

(RESEARCH ARTICLE)



Sub-chronic toxicity study of palm kernel oil and soya oil in albino Wister rats

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International Journal of Biological and Pharmaceutical Sciences Archive, 2022, 03(02), 009–019

Publication history: Received on 16 February 2022; revised on 20 March 2022; accepted on 22 March 2022

Article DOI: <https://doi.org/10.53771/ijbpsa.2022.3.2.0030>

Abstract

Background: Vegetable oils are commonly used food additives.

Aim: To characterize and observe the toxic effect of sub chronic consumption of heated and unheated Palm Kernel oil (PKO) and Soya Oil (SO) after heating at 180 °C and at room temperature in albino Wistar rats.

Methodology: The physicochemical properties were analyzed, sixty rats were divided into five groups of twelve rats and their baseline weight recorded. Group 1 (controls), received water and feed only, while 10mls of each oil was added (add libitum) to 100 grams of feed in each test group for six weeks and twelve weeks. Group 2 received feed, water and unheated PKO and Group 3 received feed, water and heated PKO. Group 4 were given feed, water and unheated SO while group 5 had feed, water and heated SO. The lipid profile and blood glucose were measured at six weeks exposure while the renal function and liver function were determined at twelve weeks exposure.

Result: Heating the oils at 180 °C for 15 minutes caused a decrease in relative density, peroxide values, and iodine values in both the PKO and SBO and increased acid values and free fatty acids in both with no effect in the refractive index. Biochemical result showed that both heated and the unheated oil samples significantly increased the LDL levels, HPKO and UHSO caused increase in total cholesterol levels, UHPKO significantly decreased the TAG, HPKO significantly increased the ALT, HPKO significantly decreased the urea level and HSO increased the BG significantly.

Conclusion: Heating alters the physicochemical properties of the oils thereby decreasing their qualities, while the oils also demonstrated evidence of hyperlipidemia.

Keywords: Sub Chronic; Toxicity; Palm Kernel Oil; Soya Oil

1. Introduction

Chronic diseases are the leading cause of mortality, morbidity, disability and decrease quality of life [1]. It accounts for at least 65% of all death and about 84% of health spending especially in the United States. A joint WHO/FAO expert consultation on diet, nutrition and prevention of chronic disease recognized that the growing epidemic of chronic diseases affecting both developed and developing countries were related to dietary and life style changes [2].

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Furthermore, rapid changes in diet and life style that have occurred with industrialization, urbanization, economic development and market globalization have accelerated over the past decade. This is having significant impact on health and nutritional status of populations, particularly in developing countries and countries in transition. While standard of living improved, food availability has expanded and become more diversified, and access to services has increase, there have also been significant negative consequence in terms of inappropriate dietary patterns, decreased physical activities, increase tobacco use, and a corresponding increase in diet related chronic disease, especially among poor people. Because of these changes in life style patterns, chronic non communicable diseases (NCDs) – including obesity, diabetic mellitus, cardiovascular diseases, hypertension and stroke, and some types of cancer are becoming increasingly significant cause of disability and premature death in both developing and newly developed countries.

The burden of chronic disease is rapidly increasing worldwide. It has been calculated that in 2001, chronic diseases contributed approximately 60% of the 56.5 million total reported deaths in the world and approximately 46% of the global burden of death [2]. The proportion of the burden of NCDs is expected to increase to 57% by 2020, chronic disease will account for almost three quarters of all death worldwide, and that 71% of deaths due to stroke and 70% of death due to diabetes will occur in developing countries. The number of people in the developing world with diabetes will increase by more than 2.5 fold from 84million in 1995 to 228million in 2025 [3].

This increasing pandemic of non-communicable/chronic diseases mandates urgent research into possible aetiopathogenic influences. Epidemiological studies show association to entity called metabolic syndrome and this suggests that chronic diseases may to an extent share common genetic and/or environmental predisposing factors [4].

While fats are essential parts of balanced diet, there is an evidence to show that limiting hydrogenated and trans-fats intake is important. Too much saturated and Tran's fat contributes to the buildup of plaques inside the blood vessels via atherosclerosis and is a major cause of heart diseases.

