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ORIGINAL ARTICLE

Deep-Frying with Thermoxidized Groundnut Oil Is a Potential Source of Free Radicals with Health Hazards

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Abstract

Groundnut oil is one of the most commonly used vegetable oils in the household and industry in Nigeria for deep frying purposes. This research work aimed to evaluate the effects of thermoxidized and fresh groundnut oils on lipid peroxidation, *in vitro* radical scavenging and *in vivo* antioxidant activities. The study comprised thirty-five (35) wistar albino rats divided into seven (7) groups of five (5) rats each. The different groups excluding group one (1) were fed with animal feeds containing various doses of the oils - high (50.52g/100g/rat/day), intermediate (31.28g/100g/rat/day) and low (16.56g/100g/rat/day) in their feed for six (6) weeks. Different methods of analyses, ranging from colorimetric, titrimetric, spectrophotometric, and hyphenated mass-spectroscopy were employed to obtain the results. The study showed that the peroxide and acid values (responsible for the off-flavour due to rancidity), 2,2- diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) (which give a measure of the pro-oxidant capacity) were significantly ($p<0.05$) increased in the thermoxidized oils when compared to the fresh edible oils. Also, superoxide dismutase (SOD), catalase (CAT) (responsible for the antioxidant activity) and malondialdehyde (MDA) values (a measure of peroxidation of a sample) increased significantly ($P<0.05$) in the thermoxidized groups. Gas Chromatography-Mass Spectroscopy analyses also revealed that thermoxidized oil samples produced compounds which are toxic or carcinogenic, like Butane, 1-[(2-methyl-2-propenyl)oxy]-, 4-n-Hexylthiane, (R)-,1,6-Octadiene, 5,7-dimethyl-, (R)- and Cyclododecanol, 1-aminomethyl and (R)-(-)-14-Methyl-8-hexadecyn-1-ol. The above findings suggest that there are potential health hazards associated with the consumption of oxidized products resulting from these oils used repeatedly in frying processes. In light of these findings, it is recommended that consumption of groundnut oils subjected to heating for long periods of time should be discouraged in Nigeria and other Low and Medium Income Countries (LMIC) where such practices are common.

Keywords: Edible oils, Deep-frying, Antioxidants, Radical scavenging, Lipid peroxidation.

Introduction

Deep frying is one of the most common and oldest methods of food preparation worldwide and deep fried foods are increasingly being consumed by the public (Chacko and Rajamohan, 2011).

Deep-frying/heating causes the oil to undergo a series of chemical reactions like oxidation, hydrolysis and polymerization which produce volatile and non-volatile components having important physiological effects. During this process, many primary and secondary oxidation products are produced, which lead to undesirable taste and decomposing nutritional quality as degradation of tocopherols, essential amino acids and fatty acids present in food. Fried products absorb a large quantity of its frying oil, thus eventually accumulating degradation products to become part of the diet (Halvorsen and Blomhoff, 2011; Ghidurus et al., 2011). The final products of oil frying are dependent on the time/temperature of frying, the frying method (deep-fat frying or shallow-frying), the type of oil, the saturation ratio of the oil, and the presence of a catalyst/antioxidant and oil family (Gupta, 2005; Halvorsen and Blomhoff, 2011) and could have a strong effect on levels of oil oxidation in the products (Koh and Surh, 2015).

Cooking oil are vegetable oils, which are obtained from coconut, groundnut, sunflower seeds, palm, cottonseeds and are extensively used in the preparation of diet. These fats and oils are the esters of glycerol and various straight chain mono-carboxylic acids, known as fatty acids. These fatty acids may be saturated, monosaturated or poly unsaturated (Chong and Ng, 1991). Unsaturated fatty acids are more susceptible to lipid oxidation than saturated fatty acids and for this reason they are good source of free radicals (Chacko and Rajamohan, 2011). Several chemical reactions take place during frying, e.g. oxidation, polymerization, hydrolysis, pyrolysis, isomerization and cyclisation. The various chemical compounds arising from these reactions are responsible for the unique flavour, colour, texture, taste, and of course off-flavour of frying oil and food. Increasingly, over the past 40 years, the conception of diet has undergone major changes. Many of these changes involve changes in dietary intake of fats and oils (Mukherjee and Mitra, 2009). Consumption of foods, which have been subjected repeatedly to deep-frying using various edible oils, is a common feature in Nigeria, although the type and composition of edible oil commonly used for cooking and frying in Nigerian kitchens vary greatly from region to region. These used cooking oils constitute a waste generated from activities in the food sector (industries and restaurants), which has greatly increased in recent years. The main use of reused oil at present is in animal feed and in a much smaller proportion in the manufacture of soaps, biodegradable lubricants or for combustion (recovery of energy in industrial plants). As a consequence, the use of recycled cooking oils in animal feed must be studied from the point of view of safety as the fats and oils which are heated at high temperature during deep frying (which is a popular food preparation method in Nigeria) may generate high levels of cytotoxic products. It may promote the induction, development and progression of cardiovascular diseases (Quiles et al., 2002). Moreover, since groundnut oil is one of the commonest used in cooking/frying, its repeated heating produces various peroxidative changes. Peroxidation of biological systems is regarded to be associated with a number of pathological manifestations. Effects of various types of dietary fats including vegetable oils on plasma lipid and lipoprotein concentrations and the influence of their constituent's fatty acids on incidence of coronary heart disease have been reported (Ramachandran et al., 2002). Hence we have undertaken the present study to evaluate the effect of reused or thermoxidized groundnut oils on some biochemical parameters in rats by assessing their physico-chemical properties, lipid peroxidation ability, pro-oxidant and antioxidant capacities.

