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DETERMINATION OF CONCENTRATIONS OF LIPID PEROXIDATION PRODUCTS IN ROASTED AND DEEP FRIED FAST FOODS SOLD IN SOUTH-EASTERN NIGERIA

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AUTHOR'S CONTRIBUTION

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ABSTRACT

Within a space of three decades, after fast foods consumption became so rampant in southeastern Nigeria, there exists a conspicuous sharp increase in undetailed/unrecorded health complications such as diabetes, hypertension, stroke, cardiovascular disorders, cancer, vascular inflammation, hematological complications and worst of it all untimely death. These have necessitated the need for an in-depth research on any link between these health impediments and the consumption of highly oxidized fatty acids via intake of roasted and ready-made deep-fried fast food as the root cause. The aim of this present research work is to determine the levels of lipid peroxidation products in most roasted and deep-fried fast foods eaten in southeastern Nigeria using thiobarbituric acid reactive substances (TBARS), conjugated dienes, and hydroperoxide as bio-markers. Our observation reveals that the concentrations of products of lipid peroxidation in deep-fried fast foods were significantly higher than that of roasted fast foods. Our results further depict that fried egg recorded the highest mean value of TBARS among fried food samples, but a non-significant decrease in comparison with the used frying oil, while the other samples exhibited varied values of TBARS in increasing order of fried egg > plantain chips > akara > yam chips>chin-chin > bonus > potatoes chips> doughnut > puff-puff. Moreover, among roasted foods suya meat exhibited the highest concentration of TBARS followed by groundnut and pork meat. This research work indicates that glacial acetic acid/water solvent mixture extracted a significant concentration of conjugated dienes from roasted bread fruit, baked cake, and fried doughnut, in comparison with methanol/chloroform extracts that gave the highest extractive mean values of conjugated dienes for roasted bambara nut, groundnut, and suya meat.

Keywords: Lipid peroxidation; roasted; deep fried fast foods; malondialdehyde; thiobarbituric acid reactive substances; lipid hydroperoxides; conjugated dienes.

1. INTRODUCTION

Consumption of highly oxidized fatty acids unknowingly via intake of ready-made deep-fried fast food may be responsible for the conspicuous sharp increase in undocumented health complications such as diabetes, hypertension, stroke, cardiovascular disorders, cancer, and vascular inflammatory diseases,

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and untimely death that is now common in the southeastern part of Nigeria. Roasting and frying of food are aged known faster ways of cooking, which are processes of subjecting raw food materials (plant and animal sources) directly to an extremely high temperature greater than 100ºC, which is the boiling point of water. However 300 and $(600\text{-}1400)^0$ C, vegetable oil, and temperature of flame respectively justify the reason frying and roasting are faster means of food preparations than boiling with water. These processes are aimed at increasing the palatability, digestibility, availability of nutrients and calories; sterilization, improvement/enhancement of food texture and flavor, and formulation of different recipes from the same foodstuff, all geared towards increasing food safety, preservation and consumption. Roasting enhances food flavor through caramelization and browning on the surface of the food. Although, beneficial nutrients, antioxidants, and phytochemicals found in both foodstuffs and many unrefined oils degenerate when overheated and worst still even at smoke point, leading to the generation of free radicals. Cooking oil acts as a medium for high heat energy transfer to the food materials and this high thermal energy initiates the formation of new compounds via hydrolysis, oxidation, and thermal alteration [1]. Heating or repeated heating of cooking oils during the frying process exposes cooking oil to an extremely high temperature, which in the presence of air (oxygen), moisture from the foodstuff, and possibly trace metals triggers the hydrolysis of the ester bonds resulting in the release of free fatty acids, monoacylglycerols and diacylglycerols [1]. These metals are traceable to wear and tears from the grinding process. Since most of the foodstuffs are salted before frying, salt increases the breakdown of chemical bonds in the fatty acid molecules, speeds up deterioration [2] and lowers the smoke point of the oil. Polyunsaturated fatty acids, which mainly consists of omega-9, -6 and -3 series are highly susceptible to oxidation [3]. Oxidation and thermal alterations of unsaturated fatty acids require the presence of both air (oxygen) and extremely high temperature to modify triacylglycerol (TAG) with at least one of the three fatty acyl chains altered [4], which generates hydroperoxides. Hydroperoxides are unstable intermediates and rapidly break down into reactive free radicals to initiate autoxidation, generally through a three-phase process (initiation, propagation and termination) [5]. At maximum concentration of hydroperoxide the double bond adjacent to the hydroperoxyl group is broken down to yield hydrocarbons, aldehydes, alcohols and ketone [5]. Their consumption accelerates oxidative degradation of membrane lipids, forming hazardous reactive oxygen species and impairs the membrane functions, inactivates membrane-bound receptors or enzymes, and disturbs ions permeability and fluidity, which eventually leads to membrane rupture [6]. These lipid oxidation products have been established to have mutagenic [7], carcinogenic [8] and cytotoxic, genotoxic [9, 10] properties and constitute a risk factor to human health. Some researchers have attributed degraded compounds in the frying oil to heterocyclic amines or polycyclic aromatic hydrocarbons formed from meat [11] or acrylamide formed in carbohydrate-rich foods [8].