Saturated fatty acids (SFA) and Tran's fats increase low density lipoprotein (LDL) cholesterol in blood, which lead to plaque formation. Polysaturated fatty acids (PUFAs) and monosaturated fatty acids (MUFAs) reduce LDL cholesterol and increase high density lipoprotein (HDL) cholesterol. It has been noted that LDL particles play a causal role in the development of CVD [5, 6] and that, in general, there is a relationship between lowering of LDL cholesterol and CVD benefit [6]. Lowering the consumption of saturated fat has been a central theme of U.S dietary goals and recommendations since the late 1970s [7]. It has been recommended that saturated fatty acid (SFA) intake be limited to <10% of total calories as a means of reducing risk for cardiovascular disease (CVD) [7]. In 2018, the U.S. Departments of Agriculture and Health and Human Services asked for public comments in response to the following question: "What is the relationship between saturated fat consumption (types and amounts) and risk of CVD in adults? [8]. In the Physician's Health Study, an increase in plasma palmitoleic acid concentration by 1 SD was associated with a 19% greater odds ratio for coronary artery disease [9].

The information emerging from these studies suggests that genetic variants may modulate the relationship between dietary SFAs and CVD-related biomarkers [10]. A major chemical alteration in the oil was the increase in both glycidyl and MCPD esters. Remarkably, addition of either glycidol or MCPD to virgin coconut oil partially recapitulated the effects on cellular cholesterol metabolism [11]. Experimental rodent studies using oxidation-resistant linoleic acid, di-deuterated in the bis allylic position, support the hypothesis that oxidation products and not specific fatty acids cause plaque formation in transgenic mouse models [12].

MUFAs are beneficial in that they increase cholesterol esterification in the liver, thereby reducing the free cholesterol pool and increasing receptor mediated uptake of LDL cholesterol, resulting in a decrease in blood cholesterol level as reported by the dietary Guidelines Advisory Committee (DGAC) on the dietary Guidelines for Americans 2010. Evidence from controlled clinical studies has shown that MUFA favourably affect a number of risk factors for coronary heart diseases (CHDs).

In America, one out of four, have multiple chronic conditions with hypertension being the most condition among Medicare beneficiaries with multiple conditions.

Additionally chronic diseases are responsible for the widest health disparity gap among racial/ethnic groups. There are many known risk factors for chronic diseases, such as smoking unhealthy diet, and physical in activities. Mediterranean-style dietary interventions were noted to reduce CVD risk [13]. The increasing expansion of our drug arsenals and use of radical surgical intervention to combat these health problems is far more expensive and less rewarding than a strategy which addresses the gene environment interactions leading to immune dysfunction and hence chronic

diseases, cancers and increased severity of infections. Thus identifying and avoiding genetically predisposed toxic environmental exposure is the key to improving the functions of the immune system.

2. Material and methods

2.1. Materials

2.1.1. Reagents/ instruments /equipment

The materials used include: experimental animals (albino Wistar rats) Solive oil (batch number 25530), Grand Soya oil (batch number 160518), weighing balance, oven, Refrigerator, UV Spectrophotometer, centrifuge, dissecting set, microtome, light microscope, and Plain bottles, growers' mash (Top Feed), accucheck glucometer, glucose strips, hand gloves, commercial kits Randox reagents.

2.2. Experimental Animals

Sixty (Male and Female) albino wistar rats weighing between (75-160g) were purchased and housed in cages at room temperature at university of Nigeria Enugu campus animal house where the experiment was carried out. After two weeks for acclimatization, they were randomly distributed into five groups each containing 12 rats and were fed with growers' mash- and water was given to them *ad-libitum*.

The principles of laboratory animal care were followed.

2.3. Experimental Design

Solive oil (PKO) and Grand Soya oil (SO) were purchased at Ogbete main market, Enugu state Nigeria. One liter of each sample was heated in an electric oven at temperature of 180 °C for 15 minutes. 200mls of each of the unheated and heated oil samples were sent to National Research Institute for Chemical Technology Zaria, where the physicochemical analysis was determined. Sixty albino Wister rats were grouped into five different groups:

- Group 1: Control Group; they received feed +water only.
- Group 2: They received feed + water + unheated PKO
- Group 3: They received feed + water + heated PKO
- Group 4: They received feed + water + unheated SO
- Group 5: They received feed + water + heated SO

To each group, 10mls of respective oil samples were added to 100g of feed +water (*ad libitum*). Baseline weights were measured before exposing them to the oil meals. After six weeks exposure, they were fasted overnight and reweighed and blood samples were collected from the peri orbital surface for determination of the serum lipid profile and from the tail vein for blood glucose. Further exposure for another six weeks (total of 90 days exposure), they were reweighed and blood samples were collected into plain bottles for assay of the liver function and renal function tests.

2.4. Blood Sample Collection

3mls of blood samples were collected from the peri orbital plexus into plain bottles and were centrifuged at 2500 rpm for 20 minutes. The serum obtained was stored in a refrigerator at 4 °C and all analysis completed within 48 hours of samples collection.