Materials and Methods

Sample collection and preparation

Sample oils

Six (6) litres of fresh groundnut oil were purchased from a local market (Ogbete) in Enugu State, Nigeria. The oil was divided into two equal portions. One portion was thermally oxidized while the

other was used in its fresh form. The physicochemical characteristics of the oils such as acid value, peroxide value and the fatty acid composition (GC-MS) were determined according to the standard procedures.

Boiling Procedure (Thermoxidation)

The oils were heated according to the method of Owu *et al*. (1998). Three (3) litres of groundnut oils were boiled for 10 minutes at 180^0C in a stainless pot. Upon completion of the boiling process, once heated oil was obtained, the process was repeated four times to obtain five times heated oil with a cooling interval of at least five hours. After being heated, small quantities of the repeatedly boiled oils were extracted to test for the rancidity using markers like the physical appearance or colour, taste and odour. Also, the acid and peroxide values were measured. Fresh (non-heated) oil was also kept to serve as control.

Formulation of Diet

The level of oils in most West African dishes is about 15% (Shastry *et al*., 2011). The oils were heated according to the method of Owu *et al*. (1998) as described above.

The fresh and thermoxidized groundnut oils were mixed with the animal feed which has the ingredients cereals, vegetable protein, premix (vitamins and minerals) essential amino acids, sal, antioxidant, antitoxins, prebiotic and enzymes according to the manufacturer's (VITAL FEED® – Grand Cereals Nigeria Limited) specifications. The nutrient composition of the feed was crude protein (14%), fat (7%), crude fibre (10%), calcium (1.0%), available phosphorus (0.35%) and metabolisable energy (2550Kcal/kg). The feed was administered/fed to the rats at different formulated dose levels - high (50.52g/100g/rat/day), intermediate (31.28g/100g/rat/day) and low (16.56g/100g/rat/day). The diets were also stored in black containers at 4^0C to prevent further oxidation of the oil component.

Measurement of Chemical Properties

Determination of the Peroxide Value of Oil Samples (Ekwu and Nwagu, 2004)

The peroxide values of the oil samples were determined by titration method. One gram of oil and one gram of potassium iodide were weighed into a clean dry boiling tube. Twenty (20) ml of solvent mixture (two volumes of glacial acetic acid with one volume of chloroform) was added to the sample. The boiling tube was placed in a water bath and the liquid boiled for 30 seconds.

The content of the tube was quickly transferred into a conical flask containing 20 ml of 5% potassium iodide solution. The tube was washed twice with 25 ml of distilled water and collected into the conical flask. The content of the conical flask was titrated against 2 X 10^{-3} N Sodium thiosulphate solution until yellow colour of the sample almost disappeared. A 0.5ml of starch solution was added to the sample, shaken vigorously and titrated against the 2 X 10^{-3}N Sodium thiosulphate solution until the blue colour disappeared. A blank was also set at the same time. The peroxide values of the oil samples were calculated using the following formula:

$$Peroxide\ Value\ \left(\frac{mEq}{kg}\right) = \frac{(S\ x\ N\ x\ 1000)}{W(g)}$$

Where,
S = titration of sample (ml)
N = Normality of the sodium thiosulphate
W = weight of the oil sample (g)
This PV measuremet was run in triplicates.

