress and consequently reduced the life span of the flies.

Acknowledgement

Special thanks to Centre for Biocomputing and Drug Development Unit AAUA, Dr. Omotuyi IO, Metibomu DS, Eniafe G. and Adelakun N. for their support.

Bibliography

1. Mba OI., *et al*. "Palm oil: processing, characterization and utilization in the food industry - A review". *Food Biological Sciences* 10.1 (2015): 26-41.

2. Oil World, Oil World Annual, (2013).

3. Oyewole OE and Amosu AM. "Public health nutrition concerns on consumption of red palm oil (RPO): The scientific facts from literature". *African journal of medicine and medical sciences* 39.4 (2010): 255-262.

4. Mukherjee S and Mitra A. "Health effects of palm oil". *Journal of Human Ecology* 26.3 (2009): 197-203.

5. Chong YH and Ng TK. "Effects of palm oil on cardiovascular risk". *The Medical Journal of Malaysia* 46.1 (1991): 41-50.

6. Katragadda HR., *et al*. "Emissions of volatile aldehydes from heated cooking oils". *Food Chemistry* 120.1 (2010): 59-65.

7. Fullana A., *et al*. "Comparison of volatile aldehydes present in the cooking fumes of extra virgin olive, olive, and canola oils". *Journal of Agriculture in Food Chemist* 52.16 (2004): 5207-5214.

8. Farmer EE and Davoine C. "Reactive electrophile species". *Current Opinion Plant Biology* 10.4 (2007): 380-386.

9. Halliwell Barry. "Oxidative stress and cancer: have we moved forward?". *Journal of Biochemistry* 401.1 (2007): 1-11.

10. Pohanka M. "Alzheimer's disease and oxidative stress: a review". *Current Medicinal Chemistry* 21.3 (2014): 356-364.

11. Ramond A., *et al*. "Oxidative stress mediates cardiac infarction aggravation induced by intermittent hypoxia". *Fundamental and Clinical Pharmacology* 27.3 (2013): 252-261.

12. Massy ZA., *et al*. "The role of oxidative stress in chronic kidney disease". *Seminar in Dialysis* 22.4 (2009): 405-408.

13. Yang H., *et al*. "Oxidative stress and diabetes mellitus". *Clinical Chemistry and Laboratory Medicine* 49.11 (2011): 1773-1782.

14. Giacco F and Brownlee M. "Oxidative stress and diabetic complications". *Circulation Research* 107.9 (2010): 1058-1070.

15. Cristina B., *et al*. "Fatty Acids Composition by Gas Chromatography-Mass Spectrometry (GC-MS) and most important physical-chemicals parameters of Tomato Seed Oil". *Journal of Agroalimentary Processes and Technologies* 18.1 (2012): 89-94.

16. Abolaji Amos Olalekan., *et al*. "Involvement of oxidative stress in 4-vinylcyclohexene-induced toxicity in Drosophila melanogaster". *Free Radical Biology and Medicine* 71 (2014): 99-108.

17. Omotuyi OI., *et al*. "Flavonoid rich extract of chromolaena odarata modulate circulating GLP-1 in wistar rats: Computational evaluation of TGR5 involvement". *3 Biotech* 8.2 (2018): 124.

18. Choe E and Min DB. "Chemistry of deep-fat frying oil". *Journal of Food Science* 72.5 (2007): 77-86.

19. Tompkins C and Perkins EG. "Frying performance of low-linolenic acid soybean oil". *Journal of the American Oil Chemists' Society* 77.3 (2000): 223-229.

20. Periasamy AP., *et al*. "Nanomaterials-acetylcholinesterase enzyme matrices for organophosphorus pesticides electrochemical sensors: A review". *Sensors Basel* 9.6 (2009): 4034-4055.

21. Akinyemi AJ., *et al*. "Curcumin administration suppress acetylcholinesterase gene expression in cadmium treated rats". *Neurotoxicology* 62 (2017): 75-79.

22. Dobson CM and Ellis RJ. "Protein folding and misfolding inside and outside the cell". *The EMBO Journal* 17.18 (1998): 5251-5254.

23. Beckman JS., *et al*. "Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide". *Proceedings of the National Academy of Sciences of the United States of America* 87.4 (1990): 1620-1624.

24. Chiang HL., *et al*. "A role for a 70-kilodalton heat shock protein in lysosomal degradation of intracellular proteins". *Science* 246.4928 (1989): 382-385.

25. Hill K., *et al*. "Yeast KRE2 defines a new gene family encoding probable secretory proteins, and is required for the correct N-glycosylation of proteins". *Genetics* 130.2 (1992): 273-283.

26. Mazari MA Aslam. "Studies on Human and Drosophila melanogaster Glutathione Transferases of Biomedical and Biotechnological Interest". *Stockholm University* (2016).

27. Nioi P and Hayes JD. "Contribution of NAD (P)Hydroquinone oxidoreductase 1 to protection against carcinogenesis, and regulation of its gene by the Nrf2 basic-region leucine zipper and the arylhydrocarbon receptor basic helix-loop-helix transcription factors". *Mutation Research* 555.1-2 (2004): 149-171.

28. Miller DF., *et al*. "Homeotic Complex (Hox) gene regulation and homeosis in the mesoderm of the Drosophila melanogaster embryo: the roles of signal transduction and cell autonomous regulation". *Mechanism Development* 102.1-2 (2001): 17-32.

29. Kumar KV., *et al*. "Asparagus racemosus extract increases the life span in Drosophila melanogaster". *Journal of Applied Biology and Biotechnology* 3.4 (2015): 49-55.

Volume 3 Issue 6 June 2019