2.5. Determination of Physicochemical Properties

2.5.1. Determination of acid value

The acid value was determined using IUPAC (1979) method. A solvent mixture of 25ml 95% ethanol and 25ml diethyl ether was neutralised with 0.1M ethanolic KOH using phenolphthalein as indicator. 4g of the oil sample was dissolved in the neutralised solvent mixture and titrated with 0.1M KOH

2.5.2. Determination of Peroxide Value

Peroxide value was determined using IUOAC (1979) method.

2-4g of oil sample was weighed into a ground neck conical flask and 10ml chloroform was added to dissolve the oil sample. This was followed by the addition of 15ml acetic acid and 1ml 5% KI solution. The mixture was shaken and kept in the dark for 5 minutes. After which 75ml of water was added and titrated with 0.002M sodium thiosulphate,

2.6. Determination of Lipid Profile

2.6.1. Determination of Total Cholesterol Concentration

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. Three test tubes were labelled blank, standard and sample respectively. Into the blank were added 10 µl of distilled water and 10 µl of standard to the labelled test tubes. Sample serum (10 µl) was added to the appropriately labelled test tube. Reagent (1 ml) was added to the three sets of the tubes, mixed and incubated at 37 °C for 5 minutes. The absorbance of the sample (A sample) was measured against the reagent blank within 60 minutes at 500 nm

2.6.2. Determination of Triacylglycerol Concentration

One hundred micro liter (100 l) of the reagent-1 was pipette into the reagent blank tube, standard tube and the sample tubes. In the standard test tube was added 10 l of the standard (CAL) while 10 l of the sample was pipette into the sample tube mixed thoroughly and incubated for 10 minutes at 20 – 25 °C. Absorbance of the sample and the standard were measured against the reagent blank within 60 minutes at 546 nm.

Triacylglycerol Concentration = $(\Delta A \text{ sample} / \Delta A \text{ standard} * \text{Conc. Of standard in mg/dL})$

2.6.3. Determination of Low Density Lipoprotein (LDL) Cholesterol Concentration

Cholesterol Concentration

Low density lipoprotein Cholesterol (LDL) level was determined using BioSystems kit. LDL-Cholesterol in the sample precipitate with polyvinyl sulphate. Their concentration is calculated from the difference between the serum total cholesterol and the cholesterol in the supernatant after centrifugation. The cholesterol is spectrophotometrically measured by means of the coupled reactions described below.

The procedure involved two steps.

- Precipitation Step

The serum sample (0.2 ml) was pipetted into labeled centrifuge tubes. A quantity (0.2 ml) of the precipitant solution or reagent was added to each of the centrifuge tubes. The contents in the various tubes were thoroughly mixed and allowed to stand for 15 minutes at room temperature (20 -25 °C), then centrifuged at 4000 rpm for 15 minutes.

- Determination of cholesterol concentration in the supernatant

Three test tubes were labeled blank, standard and sample respectively. Into the blank were added 20 µl of distilled water and 20 µl of standard to the labeled test tubes. Sample serum (20 µl) was added to the appropriately labeled test tube. Reagent (1 ml) was added to the three sets of the tubes, mixed and incubated at 37 °C for 5 minutes. The absorbance of the sample (A sample) was measured against the reagent blank within 60 minutes at 500 nm.

2.6.4. Determination of High Density Lipoprotein (HDL)

Cholesterol Concentration

High density lipoprotein Cholesterol (HDL) level was determined using Randox kit.

Low density lipoproteins (VLDL and LDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, is determined. The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

Cholesterol ester +H₂O -----→ Cholesterol +fatty acid

Cholesterol + O₂ -----→ Cholestene-3-one + H₂O₂

2H₂O₂ + Phenol + 4 Aminoantipyrine -----→ Quinoneimine + 4H₂O

Reagents used are commercial Kit obtained from Randox Lab Limited, UK.

HDL Reagent		
R1	Phosphotungstic Acid	0.55 mmol/l
	Magnesium Chloride	25 mmol/l

The procedure involved two steps.

- Precipitation Step

The serum sample (0.3 ml) was pipetted into labeled centrifuge tubes. A drop of the precipitant solution or reagent was added to each of the centrifuge tubes. The contents in the various tubes were thoroughly mixed and allowed to stand for 15 minutes at room temperature (20 -25 °C), then centrifuged at 2000 rpm.