Determination of Acid Value of the Oils (WHO, 2015)

The acid value of the oil samples was determined by titration method (WHO, 2015). Two grams (2g) of the oil was weighed into a 250 ml conical flask. Fifty (50) ml of neutralized ethyl alcohol was added to the oil sample. The mixture was then heated in a water bath. The solution was titrated against 0.1 M potassium hydroxide (KOH) using phenolphthalein as indicator. The acid value was calculated using the following formula:

$$Acid\ Value = \frac{(A\ x\ M\ x\ 56.1)}{W}$$

Where,
A = Amount (ml) of 0.1M KOH consumed by sample
M = Molarity of KOH
W = weight (g) of oil sample
56.1 = Molecular Weight of KOH (Factor)

Identification of Compounds Using Gas Chromatography-Mass Spectrometry (GC-MS) (Stein, 1999)

Agilent – 7890B GC-MS was used. The GC-MS was equipped with a split injector and an ion – trap mass spectrometer detector together with a fused – capillary column (agilent Hp.5ms ultra inert) having a thickness of 3µm, 250µm, x 0.25µm, and temperature limits of 60^{o}C to 325^{o}C. The column temperature was programmed between 60^{o}C and 250^{o}C and flow rate of 3.0ml/min, pressure; 4.4867psi. The temperature of the injector and detector were at 250^{o}C and 200^{o}C respectively. Helium gas was used as a carrier gas; methanol was used as the solvents to dissolve the sample. Split ratio of 20:1 and Split flow of 14ml/min was also used.

Interpretation of mass spectrum of GC-MS was done using computer-aided device called MSD (Mass hunter) and matching of the unknown spectra with spectra of known compounds from the Library of spectra from the National Institute of Standards (NIST14.L), Washington, USA having more than 62,000 patterns. The fragmentation patterns of the identified compounds were then examined for consistency with known data from literature. In addition, the hit quality (which indicates how closely matched the compound is with the Library data) was used to further verify the identity of the compounds in the sample. The name, molecular formula, molecular weight, mass to charge ratio, retention time and the relative percentage composition of the components of the test materials were determined.

Estimation of Radical Scavenging and Antioxidant Activities

DPPH free radical scavenging assay (Deepa et al., 2013)

The free radical scavenging activity of the oils was measured using DPPH according to Deepa *et al* (2013). A 0.1 mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of various concentrations (0.2 to 1.0 mg/ml) of sample dissolved in methanol to be tested. After 30 minutes, absorbance was measured at 517 nm. Ascorbic acid was used as a reference material. All tests were performed in triplicate. The radical scavenging activity of DPPH was calculated as follows:

$$DPPH\ (\%\ inhibition) = [\frac{(Absorbance\ of\ control - Absorbance\ of\ sample)}{(Absorbance\ of\ control)}]x100$$

The IC_{50} values were estimated from the % inhibition vs. Concentration plot using Non-linear regression algorithm.

Reducing power (Benzie and Strain, 1996)

The reducing power of the oil samples was determined also according to the method of Benzie and Strain (1996). A 1.0 ml of different concentrations of sample (0.2 to 1.0 mg/ml) was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of a 1% (w/v) solution of potassium ferricyanide. The mixture was incubated in a water bath at 50°C for 20 minutes. Afterwards, 2.5 ml of a 10% (w/v) trichloroacetic acid solution was added and the mixture was then centrifuged at 3000 rpm for 10 min. A 2.5 ml aliquot of the upper layer was combined with 2.5 ml of distilled water and 0.5 ml of a 0.1% (w/v) solution of ferric chloride, and absorbance was measured at 700 nm. All tests were performed in triplicate and the graph was plotted with the average of the three determinations.

Calculation: Results were calculated as follows:

$$FRAP_{sample}\ (\mu M) = \frac{(\Delta A\ of\ sample\ from\ 0\ to\ 4\ minutes)}{(\Delta A\ of\ standard\ from\ 0\ to\ 4\ minutes)} x FRAP\ value\ of\ standard\ (\mu M)$$

Where,
ΔA = change in absorbance; FRAP value of standard = 1000.

Catalase Method*:*

Essentially described by Beers and Sizer (1952) in which the disappearance of peroxide is followed spectrophotometrically at 240 nm. One Unit decomposes one micromole of H_2O_2 per minute at 25°C and pH 7.0 under the specified conditions. Briefly, the blank in spectrophotometer was incubated for 4-5 minutes to achieve temperature equilibration and to establish blank rate. Secondly, 0.1 M Hydrogen peroxide (2.95 ml) and enzyme tissue (3.0 ml) were added.

Calculations: Results were calculated as follows:

$$[CAT]\left(\frac{Units}{mg}\right) = [\left(\frac{\Delta A_{240}}{min}\right) x1000]/(43.6x\ mg\frac{enzyme}{ml}reaction)$$

Where,
Δ A_{240}/min = initial 60 sec.
1000 = Conversion factor in gram
43.6 = dilution factor.
mg enzyme/ml reaction mixture = 1/3

Malondialdehyde (MDA) Estimation:

Malondialdehyde (MDA) concentration was determined using the method of Ohkawa *et al.,* (1979). MDA is a secondary product of Lipid peroxidation and is used as an indicator of tissue damage. The MDA form a 1:2 adduct with thiobarbituric acid (TBA) and produces a pink coloured product which has absorbance maximum at 532nm.

