



## Histopathological consequences of dietary intake of thermally oxidized Soybean oil

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### Abstract

#### Keywords:

Soybean oil, thermal oxidation, peroxide value, acid value, histopathology.

*Thermal oxidation of unsaturated fatty acids is known to result in significant generation of dietary advanced lipid oxidation end products which are in part cytotoxic and genotoxic compounds. This present study was aimed at investigating the effects of these oxidized products on the histoarchitecture of liver and kidney of wistar albino rats. Soybean oil used to fry bean cake (akara) and sweet potato chips twice daily (150°C for 30 min) for five consecutive days were assessed for their physico-chemical stability using acid values, peroxide value and saponification value as indicator of level of deterioration. Alteration in the histology of the liver and kidney of rats placed on the oxidized oil diets were determined. Increase in the acid and peroxide values of the oxidized oils compared to the fresh oil confirmed chemical deterioration of the heated cooking oils while gross alterations in the normal architecture of the liver and the kidney revealed the possible health hazards to these vital organs. There is need for culinary activity to be carried out at low temperatures and for short periods to prevent generation of toxic oxidation products which may have adverse health effect.*

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### INTRODUCTION

Soybean oil is chiefly a poly-unsaturated fats rich vegetable oil, its fatty acid composition is its selling point as it contains a high proportion of unsaturated fatty acids, comprising of alpha-linolenic acid, C-18:3 (7–10%), linoleic acid, C-18:2 (51%) and oleic acid, C-18:1 (23%) (Ivanov *et al.*, 2010). Fat is an essential macronutrient of the human diet and vegetable oils represent a more highly consumed fat. Cooking oils are an indispensable part of our daily diet because they serve as sources of lipid which is an important source of energy, a major part of bio-membrane, and serve as building blocks for several hormones (Jan, 2014; Vaskova and Buckova 2015). When good cooking oils are used to fry foods, a desirable flavour, colour, taste and textural attributes that give a feeling of satiety to consumers is always achieved. Lipid oxidation is one of the major chemical changes that occur during processing,

storage, shipment, and final preparation of food stuffs containing lipids. The degree of changes in the physical and chemical properties of the oil dictates the quality of the oil for human consumption (Gupta, 2005). Unfortunately, most people use vegetable cooking oil numerous times for frying before discarding so as to cut down on cost. Research studies have indicated that vegetable cooking oils are used 3-6 times before being discarded as waste (Mensah and Obeng, 2013). Repetitive use of vegetable oil lowers the smoke point, which is the temperature at which the oil breaks down creating acrolein, an obnoxious smelling compound; and visible fumes are given off (Good, 2012). This makes the oil smoke at a lower temperature on heating (Rossell, 1998). Several compounds in the thermally oxidized oils induce oxidation of polyunsaturated fatty acids (PUFAs) and increase oxidative stress (Olivero *et al.*, 2010). Formation of polar compounds is strongly related with the primary and secondary

oxidation that takes place during frying. Some of these compounds may be harmful to human health (Karakaya and Simsek, 2011).

Epidemiological studies have shown that diet contributes to 80% of the known health challenges affecting man (Bingham, 2000; Venkata and Subramanyam, 2016). The gastrointestinal tract is constantly exposed to dietary oxidized food compounds which when absorbed, seems to act as injurious chemicals that activate an inflammatory response which affects not only circulatory system but also organs such as liver, kidney, lung, and the gut itself. Two principal organs responsible for excretion of these harmful substances are the kidney and the liver. When the kidneys fail, a host of problems ensue. Chronic kidney disease is usually progressive and may lead to renal failure. Common causes include diabetes mellitus, hypertension, inflammation of the glomeruli (glomerulonephritis), urinary reflux and infections (pyelonephritis), and polycystic kidney disease. Common clinical conditions involving the kidney include the nephritic and nephrotic syndromes, renal cysts, acute kidney injury, chronic kidney disease, urinary tract infection, kidney stones, and urinary tract obstruction (Cotran *et al.*, 2005). Renal damage may occur over many years and may be undetected until a considerable loss of functioning nephrons has occurred. Renal transplantation is the only real cure for patients with end-stage renal failure. It may restore complete health and function. In 2003, about 15,000 kidney transplantation operations were performed in the United States. Also, about 95% of kidneys grafted from a living donor related to the recipient function for 1 year; about 90% of kidneys from cadaver donors function for 1 year (Rhoades and Tanner, 2003).

Liver plays a central role in the metabolism and excretion of xenobiotics which makes it highly susceptible to their adverse and toxic effects (Singh *et al.*, 2011). Although, the liver has the capacity to regenerate, Symptoms of liver damage are usually not apparent until extensive, irreversible damage has been done. This may take many years or it may only take several months if the liver suffers acute damage. Most of earlier studies carried out on thermally oxidized oil were done at temperature between 170°C-220°C (Jimoh and Odutuga, 2004; Shastry *et al.*, 2011; Farah *et al.*, 2012). Recent study in our laboratory showed that most of the secondary oxidation products are generated at a temperature of 150°C for 30mins. This present study was carried out to investigate the consequences of consumption of thermally oxidized soybean diet on the histopathology of wistar albino rats.

## MATERIALS AND METHODS

Commercially refined, Executive Chef Soybean oil was purchased from JOF IDEAL Company, Owo, while fresh tubers of potato (*Ipomea batata*) and clean grains of white cowpea (beans, *Vigna unguiculata*) were purchased from Okusa market in Akungba, Ondo state. Wistar albino rats were sourced from the Animal House unit, Department of Biochemistry, University of Ibadan, Oyo state.