- Determination of cholesterol concentration in the supernatant

Three test tubes were labeled blank, standard and sample respectively. Into the blank were added 10 µl of distilled water and 10 µl of standard to the labeled test tubes. Sample serum (10 µl) was added to the appropriately labeled test tube. Reagent (1 ml) was added to the three sets of the tubes, mixed and incubated at 37 °C for 5 minutes. The absorbance of the sample (A sample) was measured against the reagent blank within 60 minutes at 500 nm.

2.7. Determination of Liver Function

2.7.1. Determination of Aspartate Aminotransferase (AST)

The serum AST concentration was determined using enzymatic colourimetric method which is based on the principle that Aspartate aminotransferase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine. L-aspartate and α-oxoglutarate are converted by Aspartate aminotransferase to L-glutamate and oxoglutarate. The liberated oxoglutarate then react with 2, 4-dinitrophenylhydrazine to form oxoglutarate hydrazone which absorbs monochromatic light at 546 nm. Provided are R1: Buffer (Phosphate buffer (100 mmol/l, pH 7.4), L-aspartate (100 mmol/l), α-oxoglutarate (2.0 mmol/l), and R2: 2, 4-dinitrophenylhydrazine (2.0 mmol/l)) the set-up consists of test tubes labeled reagent blank and sample placed in a test tube rack. The reagent blank test tube contained 0.5 ml of solution R1 and 0.1 ml of distilled water, while the sample test tube contained 0.5 ml of solution R1 and 0.1 ml of serum. They were properly mixed and incubated for 30 min at 37 °C before aliquot of (0.5 ml of solution R2) was added to both the reagent blank and sample respectively. All the test tubes were properly mixed and incubated for 20 min at 25 °C, after which (5.0 ml) of sodium hydroxide was added to all the test tubes mixed properly and the absorbance of the sample (A sample) was read against the sample blank at

2.7.2. Determination of Alanine Aminotransferase (ALT)

The serum ALT concentration was determined using enzymatic colourimetric method which is based on the principle that Alanine aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine. L-alanine and α-oxoglutarate are converted by Alanine aminotransferase to L-glutamate and pyruvate. The liberated pyruvate then react with 2,4-dinitrophenylhydrazine to form pyruvate hydrazone which absorbs monochromatic light at 546 nm. Provided are R1: Buffer (Phosphate buffer (100 mmol/l, pH 7.4), L-alanine (200 mmol/l), α-oxoglutarate (2.0 mmol/l), and R2: 2,4-dinitrophenylhydrazine (2.0 mmol/l)) the set-up consists of test tubes labeled reagent blank and sample placed in a test tube rack. The reagent blank test tube contained 0.5 ml of solution R1 and 0.1 ml of distilled water and sample test tube contained 0.5 ml of solution R1 and 0.1 ml of serum. They were properly mixed and incubated for 30 min at 37 °C before aliquot of (0.5 ml of solution R2) was added to both the reagent blank and sample respectively. All the test tubes were properly mixed and incubated for 20 min at 25 °C, after which (5.0 ml) of sodium hydroxide was added to all the test tubes mixed properly and the absorbance of the sample (A sample) was read against the sample blank at 546 nm.

2.8. Renal Function Test

2.8.1. Determination of Serum Urea Concentration

The serum urea concentration was determined using enzymatic colourimetric method which is based on the principle that urea is converted by urease to ammonia and carbon dioxide. The liberated ammonia is then measured photometrically by Berthelot's reaction (the reaction of NH_3 , hypochlorite and phenol to form a blue coloured complex compound indophenol that absorbs monochromatic light at 546 nm. Provided are R1: EDTA (116 mmol/l), Sodium nitroprusside (6 mmol/l), Urease (1 g/l), R2: Phenol (diluted) (120 mmol/l), R3: Sodium hypochlorite (diluted) (27 mmol/l), Sodium hydroxide (0.14N) and Standard. One bottle of diluted phenol was diluted with 660 ml of distilled water. One bottle of enzyme reagent was reconstituted with one bottle of diluted phenol, while one bottle of sodium hypochlorite was diluted with 750 ml of distilled water. The set-up consists of test tubes labeled reagent blank, standard and sample placed in a test tube rack. The reagent blank test tube contained 10 μl of distilled water, standard test tube contained 10 μl of uric acid and sample test tube contained 10 μl of serum. An aliquot of 100 μl of R1 diluted enzyme reagent was added to all the test tubes and were properly mixed and incubated for 15 min at 37 °C, after which (2.50 ml) of both reagents 2 and 3 were added to all the test tubes mixed properly and incubated at 37 °C for 15 min. The absorbance of the sample (A sample) and of the standard (A standard) was read against the reagent blank at 546 nm. The urea concentration was calculated using the relation.