A 0.2 ml of normal saline was pipetted into test tubes labelled as 'sample test' and 'sample blank' while 0.2 ml of serum was pipetted into the 'sample' test tubes only. A 0.5ml of TCA solution was added to each of the sample test/blank tubes. Then 0.1ml of TBA was added to the mixtures in each of the tubes. The mixtures were heated in a water bath for 60 minutes at 95°C. After cooling to room temperature, 3ml of n-butanol was added and mixed vigorously and centrifuged at 1000 rpm for 5 minutes. The butanol phase was then separated and its absorbance was taken against sample blank at 532nm.

Calculation: The Concentration of TBARS is expressed in term of MDA in µmol/mg of tissue.

$$[MDA]\left(\frac{\mu mol}{mg} of\ tissue\right) = \frac{ABS}{(1.56X10^5 cm^{-1}m^{-1})} x1000$$

Where,
1.56 X 10^5 $cm^{-1}m^{-1}$ is the Molar extinction coefficient of MDA; 1000 is the conversion factor.

Superoxide Dismutase (SOD) assay* (WST-1 method; ElabScience kit, USA)*:
Water-soluble tetrazolium (WST-1), the sodium salt of 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-13-benzene disulfonate, was used as a detector of superoxide radical generated by xanthine oxidase and hypoxanthine. The rate of the reduction with a superoxide anion is linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD.

The inhibition activity of SOD can be determined by a colorimetric method as tabulated below. In separate test tubes labelled "blank 1", "blank 2", "sample" and "blank 3" the rat liver homogenate (sample), ddH_2O, enzyme working solution, dilution buffer and WST working solution were added as indicated by the figures in the respective columns of the Table 1.

Table 1: Procedures for SOD

Kit Components	blank 1	blank 2	sample	Blank3
Sample (µL)			20	20
ddH_2O (µL)	20	20		
Enzyme working solution (µL)	20		20	
Dilution buffer (µL)		20		20
WST working solution (µL)	200	200	200	200

The solutions were then mixed, incubated for 20 minutes at 37°C, and absorbance measured at 450 nm.

Calculations:

$$SOD\ ACTIVITY(Inhibition\ ratio\ \%) = \frac{[(A\ blank\ 1 - A\ blank\ 2) - (A\ sample - A\ blank\ 3)]}{(A\ blank\ 1 - A\ blank\ 2)}(x100)$$

Where,
A = Absorbance (respectively)

Tissue sample:

$$SOD\ ACTIVITY\left(\frac{U}{gtissue}\right) = \frac{inhibition\ ratio\ of\ \frac{SOD}{50}\%}{Concentration\ of\ homogenate}$$

Experimental Animals
Ethical clearance for all the experimental procedures was approved by College of Health Sciences Research Ethics Committee, Bayero University Kano with reference number BUK/CHS/HREC/VII/62. Thirty-five (35) apparently healthy, wistar albino rats (*Rattus norvegicus domestica*) of both sexes weighing 180g to 200g (7 to 8 weeks) were obtained from the animal

house of the Department of Biological Sciences, Bayero University Kano, Nigeria. The animals were housed in plastic cages at room temperature with 12 hours day & light cycle at 64% humidity. The animals were acclimatized for one week (7days) and allowed access to food and water *ad libitum*. The rats were randomly assigned into seven (7) groups and were fed (see experimental design) for six (6) weeks. Animals were allowed access to food and water *ad libitum* and experimentally handled according to the OECD's (Organization of Economic Corporation and Development's) guidelines of 2005.

Experimental Design

Thirty-five (35) wistar albino rats of both sexes were used for the study. They were divided into seven (7) groups of five (5) rats each. The different groups excluding group one (1) were fed with animal feeds containing various doses of oils in their feed as shown below:

Group 1: Control (Normal feed-100g and water) (5 rats)
Group 2: Fresh Groundnut Oil High Dose (FGOH) – groundnut oil (35ml) mixed with 65g of rats' feed (5 rats)
Group 3: Fresh Groundnut Oil Intermediate Dose (FGOI) – groundnut oil (25ml) mixed with 75g of rats' feed (5 rats).
Group 4: Fresh Groundnut Oil Low Dose (FGOL) – groundnut oil (15ml) mixed with 85g of rats' feed (5 rats).
Group 5: Thermoxidized Groundnut Oil High Dose (TGOH) – groundnut oil (35ml) mixed with 65g of rats' feed (5 rats).
Group 6: Thermoxidized Groundnut Oil Intermediate Dose (TGOI) – groundnut oil (25ml) mixed with 75g of rats' feed (5 rats).
Group 7: Thermoxidized Groundnut Oil Low Dose (TGOL) – groundnut oil (15ml) mixed with 85g of rats' feed (5 rats).