Crispy, flavor, golden texture, and extension of shelf-life of food items which are the characteristic features of roasting and frying are seriously jeopardized/antagonized by lipid peroxidation and its by-products. Deleterious molecular changes in food caused by oxidation of lipid include; loss of flavor, loss of colour, loss of nutrients/nutritional value and functionality, and finally the accumulation of toxic products. Food intake pattern of an average Nigerian is determined by socioeconomic status and the level of education/exposure. In Nigeria, the rate of dependence on deep-fried fast foods from (eateries and restaurants) and (roadside stalls and food outlets in markets) both by high income and low-income groups respectively, are on the increase. It is also a common fact that most snacks used as morning breakfast are prepared on daily basis by Nigerian housewives, who deploy these food preparation techniques in an attempt to overcome the early morning rush to school and work. However, both snacks in school children's lunchboxes and those eaten by bricklayers account for 2/3 of all meals consumed per individual per day. Various techniques are available for the detection and measurement of lipid peroxidation, which include estimation of conjugated dienes, quantification of lipid hydroperoxide. The thiobarbituric acid reactive substances (TBARS) assay is most commonly used to quantitate malondialdehyde, which is the end product of lipid peroxidation.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Thiobarbituric acid (TBA) 99% pure was purchased from Molychem (India); malondialdehyde tetrabutyl ammonium salt (MDA salt) 96% pure, Methanol 99.8% and Chloroform 99.5% purities were purchased from CDH. However, deionized/double distilled water was used. Butylated Hyroxy Toluene 99% was purchased from Lobachemie.com. All other chemicals and reagents were of an analytical standard with high purity.

2.2 Reagent Preparations

2.2.1 Preparation of TBA reagent

A standard solution of thiobarbituric acid with a concentration of 4.0mM was freshly prepared each day in 1:1 mixture of methanol and chloroform. In this current experiment, 115.32mg of TBA was dissolved in 200 mL of 1:1 mixture of methanol and chloroform.

2.2.2 Preparation of MDA and calibration standards

Standard stock solution of MDA (1mM) was prepared in glacial acetic acid. MDA (31.35mg) was accurately weighed and dissolved in 100 mL solvent. From the stock solution, different concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0mM were prepared. The calibration curve was plotted in the concentration range of 0.2 to 1.0mM.

2.2.3 Preparation of butylated hyroxy toluene

A known concentration (0.01%) of BHT was prepared by dissolving 0.01g of BHT and making it up to 100 mL mark in a graduated measuring cylinder. This was used to prevent further oxidation of the medium during the actual analysis.

2.3 Food Sample Collection

Both roasted and deep fried food samples were bought from different eateries, restaurants, roadside stalls and food outlets in markets within the South Eastern Nigeria in the month of August 2021. Each individual food product samples were aseptically collected approximately 50g and properly sealed in a transparent plastic container to ensure no oil leakage occurred during transportation.

2.4 Preparation of Food Samples by Local Vendors

2.4.1 Fried food samples

2.4.1.1 Yam chips, riped plantains chips and potatoes chips

Yam tubers, riped plantains and potatoes tubers. These samples are washed with water and their back covers peeled out with sharp knife. Finally, they are sliced into pieces and salted before introducing them into a boiling vegetable oil.