2.8.2. Determination of creatinine concentration

Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration.

Standard solution (0.1ml) was added to set of test tubes labeled ST (standard), while (0.1 ml) of the sera from the different rats were added to different test tubes labeled SA (sample). Working reagent gotten by mixing equal volumes of picric acid and sodium hydroxide (1.0ml) was added to all the test tubes. The content was mixed thoroughly and the first absorbance A1 was read 30 seconds after mixing. Exactly 2 minutes later the second absorbance A2 was read at a wavelength of 492 nm

2.9. Statistical Analysis

The data obtained from the experimental measurements were analyzed using statistical package for social sciences (SPSS version 21). Further analysis was done using one way analysis of variance (ANOVA) to determine the significant differences among the treatments and compared with Bonferroni post Hoc tests. The data were expressed as mean \pm the standard error of mean (SEM) and considered significant at $p < 0.05$

3. Results

In table 1 below, both PKO and SO when subjected to high temperature of 180-200 °C showed decrease in their iodine values, relative density, and peroxide values while there was increase in the free fatty acid and acid values, but heating has no effect on their refractive index. However the saponification number was slightly decreased in the PKO oil but appears markedly increased in SO.

Table 1a Effects of heat on physicochemical properties of PKO and SO and compared with Codex Stan

Samples	Relative density (X °C/H ₂ O)			Refractive index (ND 40 °C)			Saponification number (mgKOH/gml)		
	Codex Stan.	Room Temp.	180 °C	Codex Stan.	Room Temp.	180 °C	Codex Stan.	Room Temp.	180 °C
PKO	0.899-0.914	0.973	0.960	1.448-1.452	1.474	1.472	230-254	138.850	133.240
SO	0.913-0.919	0.965	0.941	1.466-1.470	1.456	1.456	189-195	100.980	133.240

Table 1b Effects of heat on the physicochemical properties of PKO and SO and compared with Codex Stan

Samples	Peroxide values (Meq/kg)			Acid values (mgKOH/g)		
	Codex Stan.	Room Temp.	180 °C	Codex Stan.	Room Temp.	180 °C
PKO	<10.000	4.400	4.000	<2.000	7.150	9.820
SO	<10.000	4.400	0.800	<0.600	7.010	9.120

Table 1c Effects of heating on the physicochemical properties of PKO and SO and comparison with Codex Stan

Samples	Iodine values(g/100g)			Free fatty acid values(percent)		
	Codex Stan.	Room Temp.	180 °C	Codex Stan.	Room Temp.	180 °C
PKO	14.100-21.000	82.490	75.500	2.400-6.200	3.525	4.900
SO	124.000-139.000	88.830	67.790	48.000-59.000	3.500	4.560

Table 2 The effect of Unheated and Heated PKO and SO on Lipid profile of albino Wister rats

Parameters/Groups	CONTROL	UHPKO	HPKO	UHSO	HSO
Total Cholestrol (Mg/Dl)	52.420±5.620	64.820±10.70	*88.360±14.830	*80.580	76.140±10.230
Triacylglycerol(Mg/Dl)	59.810±9.560	*39.050±2.840	86.980±14.820	93.310±11.200	78.030±10.860
Low Density Lipoproteins(Mg/Dl)	*81.580±12.470	*149.500±16.400	*152.230±15.040	*128.08±17.030	*142.250±7.660
High Density Lipoproteins(Mg/Dl)	55.670±6.030	47.760±6.540	51.330±6.540	61.580±36.400	52.670±4.820

Results are expressed as Mean± SEM (N=12). *Significant at p<0.05 compared with control using analysis of variance.

Table 3 The effect of Unheated and Heated PKO and SO on Liver function parameters of albino rats

	CONTROL	UHPKO	HPKO	UHSO	HSO
Albumin (U/L)	3.770±0.080	3.680±0.080	3.810±0.070	3.860±0.050	3.760±0.040
Alanine aminotransaminases (U/L)	39.900±5.270	61.470±13.040	*58.850±6.270	50.360±1.970	40.350±2.210
Aspartate aminotransferases(U/L)	28.710±0.076	28.740±0.080	28.850±0.039	28.900±0.086	28.660±0.042

Results are expressed as Mean± SEM(N=12). *Significant at p<0.05 compared with control using analysis of variance.