Group one (1) animals served as the control group consisting of 5 rats and the animals were given normal feed and water. Groups 2 - 4 animals were fed with fresh groundnut oil-rich diet containing varying concentrations of the oil. Groups 5 - 7 were fed thermoxidized groundnut oil-rich diet at varying doses. Therefore, a total of thirty-five (35) albino rats were used for this research work and the feeding duration was six (6) weeks.

Tissue Sample Collection

At the end of the experiment, the animals were sacrificed humanely by decapitation for tissue samples (Shanmugasundaram *et.al.*, 1990). Rats were dissected and the liver of each rat collected and weighed separately. The tissue samples of the liver were homogenized immediately after sacrifice in ice-cold 10% sucrose solution for the MDA, CAT and SOD assays.

Statistical Analysis

Data were expressed as mean ± SEM (standard error of mean). Data were analysed using one way ANOVA (analysis of variance), followed with a post hoc (LSD) test for significant values. P-values of less than 0.05 were considered statistically significant. Correlation was run between groups to establish relationships or otherwise. These analyses were done using the SPSS (20.0) Software package.

Results

Chemical Analysis of the Thermoxidized and Fresh Oils

The chemical properties of the thermoxidized and fresh edible oil were determined. The results obtained were compared with international standards (Codex Alimentarius Commission, 1987).

With the exception of the fresh oil samples, there was significant deviation from the standard set by the Codex Alimentarius Commission. From the Table 2, it can be seen that for the peroxide value, there is a significant difference ($p < 0.05$) between FGO and TGO. For the acid value, there were significant differences ($p < 0.05$) between all the groups i.e. FGO and TGO.

Table 2: Chemical Properties of Locally-Produced Thermoxidized Groundnut Oils

Groups	Peroxide value (meqO2/kg)	Acid Value (mgKOH/g)
FGO	1.72 ± 0.72^{a}	1.82 ± 0.04^{a}
TGO	16.2 ± 0.84^{b}	9.39 ± 0.14^{b}

Values are expressed as mean ± SEM (n= 3). Means with different superscripts within columns are significantly different at $p < 0.05$. Key: FGO (Fresh Groundnut Oil); TGO (Thermoxidized Groundnut Oil).

Gas Chromatography Mass Spectrometry (GC-MS) Analysis

GC-MS reveals the compounds eluted at different retention times of the thermoxidized and fresh groundnut oils. Results from the GC-MS analysis of the Fresh Groundnut oil from the Library of National Institute of Standards (NIST14.L) revealed different compounds, some of which are very rich in offering different kinds of protections to the body. Compounds with anti-inflammatory and antioxidant properties were identified. For the GC-MS of Thermoxidized Groundnut oil in Table 3 and 4, most of the phytoconstituents have been denatured by high temperature and most of the compounds are either toxic or carcinogenic.

Table 3: Compound Composition of Fresh Groundnut Oil using GC-MS

No	RT	Name of Compound	Molecular formular	MW(g/mol)	Area %	m/z
1.	1.704	(Z,Z)-.alpha.-Farnesene	$C_{15}H_{24}$	204.351	0.100	204.000
2.	1.789	2-Ethyl Hexanal	$C_8H_{16}O$	128.215	0.720	128.000
3.	14.486	1,2,3-Benzenetriol	$C_6H_6O_3$	126.111	3.710	126.000
4.	15.575	9,17-Octadecadienal, (Z)	$C_{18}H_{32}O$	264.446	0.290	264.000
5.	17.966	9,12-Octadecadienoic acid (Z,Z)-	$C_{19}H_{34}O_2$	294.479	18.110	280.000
6.	19.044	Tetradecanoic acid	$C_{14}H_{30}$	198.394	0.260	198.000
7.	20.390	Neophytadiene	$C_{20}H_{38}$	278.000	0.760	278.000
8.	21.085	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280.488	0.130	280.276
9.	21.295	n-Hexadecanoic acid	$C_{16}H_{32}O$	256.424	12.780	256.000
10.	22.484	cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	324.549	0.090	324.000
11.	22.631	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308.498	0.890	308.000
12.	23.724	Oleic Acid	$C_{18}H_{34}O_2$	282.468	4.130	282.000

RT: Retention Time, **MF:** Molecular formular, **MW:** Molecular weight, **m/z:** mass to charge.