2.4.1.2 Chin-chin, Puff-puff and Bons

These samples are prepared from the same all-purpose flour. For Chin-chin, a known quantity of flour is introduced into a clean bowl followed by the addition of the baking powder, salt and sugar. Extra water, margarine and nutmeg are added, these are thoroughly mixed and blend to remove lumps. Moreover, eggs and more water can be added to give the dough the desirable thickness. The dough is stretched over a smooth surface with the help of a roller, cut into pieces of any shape of your choice. Finally, They are introduced into a boiling vegetable oil. `

2.4.1.3 Doughnut

The sample is prepared by adding yeast and 3 tablespoonful of flour, 5 tablespoonful of warm water in a bowel together, properly mixed and covered with alumininm foil to allow formation of bubbles. In another bowl, flour, sugar and salt are properly mixed. Egg, warm milk, melted butter and yeast are thoroughly mixed and allowed to ferment for ten minutes. Then, the sticky dough is folded into a rough ball and placed in a greased bowl. It is covered and allowed to stand for some time. This is then placed on a floured table, flattened with palms and rubbed a little flour on the sticky flattened dough. Biscuit cutter is used to cut the flattened dough into doughnuts rounds. Finally, they are introduced into a boiling vegetable oil.

2.4.1.4 Akara

Akara is a popular Nigerian snack made with beans, also known as bean cakes, bean-balls or bean fritas. This sample is prepared by socking the bean seeds in water for 2 hours to soften it and make it easy for the coat layer to be washout. The next step is grinding with little water in a blender. Onions, salt, and pepper are added into a mortar containing the beans puree. The beans puree is stirred with pestle in a continuous circular motion. Finally, the mixture is scooped with a tablespoon and slowly pour into a boiling vegetable oil. The underside is fried till it turned brown and flip to fry the top side too. When the akara balls turns brown all over, they are removed and placed in a sieve lined with paper towels.

2.4.2 Roasted Food Samples

2.4.2.1 Roasted groundnuts, roasted bambaranuts, roasted cashewnuts and roasted breadfruits

These samples are prepared by wetting them with salty water and sundried. Small quantity of sand is added to the frying pan on the stove, it is allowed to heat up for about 10 minutes, before the sample is introduced into the heated sand in the frying pan. It is continuously stirred with a spatula or wooden spoon until it turns brown or golden. After cooling, the crunchy sample is stored in airtight containers.

2.4.2.2 Roasted Pork meat and roasted suya (beef)

Sharp knife is used to produce a thin slice of steak of high quality meat samples. Peanuts are ground into paste with blender. The peanut paste is mixed with little salt and a drizzle of olive oiland further ground to form a thick cream. Lime juice, chill powder, ginger, onion powder, sea salt and paprika are further added. The sample (beef or pork) is placed in a large bowel and peanut sauce poured over it. This is properly mixed with hands, covered and refrigerated for 2 hr. The sample strips are threaded onto soaked wooden skewers accordion-style, to stretche out the meat well. The staeks samples are placed on the grill and cooked until it turns golden brown. Brush the grill rack with oil and carefully place the skewers on the rack. However, you can move them to grill over direct haet until the meat soften and tender.

2.5 Extraction of Lipid Peroxidation Products from both Fried and Roasted Food Samples Using Different Solvents Mixtures, Methanol: Chloroform (1:2) and Glacial Acetic Acid:Water (1:1)

One gram of each of both fried and roasted ground samples (roasted breadfruit, roasted Bambara nut, roasted chin-chin, fried Akara, puff-puff, fried bons, fried egg, roasted cashew, plantain chips, yam chips, potatoes chips, roasted groundnut, cake, doughnut, roasted pork meat, and suya meat) were mixed with 5mL of the solvent. However, 0.01% of BHT was used to prevent further oxidation of the medium in the course of this analysis. With the help of a vortex mixer, the samples were shaken for 1 h and filtered. The filtrates were centrifuged at 4000 rpm for 30 minutes and the clear supernatants were used for analyses.