Table 4 The effect of Unheated and Heated PKO and SO on Renal function parameters of albino rats

	CONTROL	UHPKO	HPKO	UHSO	HSO
Creatinine (Mg/dl)	0.072±0.020	0.0710±0.090	0.790±0.040	0.660±0.040	0.720±0.030
Urea (Mg/dl)	29.270±910	27.410±1.95	*22.720±0.910	32.370±1.320	26.440±1.680

Results are expressed as Mean± SEM (N=12). *Significant at p<0.05 compared with control using analysis of variance.

Table 5 The effect of Unheated and Heated PKO and SO on Blood glucose on albino Wister rats

	CONTROL	UHPKO	HPKO	UHSO	HSO
Blood glucose (Mg/dl)	94.250±5.260	90.580±0.720	101,750±10.400	105.330±6.350	*110.830±4.760

Results are expressed as Mean± SEM (N=12). *Significant at p<0.05 compared with control using analysis of variance.

4. Discussion

The quality of the PKO and the SO were analyzed by evaluating physiochemical properties such as Relative density, Refractive index, Saponification value, Peroxide value, Acid value, Iodine value and FFA value and the effect of temperature on them as shown in table 1a-c

The relative densities of PKO and SO at room temperature were 0.973 and 0.963x °C/H₂O respectively which is higher than the normal CODEX STAN range. This parameter for both PKO and SO were decreased following the heating of the oils. The density of PKO and SO were temperature dependent and decreased in value when temperature increased. Density has been described as one of the most basic or important parameters of fuel as certain performance indicators such as heating value and cetane number are correlated with it [14]. During heating, thermo oxidation or lipid oxidation and hydrolytic reactions take place that result in deterioration in quality of the heated oil. Normally, oils undergo extensive degradation and complex chemical transformation when heated. The refractive index for Palm Kernel and Soya Oils at room temperature were 1.474 and 1.456 ND 40 °C respectively which is higher than the CODEX STAN range. However heating did not affect it. The saponification numbers of both oils at room temperatures were 138.850 and 100.98mgKOH/g which is below the normal CODEX STAN range. The low saponification suggests that the mean molecular weights of fatty acid or that the number of ester bond is less. The results showed increase in the saponification value during heating. High saponification value indicates that oils are very useful in production of liquid soap and shampoo industries [15]. The saponification value gives an idea about the number of ester equivalents per unit mass of the oil or biodiesel. Heating increased the parameter. The acid values were 7.150 and 7.010mgKOH/g, both oil demonstrated increase as a result of heating. The peroxide values at room temperature were 4.400Mequiv/g for both PKO and SO, this was noted to be below CODEX STAN (normal range= 10 Mequiv/g). However at high temperature the values were further decreased.

The iodine values for the PKO and SO are 82.49 and 88 I₂ g/100g oil respectively compared with the CODEX STAN, the PKO was higher while the SO was low and when they were subjected to heat, were further decreased. The decrease of iodine value correlated well with the decrease of unsaturated fatty acids. Iodine value decrease is indicative of the increased rate of oxidation during heating and could be attributed to oxidation and polymerisation reactions involving the double bonds [16]. Unsaturation to limited extent is desirable in biodiesel feedstock to meet the requirements for cold weather conditions [17]. Unsaturation reduces cloud point, pour point and cold filter plugging point to make biodiesel suitable for cold weather conditions [17]. The free fatty acid values at room temperature were 3.525 and 3.500 percent respectively and were increased with high temperature. The increase in FFA could be attributed to oxidation and hydrolysis that produces Free Fatty Acids. However, the PKO was within standard range but the SO was lower than the standard. Alterations in the concentrations of major lipids can give useful information on the lipid metabolism and predisposition to cardiovascular risk. From the findings obtained in this study, HPKO and UHSO significantly increased the total cholesterol (p<0.05) in the test animals.

An increased level of cholesterol in the blood (Hypercholesterolemia) as observed in this result has been implicated in cardiovascular diseases. Hence, thermally oxidized PKO may increase the risk of cardiovascular diseases

Only the UHPKO showed significant decrease (p<0.05) in the level of serum TAG, Ingestion of oxidized lipids rich in linoleic acid causes profound alteration in membrane composition fluidity and function which is likely to be associated with an enhanced cholesterol turn over [18]. Others added that oxidation process of heated oil causes changes in fatty acid configuration from the cis isomer to the Trans.