Table 4. Compound Composition of Thermoxidized Groundnut Oil using GC-MS

No	RT	Name of Compound	Molecular formular	MW(g/mol)	Area %	m/z
1	1.802	Butane, 1-[(2-methyl-2-propenyl) oxy]-	$C_8H_{16}O$	128.212	1.090	128.000
2.	20.385	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	$C_{17}H_{32}$	252.435	0.220	252.000
3.	20.843	4-n-Hexylthiane, S,S-dioxide	$C_{11}H_{22}$	218.356	0.110	218.000
4.	25.265	1,6-Octadiene, 5,7-dimethyl-, (R)-	$C_{10}H_{18}$	138.250	0.500	138.000
5.	25.771	Cyclododecanol, 1-aminomethyl-	$C_{13}H_{27}NO$	213.360	0.260	213.000

RT: Retention Time, **MF**: Molecular formular, **MW:** Molecular weight, **m/z:** mass to charge.

Liver Antioxidant Values

Liver Antioxidant Activities of rats administered with feeds rich in Groundnut oil seen in Table 5 showed that for SOD activities, that there was no significant difference between control and TGOL, FGOI, FGOH, TGOL, TGOI and TGOH at $p<0.05$, but for CAT there was significant difference between control and TGOL. For MDA, the difference was significant between control and FGOH, TGOL, TGOH, all at $p<0.05$. There was also a significant difference between FGOI and TGOH.

Table 5. Liver Antioxidant Activities and MDA Levels of Rats Administered Feeds Supplemented with Groundnut Oil

Groups	SOD (µ/mg of tissue)	CAT (Unit/mg)	MDA (µmol/mg of tissue)
I (Control)	13.072 ± 0.18	23.74 ± 0.10[a]	0.04 ± 0.001[a]
II (FGOL)	21.17 ± 0.20	39.40 ± 0.11	0.03 ± 0.002
III (FGOI)	21.22 ± 0.12	35.78 ± 0.11	0.05 ± 0.003
IV (FGOH)	23.46 ± 0.33	39.69 ± 0.07	0.02 ± 0.01[b]
V (TGOL)	36.15 ± 0.09	48.60 ± 0.08[b]	0.07 ± 0.005
VI(TGOI)	61.99 ± 0.20	54.32 ± 0.08	0.11 ± 0.008[c]
VI(TGOH)	79.22 ± .16	66.75 ± 0.10	0.19 ± 0.010[d]

Values are expressed as mean ± SEM (n= 5). Means with different superscripts within columns are significantly different at $p < 0.05$. **Key: FGOL**(Fresh Groundnut Oil Low Dose; **FGOI**(Fresh Groundnut Oil Intermediate Dose); **FGOH**(Fresh Groundnut Oil High Dose); **TGOL**(Thermoxidized Groundnut Oil Low Dose). **TGOI**(Thermoxidized Groundnut Oil Intermediate Dose); **TGOH**(Thermoxidized Groundnut Oil High Dose).

The radical scavenging activity and IC_{50} of thermoxidized and fresh Groundnut Oil on DPPH radicals when compared with a Standard (Vitamin C) was determined. The IC_{50} for Ascorbic acid as shown in Table 6 was the lowest when compared with the remaining groups. This shows that ascorbic acid has the best antioxidant capacity among the groups, with TGO having the least. Moreso, the Ferric Reducing Antioxidant Power (FRAP) of both the thermoxidized and fresh Groundnut Oils when compared with ascorbic acid showed that Ascorbic acid had the highest absorbance value when compared with the remaining groups (0.298±0.01), which still established it to have the best antioxidant capacity (TGO still had the least). For DPPH (IC_{50}), significant difference was observed between control and the remaining groups. This result was not different from the FRAP as significant difference was also seen between control, FGO and TGO.

Table 6. Radical Scavenging Activity of Thermoxidized and Fresh Palm and Groundnut Oils

Treatment	DPPH (IC_{50}) (mg/ml)	FRAP (mg/ml)
Ascorbic Acid	$8.04E^{-7} \pm 0.21^{a}$	0.298 ± 0.01^{a}
FGO	$9.00E^{-5} \pm 0.03^{b}$	0.292±0.01
TGO	$8.99E^{-2} \pm 0.01^{c}$	0.239 ± 0.02^{b}

Values are expressed as mean ± SEM (n= 3). Means with different superscripts within columns are significantly different at $P < 0.05$. **Key: FGO** (Fresh Groundnut Oil); **TGO** (Thermoxidized Groundnut Oil).

Discussion

Repeatedly heated cooking oil is often used interchangeably with thermoxidized or recycled edible oil. Repeated use of this oil has become a common practice due to low level of awareness among the public about the bad effect of this practice (Abdullah *et al*., 2010). Nowadays, the consumption of deep-fried food has gained popularity which may cause increased risk of obesity and other diseases (Sayon-Orea *et al*., 2012). The chemical properties of the thermoxidized and fresh edible oils were determined and the results obtained were compared with international standards - Codex Alimentarius Commission Standards. It was shown in the present study that the peroxide values (Table 2) were increased in the thermoxidized groundnut oil. Increased values indicate increased lipid peroxidation by-product content, mainly the peroxides that were formed in the oil during heating process. Other than the peroxides, there are other oxidized components that are formed during oil heating such as oxidative dimers and oxidized triacylglycerols (Awney, 2011).