2.6 Analytical Procedure

The level of MDA were determined using the method of Zeb and Ullah [12] with slight modification. A known volume (1 mL) of standard MDA solution was mixed with 1 mL of TBA in a 10 mL test tube. The mixture was heated for 1 hour in an approximately boiling water bath at 95°C. However, after heating for 1 hour, the test tubes were cooled at room temperature and absorbance read at 532nm using UV-visible spectrophotometer. Each concentration of the standard MDA for calibration was repeated (n = 4) in line with the above procedure. A blank sample was repeated (n = 5) replacing standard or sample by equal extraction solvents. Various kinds of both fried and roasted fast foods collected from different locations within the South Eastern part of Nigeria were extracted separately with (1:2 mixture of methanol and chloroform). A known volume (1 mL) of the extract of each sample was mixed with 1mL TBA reagent and the above procedure was repeated five times (n = 5). The TBARS was calculated using the formula as μM/g of the sample:

TBARS (μM/g) = (Ac × V)/W Equation- 1

where Ac is the amount determined from the calibration curve and W is the weight of the sample taken, while V is volume in mL or dilution factor of the total extract prepared.

2.7 Lipid Peroxidation Assay (negative control)

For this assay, egg yolk homogenate was used as lipid source and free radicals were produced by Fenton reagent ($FeSO_4/H_2O_2$), a modified thiobarbituric acid reactive substances (TBARS) assay [13, 14]. In brief, 1mL reaction mixture containing 0.95 mL egg yolk homogenate (10% in distilled water, v/v), was mixed with 0.05mL $FeSO_4$ (0.07M) and incubated for 30min to induce lipid peroxidation. Free radical ruptures the lipid bilayer to form malonaldehyde as a secondary product. Immediately after incubation 1 mL of TBA was added into the mixture. The mixture was heated for 1 hour in an approximately boiling water bath at 95°C. However, after heating for 1 hour, the test tubes were cooled at room temperature and absorbance read at 532nm using UV-visible spectrophotometer model. Two molecules of thiobarbituric acid react with one molecule of MDA to form pink coloured product that absorbs monochromatic light maximally at 532nm. The TBARS was calculated using the formula in equation (1) above.

2.8 Determination of lipid hydroperoxide concentrations in samples by FOX-2 assay

Lipid hydroperoxides (ROOH) were determined by a slightly modified ferrous oxidation-xylenol orange assay method of [15] in conjunction with triphenylphosphine (TPP). The samples were extracted with methanol: chloroform (1:2) mixture using vortex mixer. These were centrifuged at 2,000 rpm for 10min. The upper aqueous layer obtained was discarded along with the protein layer. The lower chloroform layer was dried under inert atmosphere at 45 C. The residue obtained were stored in freezer for further use. Aliquots (180µl) of sample were transferred into eight centrifuge tubes (vials). Then 20 µL of 10 mmol TPP in methanol was added to four of the tubes (vials) to reduce ROOHs, thereby generating

a quadruplicate of blanks. Methanol (20 µL) was added to the remaining four (vials) to produce a quadruplicate of the test samples. All the tubes were then vortexed and incubated at room temperature for 30 min prior to the addition of 1800 µL of FOX-2 reagent (250 µM ammonium sulphate, 100µM xylenol orange, 25 mM H_2SO_4 and 4 mM BHT in 90% (v/v) methanol. The working reagent was routinely calibrated against H_2O_2 of known concentrations). After mixing, the samples were incubated at room temperature for another 30 min and the tubes were centrifuged at 8000 g for 10 min. Absorbance of supernatant was measured at 560 nm. The ROOH concentration in samples was calculated using the mean absorbance difference between quadruplicates of test samples and the blank samples. Hydroperoxide content was determined by using a molar absorbance co-efficient of 4.3 x 104 M^{-1} CM^{-1} in reference to H_2O_2 standard curve. The experiment was carried out in an inert atmosphere.