On the other hand, the entire test samples significantly (p<0.05) increased the serum LDL. This finding is in agreement with [19] where it was reported that prolonged feeding with heated soya oil and heated palm oil increased serum LDL-cholesterol. Furthermore, an increased level of LDL-cholesterol is a risk factor of cardiovascular diseases such as atherosclerosis. Earlier studies reported that serum lipids levels of rats fed repeatedly with heated oil were significantly increased

Intake of Tran's fat was found to be correlated with the increase in serum total cholesterol and LDL levels and the decrease in HDL values [20, 21]. It was proved that hydroxy fatty acids and other secondary lipid oxidation products of oil especially that contain PUSFA cause diarrhea, an impairment of liver function, elevation in serum liver enzymes with a slight hypertrophy and an increase in blood lipids and cholesterol [22].

Ingestion of thermally oxidized oil has been reported to cause a concomitant evolution of very cytotoxic and destructive by-products which may be injurious to cells, tissues and organs [23].

Liver enzymes are raised in acute hepatotoxicity and reduced in prolonged intoxication due to damage to the liver. Alanine transaminase (ALT), and Aspartate transaminase (AST) are enzymes that are located in the liver cells and that leak out and make their way into the general circulation when liver cells are injured [24]. ALT is regarded as being a more specific indicator of liver inflammation, since AST may be elevated in diseases of other organs such as heart disease or muscle disease (Johnston). In acute liver injury, such as acute viral hepatitis, ALT and AST may be elevated to very high levels, sometimes over 1000 U/L [24]. In chronic hepatitis or cirrhosis, the elevation of these enzymes may be minimal (less than two to three times normal) or moderate. Mild or moderate elevations of ALT or AST are nonspecific and may be caused by a wide range of liver diseases.

This study shows that HPKO significantly increased ($P < 0.05$) the ALT. The reason for this is not clear but it may be connected with lipid peroxidation which may be responsible for the induced enzyme changes that occurred. (Further studies are recommended to observe the effect and possible mechanism). This result is in agreement with the study done by [25] where rats fed with palm oil rich diet were noted to have significant increase in the ALT.

The significant increase in ALT activities by HPKO may therefore be indication that HPKO may have a deteriorative effect on the integrity of the hepatocytes,

The test samples (UHPKO, HPKO and HSO) showed decrease in the serum urea concentration that was significant ($p < 0.05$) only by HPKO. Clinically, measurement of blood urea nitrogen alone is less useful in diagnosing kidney diseases because its blood level is influenced by dietary protein and hepatic function but its diagnostic value is improved with serum creatinine values. In this study, the serum creatinine was not significantly ($p < 0.05$) affected thus the test samples may have no significant effect on the renal functions.

The mean values of serum glucose concentrations were significantly ($P < 0.05$) higher in HSO when compared with the control. Elevated levels of free fatty acids and triglycerides in the blood stream and tissues have been found in many studies to contribute to diminished insulin sensitivity and development of insulin resistance [26]. Reduced peripheral use of glucose and a failure to suppress glucose production and release into the blood associated with insulin resistance all contribute to elevated blood glucose levels.

5. Conclusion

Heating alters the physicochemical properties of both solive and grand soya oils, thereby lowering their qualities. Also, the lipid profile showed significant derangement, implying that consumption of these products can predispose to hyperlipidemia as demonstrated by the significantly increased LDL levels by all the test samples and the significant increase in total cholesterol levels by HPKO and UHSO. Thus consumption of these products especially when heated, may not be completely safe.

Compliance with ethical standards

Acknowledgments

Authors are grateful to all the laboratory attendants and technicians that were of help in the course of the study.

Disclosure of conflict of interest

The authors declare that they have no conflict of interests.

Statement of ethical approval

The approval of the Ethics Committee of the University of Nigeria was obtained for the study.