Peroxide values of the TGO (16.2 ± 0.84 mEq O_2/kg) deviated from the Codex standard value of 10 mEq O_2/kg (Codex Alimentarius Commission, 1987); whereas peroxide values for the FGO were in conformity with the Codex standard value of 10 mEq O_2/kg as shown in the Table 2. The deviations from the standard value could possibly be due to the continuous exposure of the oil to light, high temperatures and atmospheric oxygen, which reacts with the oil to form peroxides. Thus, the results showed degradation in the chemical properties of the vegetable cooking oil after repetitive frying. Acid value of TGO (9.39±0.14) was higher than that of the FGO. Acid value of thermoxidized oil was found to increase due to rancidity. With the exception of the fresh oil samples, the oil samples exhibited significant deviation from the standard set by Codex Alimentarius Commission (4mgKOHg-1) for refined oils for human consumption (Codex Alimentarius Commission, 1987). Studies have shown that high acid values translate to high free fatty acids. These changes in physicochemical parameters of reused oils confirm the oxidation of edible oil and formation of free radicals.

Results from the GC-MS analysis for the Fresh Groundnut oil showed, from the Library of National institute of Standards (NIST14.L) as seen in Table 3, that 12 compounds were present. These compounds are very rich in offering different kinds of protection to the body. Compounds with hepatoprotective and antioxidant properties include (Z,Z)-.alpha.-Farnesene, Neophytadiene (Sermakkani and Thangapandian, 2012), 2-Ethyl Hexanal, 9,17-Octadecadienal, (Z)- (Radhakrishnan *et al*., 2016), Tetradecanoic acid, 1,2,3-Benzenetriol and n-Hexadecanoic acid (Belakhdar *et al*., 2015). Compounds with anti-inflammatory activities includes: Oleic Acid, 9,12-Octadecadienoic acid, ethyl ester, cis-13-Eicosenoic acid, 2-Methyl-Z,Z-3,13-octadecadienol, Neophytadiene (Sermakkani and Thangapandian, 2012), 9,12-Octadecadienoic acid (Z,Z)-. Those with anti-cancer protection includes 9,12-Octadecadienoic acid (Z,Z)-, 2-Methyl-Z,Z-3,13-octadecadienol and Oleic Acid (Sermakkani and Thangapandian, 2012). Oleic acid helps to reduce the risk of breast cancer (Sarumathy *et al*., 2011).

For the GC-MS of Thermoxidized Groundnut oil in Table 4, most of the phytoconstituents have been denatured by high temperature and most of the compounds detected are either toxic

or carcinogenic. They are; Butane, 1-[(2-methyl-2-propenyl) oxy]-, 4-n-Hexylthiane and (R)-,1,6-Octadiene, 5,7-dimethyl-, (R)-, Cyclododecanol, 1-aminomethyl and (R)-(-)-14-Methyl-8-hexadecyn-1-ol (Sermakkani and Thangapandian, 2012). Most decomposition products of hydroperoxides are responsible for the off-flavor in the oxidized edible oil (Frankel, 1998). Aliphatic carbonyl compounds have more influence on the oxidized oil flavour due to their low threshold values. Threshold values for hydrocarbons, alkanols, 2-alkenals, and *trans, trans*-2,4-alkadienals are 90 to 2150, 0.04 to 1, 0.04 to 2.5, and 0.04 to 0.3 ppm, respectively (Frankel, 1998). R)-(-)-14-Methyl-8-hexadecyn-1-ol (23.5%) and 2,4-Decadienal, (E,E)- (34.3%), and trans-13-Octadecenoic acid (29.5%) and Cyclododecanol, 1-aminomethyl- (18.1%), were some of the major volatile compounds detected in the thermoxidized groundnut oil (Frankel, 1998). Frankel (1998) reported that *trans, cis*-2,4-decadienal was the most significant compound in determining the oxidized flavor of oil, followed by *trans, trans*-2,4-decadienal, *trans, cis*-2,4-heptadienal, 1-octen-3-ol, butanal, and hexanal. Hexanal, pentane, and 2,4-decadienal were suggested and used as indicators to determine the extent of the oil oxidation (Choe, 1997). *Trans*-2-hexenal, *trans, cis, trans*-2,4,7-decatrienal and 1-octen-3-one, were reported to give grass-like and fish-like flavor in oxidized soybean oil, respectively (Min and Bradley, 1992). No single flavor compound is mainly responsible for the oxidized flavor of vegetable oils.