2.9 Determination of Conjugated dienes

The concentration of conjugated dienes (CD) were determined by a slightly modified of Buege and Aust [16]. A known quantity 1 g each of test samples were treated with 10ml of methanol: chloroform mixture (1:2) and glacial acetic acid: water mixture (1:1). These samples were vigorous vortexed and finally centrifuged at 2,000 rpm for 10 min. The upper layer obtained was discarded along with the proteins, while the lower chloroform layer was dried under a stream of nitrogen at 45ºC. The residues obtained were dissolved in 2 ml of cyclohexane and absorbance was taken at 234 nm against a cyclohexane (standard O.D. = 37.5 mmoles). This assay was carried out under inert atmosphere, in the presence of nitrogen.

2.10 Statistical analysis

The data were recorded as means ± standard deviation and analyzed by SPSS. One-way analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple range tests. $p < 0.05$ was regarded as significant and $p > 0.05$ was non-significant.

3. RESULTS

Table 1 shows the group descriptive statistics for the two different modes of food preparation applied in this study. The data depict that frying exhibited significant increases in lipid peroxidation in comparison with the second mode of food preparation which is roasting.

Table 1. Comparison of thiobarbituric acid reactive substances (TBARS) concentrations in both roasted and fried fast foods samples (the two different modes of preparation of our samples, frying and roasting)

	Mode of preparation	Mean ± Std. Deviation
1.	Frying	0.6493 ± 0.2051
2.	Roasting	0.1649 ± 0.1930

Table 2. Thiobarbituric acid reactive substances (TBARS) in roasted and fried fast foods samples

S/N	Mode of preparation	Sample	Concentration of TBARS from different Methanol/Chloroform Mean ± Std. Deviation (µM/g)
1.		Bread Fruit	0.0260±0.1775
2.		Bambara nut	0.0115±0.0049
3.		Pork Meat	0.0770±0.0403
4.	Roasted	Cashew nut	0.0130±0.3493
5.		Ground nut	0.1190±0.0368
6.		Baked Cake	0.0120±0.0106
7.		Suya Meat	0.5220±0.1280
8.		Chin~chin	0.4915±0.1570
9.		Puff~puff	0.0030±0.0672
10.		Bons	0.3570±0.1209
11.		Doughnut	0.1815±0.1018
12.	Fried	Akara	0.7130±0.1365
13		Plantain Chips	0.7295±0.1259
14.		Yam Chips	0.6705±0.2029
15		Potatoes Chips	0.2765±0.1824
16.		Egg	0.8020±0.0990
17.		Used frying Oil	0.8296±0.1974
18.		Negative Control	1.2340±0.1583

The concentration of TBARS extracted from Suya meat with methanol/chloroform solvent mixture exhibited significant difference when compared with other samples. Our results showed that Suya meat exhibited the highest concentration of TBARS of (0.5220±0.1280µM/g), followed by groundnut and pork meat with mean values of 0.1190±0.0368 and 0.0770±0.0403µM/g respectively. Our observation shows that fried egg recorded the highest mean value of TBARS of (0.8020±0.0990 µM/g) among fried food samples, but a non-significant decrease in comparison with the used frying oil, while the other samples exhibited varied values of TBARS in the increasing order of fried egg > plantain chips > Akara > yam chips>chin-chin > bons > potatoes chips> doughnut > puff-puff.

Table 3. The mean ± standard deviation values of Conjugated dienes from different extractive solvents in both roasted and fried fast foods samples

S/N	Mode-of Preparation	Sample	The concentration of conjugated dienes from different Extractive Solvents	
			Methanol/Chloroform (1:2)	Glacial Acetic Acid/Water (1:1)
			Mean ± SD	Mean ± SD
1.		Bread Fruit	0.0000±0.0000	0.03750±0.0370
2.		Bambara nut	0.5753±0.0003	0.30000±0.0750
3.		Pork Meat	0.2674±0.0000	0.93750±0.0750
4.	Roasted	Cashew nut	0.0152±0.0000	0.91320±0.0761
5.		Ground nut	1.3126±0.0006	0.93000±0.0750
6.		Baked Cake	0.0000±0.0000	0.01000±0.0433
7.		Suya Meat	2.6625±0.0001	0.86300±0.0000
8.		Chin~chin	1.2002±0.0003	0.45000±0.0750
9.		Puff~puff	0.0750±0.0000	0.16250±0.0573
10.		Bons	0.0750±0.0000	0.52500±0.0750
11.		Doughnut	0.0000±0.0000	0.93750±0.0750
12.	Fried	Akara	3.1876±0.0006	0.93750±0.0750
13.		Plantain Chips	3.16250±0.075	2.66250±0.0000
14.		Yam Chips	3.55000±0.075	2.66260±0.0006
15.		Potatoes Chips	0.0375±0.0000	2.46250±0.0750
16.		Egg	1.0125±0.0000	1.61250±0.0000
17.		Used Oil	3.6502±0.0003	3.48750±0.0750