References

- [1] CSTE. Essential Functions of Chronic Disease Epidemiology in State Health Departments. September 21,2004 page iv
- [2] Guilbert JJ. The World Health report 2002- reducing risks, promoting healthy life. Educate Health (Abingdon).2003 Jul; 16(2):230.
- [3] World Health organization. (2001). Life course perspectives on coronary heart disease, stroke and diabetes: key issues and implications for policy and research: summary report of a meeting of experts, 2-4 may 2001.Worltd Health organization
- [4] Love NR, Chen Y, Bonev B. et al. Genome-wide analysis of gene expression during *Xenopus tropicalis* tadpole tail regeneration. *BMC Dev Biol.* 2011; 11: 70.
- [5] Boren J, Chapman MJ, Krauss RM, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J.* 2020; 41: 2313–30.
- [6] Ference BA, Ginsberg HN, Graham I, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J.* 2017; 38: 2459–72.
- [7] Mozaffarian D, Rosenberg I, Uauy R. History of modern nutrition science-implications for current research, dietary guidelines, and food policy. *BMJ.* 2018; 361: k2392.
- [8] Harcombe Z. US dietary guidelines: is saturated fat a nutrient of concern? *Br J Sports Med.* 2019; 53: 1393–6.
- [9] Djousse L, Matthan NR, Lichtenstein AH, Gaziano JM. Red blood cell membrane concentration of cis-palmitoleic and cis-vaccenic acids and risk of coronary heart disease. *Am J Cardiol.* 2012; 110: 539-44.
- [10] Corella D, Coltell O, Mattingley G, Sorli JV, Ordovas JM. Utilizing nutritional genomics to tailor diets for the prevention of cardiovascular/AQW disease: a guide for upcoming studies and implementations. *Expert Rev MolDiagn* 2017; 17: 495–513.
- [11] Liu R, Cheng M, Kothapalli KSD, et al. Glycerol derived process contaminants in refined coconut oil induces cholesterol synthesis in HepG2 cells. *Food ChemToxicol.* 2019; 127: 135–42.
- [12] Berbee JFP, Mol IM, Milne GL, et al. Deuterium-reinforced polyunsaturated fatty acids protect against atherosclerosis by lowering lipid peroxidation and hypercholesterolemia. *Atherosclerosis.* 2017; 264: 100–7.
- [13] Fito M, Guxens M, Corella D, et al. Effect of a traditional Mediterranean diet on lipoprotein oxidation: a randomized controlled trial. *Arch Intern Med.* 2007; 167: 1195–203.
- [14] Demirbas A. A direct route to the calculation of heating values of liquid fuels by using their density and viscosity measurements. *Energy Conversion and Management.* 2000; 41(15): 1609-1614.
- [15] GohariArdabili A, Farhoosh R, Haddad Khodaparast MH. Chemical composition and physicochemical properties of pumpkin seeds (*Cucurbita pepo* Subsp. *pepo* Var. *Styriaka*) grown inIran. *Journal of Agricultural Science and Technology.* 2011; 13: 1053-1063.
- [16] Erum Z, Rehana S, Mehwish AH, Anjum Y. Study of physicochemical properties of edible oil and evaluation of frying oil quality by Fourier Transform-Infrared (FT-IR) Spectroscopy. *Arabian Journal of Chemistry, Volume 10, Supplement 2, 2017 pages S3870-S3876.*
- [17] Ramos MJ, Fernandez CM, Casas A, Rodriguez L, Perez A. Influence of fatty acid composition of raw materials on biodiesel properties. *Bioresource Technology.* 2009; 100(1): 261-268.
- [18] Aruna K, Rukkumani R, Sureshvarma P. Role of an aminothiazole derivative on ethonal and thermally oxidized sunflower oil – induced toxicity. *Polish J pharmacol.*2004; 56: 233.
- [19] Jaarin K, Norhayati M, Norzana G, Nor Aini U, Ima-NirwanaS.Effects of heated vegetable oils on serum lipids and aorta of ovariectomized rats. *Pak J Nutr.* 2006; 5(1): 19e29.
- [20] Donfrancesco, C., Lo Noce, C., Brignoli, O. et al. Italian network for obesity and cardiovascular disease surveillance: A pilot project *BMC Fam Pract* 9,53(2008). <https://doi.org/10.1186/1471-2296-9-53>

- [21] Badlishah Sham Nurul-Iman, YusofKamisa, KamsiahJaarin, Hj Mohd Saad Qodriyah1. Virgin Coconut Oil Prevents Blood Pressure Elevation and Improves Endothelial Functions in Rats Fed with Repeatedly Heated Palm Oil. *Evidence-Based Complementary and Alternative Medicine*, Vol. 2013, Article ID 629329, 7 pages. 2013
- [22] Kanazawa K, Kanazawa E, Ntake M. Uptake of secondary autoxidation products of linoleic acid by the rat. *Lipids*. 1985;20: 412-419.
- [23] Meredith S. Free radicals: friend or foe? *Med Dig*. 1984; 10(4): 23e5.
- [24] Johnston DE. Special considerations in interpreting liver function tests. *Am Farm Physic*. 1999; 59: 2223e30.
- [25] Kamsiah J, Nor-Aini U, Siti-Aishah, Srijit Das. Palm Oil Fat Diet Consumption and its effects on Serum Liver Enzymes and Microscopic changes in Experimental Rats. *Pakistan Journal of Nutrition*. 2015; 14(9): 575-580.
- [26] Schinner S, Scherbaum WA, Bornstein SR, Barthel A. Molecular mechanisms of insulin resistance. *Diabet Med*. 2005 Jun;22(6):674-82.