The liver antioxidant activities of rats administered feed supplemented with Groundnut oil (Table 4) revealed that there was significant increase ($p<0.05$) in the levels of SOD, CAT and MDA in TGOL, TGOI and TGOH groups compared to the control group and likewise the FGOL, FGOI and FGOH groups. There was a significant ($P < 0.05$) increase in the liver MDA contents of the groups fed with thermoxidized groundnut oil diets when compared with the control. This suggests that liver is more prone to lipid peroxidation because of its role in detoxification and exposure to free radicals and other reactive species which is capable of compromising its membrane integrity thereby leading to the leakage of liver enzymes and oxidative stress. Oxidative stress plays a fundamental role in the onset and development of liver diseases (Sushma *et al*., 2013). The increased MDA content observed in this study may suggest that thermal oxidation of the oil might affect free radical formation which is in agreement with previous work reported by Nwanguma *et al*. (1999). However, the effects of thermally oxidized oil diets on liver MDA in this study is contrary to the findings of Benedetti *et al*. (1987), who reported that oxidized soy bean oil affects neither plasma nor liver MDA levels. Likewise, Izaki *et al*. (1984), found that serum and kidney TBARS level were unchanged in the rats fed with thermally oxidized rapeseed oil. The reason for the discrepancies in the result might be due to difference in the fatty acid composition of the oils. It has also been observed that for the SOD of the liver antioxidant activities of rats administered feed supplemented with Groundnut oil (Table 5), there was no significant difference between control, FGOL, FGOI, FGOH, TGOL, TGOI and TGOH groups at $p<0.05$. SOD did not show any significant difference because it is a weak oxidant (Abdullah *et al.,* 2014).

Antioxidant activity in the form of IC_{50} (DPPH radical Scavenging activity) values of the thermoxidized and fresh oils and that of the ascorbic acid were calculated as shown in Table 6. The IC_{50} value for ascorbic acid ($8.04E^{-7} \pm 0.21$mg/ml) was lower when compared with that of FGO ($9E^{-5} \pm 0.03$mg/ml) and TGO ($8.99E^{-2} \pm 0.01$mg/ml). It means for example that, FPO will require about $9.0E^{-5} \pm 0.03$ mg/ml to mop out 50% of the Free radical population and ascorbic acid will take a lesser amount (($8.04E^{-7} \pm 0.21$mg/ml)) to scavenge 50% of the radical population. Thus, ascorbic acid is a better antioxidant when compared to the fresh and thermoxidized oil samples but it should be noted that they still, to some extent, possess antioxidant capacities. The antioxidant activity of a given compound or extract is also often associated to its radical scavenging activity. In general, it is considered that the antioxidant capacity of a molecule is equivalent to its capacity to react with free radicals. DPPH is a known free radical reagent and relatively stable compared to the highly reactive superoxide and hydroxyl species that are primarily responsible for oxidative damage in biological systems, and is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors (Gao *et al*., 2006; Garcıa-Pe´rez

et al., 2008). The results of the present study indicated that the compounds found in the fresh groundnut oil are powerful scavengers of free radicals as demonstrated by a high correlation of DPPH free radical scavenging activity and MDA activity (r=0.98, p= 0.003). Similar results were obtained by Nsimba *et al*. (2008).

Conclusion

Generally, most compounds acts as antioxidants synergistically by either scavenging these free radicals such as Superoxides anion radical (O_2•−), Hydroperoxyl radical (HOO•), Hydrogen peroxide (H_2O_2), Hydroxyl radical (OH•) and Lipid peroxide radical (ROO•) or inhibiting the production of these radicals. Most of these compounds do it by donating their electrons. Their ability to donate their electrons lies in their structural orientations and their functional groups such as hydroxyl groups (-OH) and carbonyl group (C=O). Some of the compounds that were identified to have antioxidant potential from the study include 1,2,3-Benzenetriol, 2-Methyl-Z, Z-3,13-octadecadienol and 9,12-Octadecadienoic acid (Z, Z)-. The main structural feature responsible for the antioxidant and free radical scavenging activity in the case of these compounds is the hydroxyl group. They are able to donate the hydrogen atom of the phenolic OH to the free radicals, thus stopping the propagation chain during the oxidation process. This effect is modulated by the ring substituents, so that electron-withdrawing groups increase the bond-dissociation enthalpy, due to the stabilization of the phenol by a polar structure that leaves a positive charge on the OH group. Consequently, electron-donating groups produce a reduction of the bond dissociation enthalpy due to the stabilization of the phenoxyl radical by mesomeric structures bearing a positive charge on the substituent (Gao *et al*., 2006).

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