Table 4. The mean ± standard deviation values of Hydroperoxide values for the two different modes of preparation of samples, frying and roasting

S/N	Mode of preparation	Sample	Concentration of hydroperoxide Mean ± Std. Dev. (µmol/L)
1.	Frying	Doughnut	0.000±0.000
2.		Buns	1.16×10^{-6} ±0.000
3.		Akara	5.56×10^{-6} ±0.000
4.		Chin-chin	3.84×10^{-6} ±0.000
5.		Egg	1.41×10^{-6} ±0.000
6.		Oil used	5.89×10^{-3} ±0.000
7.		Oil fresh	0.000±0.000
8.		Potato Chips	0.000±0.000
9.		Puff-Puff	0.000±0.000
10.		Yam	0.000±0.000
11.		Plantain	0.000±0.000
12.	Roasting	Suya	0.000±0.000
13.		Roasted Pork	0.000±0.000
14.		Cashew	0.000±0.000
15.		Groundnut	0.000±0.000
16.		Bambara	0.000±0.000
17.		Breadfruit	0.000±0.000

Glacial acetic acid/water solvent mixture extracted a significant concentration of conjugated dienes 0.03750±0.0370, 0.01000±0.0433, and 0.93750±0.0750 from roasted breadfruit, baked cake, and fried doughnut respectively, in comparison with their methanol/chloroform extracts that gave no dictation at all. In general, the methanol/chloroform solvent mixture gave the highest extractive mean values of conjugated dienes for roasted Bambara nut, groundnut, and suya meat, with suya meat exhibiting the highest extractive yield of 2.6625±0.001. The concentration of conjugated dienes extracted with glacial acetic acid/water solvent mixture were within dictation limits for all the roasted samples tested. Methanol/chloroform solvent mixture extracted a significant concentration of conjugated dienes from fried samples in the following order fried Yam-chips > Akara > Plantain chips >Chin-Chin > Fried egg > Bons = Puff-puff > Potatoes chips, with Yam chips exhibiting the highest extractive yield of 3.55000±0.075 in comparison with glacial acetic acid/water solvent mixture extract. Glacial acetic acid/water solvent mixture exhibited significant extractive yield of conjugated dienes from fried foods in increasing order of Yam Chips > Plantain chips = Potatoes chips > Egg > Doughnut = Akara > Bons > Chin-chin > Puff-puff.

Our results in Table 4 showed that varied concentration of hydroperoxide were detected in Buns, Akara, Cin-chin, fried egg and used oil, with used oil exhibited the highest concentration of hydroperoxide level of (5.89×10^{-3} ±0.000 µmol/L). Our observation shows that Akara recorded the highest mean value of hydroperoxide level of (5.56×10^{-6} ±0.000 µmol/L) among fried food samples. Hydroperoxide were beyond dictation among the roasted food samples.

4. DISCUSSION AND CONCLUSION

The health of cells of an individual determines the general overall health of that individual which is central to the quality of life, legally viewed and upheld by many as a fundamental human right that should be central to many economic and political decisions of any rational government or policymakers. Cellular membrane lipids represent the most vulnerable targets of oxidative free radicals since they are the first line of defense/attack because they constitute the plasma membrane of the outermost part of everybody's cells. Polyunsaturated fatty acids (omega-9, -6, and -3 series) serve as excellent substrates for lipid peroxidation because of the presence of active bis-allylic methylene groups [17]. The carbon-hydrogen bonds on these activated methylene units have lower bond dissociation energies, making these hydrogen atoms more easily abstracted during free radical reaction/attack [17].

The concentration of TBARS extracted from Suya meat with methanol/chloroform solvent mixture exhibited a significant difference when compared with other samples. Our results showed that Suya meat exhibited the highest concentration of TBARS of (0.5220±0.1280µM), followed by groundnut and pork meat with mean values of 0.1190±0.0368 and 0.0770±0.0403µM respectively. Our observation shows that fried egg recorded a non-significant decrease in the mean value of TBARS of (0.8020±0.0990 µM) in comparison with the frying oil, while the other samples exhibited varied mean values of TBARS in the increasing order of fried egg > plantain chips > Akara > chin-chin > bons > potatoes > doughnut > puff-puff. [18,19] recently reported that intake of repeatedly heated palm and soybean oils significantly increased the blood pressure in experimental animals. In addition [20] reported that consumption of repeatedly heated frying oils is associated with an increased risk of hypertension. Observation from this present research reveals that glacial acetic acid/water solvent mixture extracted varied significant concentrations of conjugated dienes 0.03750±0.0370, 0.01000±0.0433, and 0.93750±0.0750 from roasted bread fruit, baked cake and fried doughnut respectively, in comparison with their methanol/chloroform extracts that were beyond dictation limits. The non-significant increase observed in the extractive yield of glacial acetic acid/water solvent mixture may be attributed to the hydrophilic (polar) protic solvent nature of the mixture with a moderate dielectric constant of 6.2 and can dissolve not only polar compounds but also non-polar compounds such as oils and elements such as sulfur and iodine. Our results regarding the non-significant increase in extractive yield of glacial acetic acid/water solvent mixture in relation to the conjugated dienes are consistent with previous studies by Zeb, and Ullah [12]. In the present study, the methanol/chloroform solvent mixture gave the highest significant extractive mean values of conjugated dienes for roasted suya meat, groundnut, and Bambara nut, with suya meat exhibiting the highest concentration of 2.6625±0.001. The concentration of conjugated dienes extracted with glacial acetic acid/water solvent mixture were within dictation limits for all the roasted samples tested. This present *in-vitro* determination of the level of lipid peroxidation in both roasted and fried fast foods indicates that methanol/chloroform solvent mixture extracted a significant concentration of conjugated dienes from fried samples in the following order fried Yam-chips > Akara > Plantain chips >Chin-Chin > Fried egg > Bons = Puff-puff > Potatoes chips, with yam chips exhibiting the highest extractive yield of 3.55000±0.075 in comparison with glacial acetic

acid/water solvent mixture extract. It was part of our observations that the mean glacial acetic acid/water solvent mixture exhibited significant extractive yield of conjugated dienes from fried in increasing order of fried Yam Chips > Plantain chips = Potatoes chips > Egg > Doughnut = Akara > Bons > Chin-chin > Puff-puff. This current observed a significant difference between roasted foods and deep-fried fast foods indicates that the oil used in frying does not only serve as the medium of heat energy transfer but also as one of the major sources of lipid peroxidation products for the food sample being fried, unlike roasting where the food sample has only direct contact with the temperature of the flame. However, the two major known sources of these toxic products of lipid peroxidation (alcohols, malondiadheyde, aldehydes, hydroperoxide, ketones, hydrocarbons, *trans* isomers, cyclic and epoxy compounds) [21,22], dictated in fried food samples may be those generated due to extreme temperature of the frying oil and those generated from the lipid peroxidation of intrinsic lipid constituent of the fried food. This can be presented by the following equation;

Total lipid peroxidation products in the fried food sample = { Total concentration of volatile and non-volatile lipid peroxidation products absorbed by the fried food sample from the frying oil } + {Total concentration of volatile and non-volatile lipid peroxidation products formed from the intrinsic lipid constituents of the fried food sample}.

{TTPP = TVVnVLPFO + TVVnLPiLFF} Equation-2

Using a constant volume of frying oil, we may assume that the total lipid peroxidation products of frying oil to be constant since the boiling point of the frying oil is the same. Therefore, the total concentration of toxic lipid peroxidation products in the fried food sample is dependent on these variable factors; Firstly, the intrinsic lipid peroxidation in the fried food sample, which invariably is dependent on the intrinsic volume of the lipid constituent of the food sample, the concentration of the antioxidants in the food sample, the overall intrinsic temperature of the food sample during frying, which depends on the food matrix, cellular location of the lipid (extracellular or intracellular), the type of cell involved, either plant cell wall or animal cell, and lipid absorptivity of the food sample. Secondly, lipid peroxidation products generated from the frying oil, which invariably is dependent on the volume of the frying oil used, presence of trace metals in the oil, presence of salt in the oil, presence of water, number of times the oil has been used, presence of impurities in the oil, duration of heating, the type of frying oil used and smoke point. The health implication of these findings is that daily consumption of highly oxidized fatty acids via intake of ready-made deep-fried fast food will lead to accumulation of lipid peroxidation products with severe health complications such as diabetes, hypertension, stroke, cardiovascular, cancer and vascular inflammatory diseases and untimely death.

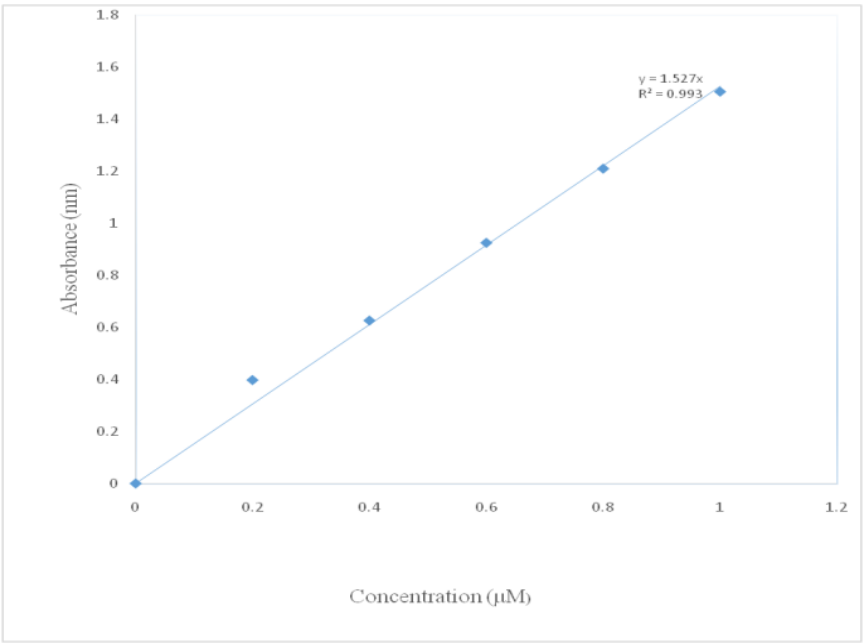

Fig. 1. A linear regression curve of the standard concentration of 0.2 – 1.2 µM with a correlation coefficient of 0.9963 and regression equation of y = 1.5278x. Each point in the regression represents the replicate measurement (n =3)

Table 5. The Standard MDA Concentrations of 0.2 – 1.0 µM and the Absorbance (nm)

S/N	Concentration (µM)	Absorbance (nm)			Average
1.	0.2	0.3978	0.3978	0.3978	0.3978
2.	0.4	0.6267	0.6267	0.6267	0.6267
3.	0.6	0.9256	0.9256	0.9256	0.9256
4.	0.8	1.2110	1.2110	1.2110	1.2110
5.	1.0	1.5068	1.5068	1.5068	1.5068

NOTE: CD levels are expressed as absorbance of a 1% solution at 234 nm ($E_{234}^{1\%}$); however, the calculation necessary for expressing them this way requires knowing the extinction coefficient of the sample, which itself requires knowledge of the specific fatty acid composition of each sample. Because these information for all the tested samples were not available for this research work, CD levels were expressed in units of raw absorbance.

HIGHLIGHTS

- ✓ The levels of lipid peroxidation products in most roasted and deep-fried fast foods eaten in south eastern Nigeria were determined.
- ✓ Extraction of TBARS from both Fried and Roasted Food Samples using different solvents mixtures, Methanol:Chloroform (1:2), and Glacial Acetic Acid:Water (1:1).
- ✓ Thiobarbituric acid reactive substances (TBARS), conjugated dienes, and hydroperoxide were used as bio-markers
- ✓ Our observation reveals that the concentrations of these products of lipid peroxidation in deep-fried fast foods were significantly higher than that of roasted fast foods.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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