### Generation of oxidized oil:

About 500 ml each of soya bean oil were heated in a clean 3litre deep fryer at varying temperatures and time ranging from 100°C to 190°C and 10 mins to 60 mins respectively, to determine the optimum temperature and time for secondary oxidation products formation (Adeniran and Onigbinde, 2018). Fresh samples of soy bean oil were used to fry sliced potatoes and milled beans at 150°C for 30mins (Adeniran and Onigbinde, 2018) to simulate the conditions for deep frying normally employed in homes and commercial outlets in Nigeria. The oils were cooled for 5 hours before the frying process was repeated with a fresh batch of sliced potato and milled beans for five consecutive days. The samples collected were subjected to physicochemical analysis.

### Physicochemical properties of fresh and oxidized oils:

Acid value (AV), peroxide value (PV), and saponification value (SV) contents were determined in the frying media according to AOAC 1995.

**Animal Treatment:** Twenty male weaning albino rats were maintained under standard laboratory conditions. The average weight of the rats was 77.0g. The rats were fed with commercial poultry feed and had free access to air and water. The feeding exercise lasted for 30 days preceded by fourteen days of acclimatization.

### Formulated Diets

Table 1: Composition of Formulated Diets (Feed) Containing Fresh Oil and Oil Used Repeatedly to Fry Different Food Items (g/kg).

Sample (g/kg)	Amount in diet		
	Fresh oil	Used oil <sup>1</sup>	
Used oil <sup>2</sup>			
Protein 200	200		200
Oil 100	100		100
Sucrose 60	60		60
Cellulose 60	60		60
Vitamin and Mineral	50	50	50
Corn Starch	530		530
	530		

Used oil<sup>1</sup> Feed with oil used to fry potatoes  
 Used oil<sup>2</sup> Feed with oil used to fry milled beans

Table 2: Animal grouping based on diet

GROUP OF ANIMALS	DIETS
GC (CONTROL)	Commercial diet
TPSO	Feed + thermally oxidized Soybean oil from potatoes
TBSO	Feed + thermally oxidized Soybean oil from milled beans
FSO	Feed + fresh Soybean oil

**Key:**

GC Animals placed on commercial diet  
 TPSO Animals placed on thermoxidized soybean oil used to fry potato chips  
 TBSO Animals placed on thermoxidized soybean oil used to fry bean cake  
 FSO Animals placed on fresh soybean oil.

**Preparation of Samples for Histopathology**

At the end of the feeding period, the rats were sacrificed and the liver and kidney were quickly removed from the sacrificed rats and cleansed of blood using 0.9% NaCl solution and dried on filter paper. They were then fixed separately in 10% formalin solution before being taken to where the histopathology was carried out according to the procedure of (Slaoui and Fiette, 2014).  
 In brief, fixed tissues were dehydrated through ascending grades of ethanol to absolute ethanol. They were cleaned in xylene, impregnated and embedded in paraffin wax (melting point 56°C). Sections were cut at 5µm on a rotatory microtome. They were flattened on warm water and mounted onto albuminised slides and dried overnight. The sections were dewaxed in xylene and hydrated through descending grades of ethanol to water. They were initially stained in Harris haematoxylin and differentiated in acid alcohol and thereafter stained with methylene blue. They were then dehydrated in 95% alcohol, stained in 10% alcohol eosin, dehydrated in absolute alcohol, cleaned in xylene and mounted in Canada balsam. The resulting slides were then viewed under the light microscope. The photomicrographs were printed at a total magnification of x100.

**RESULTS**

Results of acid value (AV), saponification value (SV) and peroxide value (PV) are presented in Table 3. There was increase in the AV values of the test groups especially TBSO compared to FSO which served as the control group. The same trend

was observed in the saponification value and the peroxide value of the three oil samples.

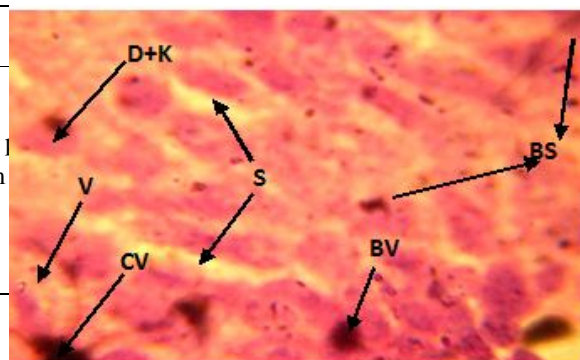


PLATE 2: FSO-LIVER (Mag: x100)  
 Plate 2: Liver section of the positive control group fed with FSO showed normal histological parameters: Sinusoids (S), Central vein (CV), although slight signs of vacuolation (V), and degeneration and karyolysis in the nuclei of the hepatocytes (D+K), Dilated blood sinusoids (BS), large hepatic portal veins/ blood vessels (BV) were also observed.

**Table 3: Physicochemical characteristics of fresh and fried oils.**

GROUP	ACID VALUE (mg KOH)	SAPONIFICATION VALUE	PEROXIDE VALUE (MeqKOH)
FSO	0.15	177.92	1.01
TBSO	4.10	195.17	3.80
TPSO	2.91	192.31	3.20

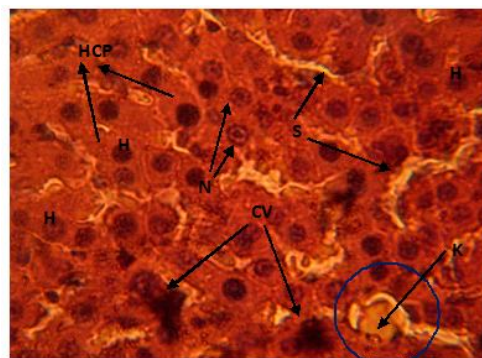
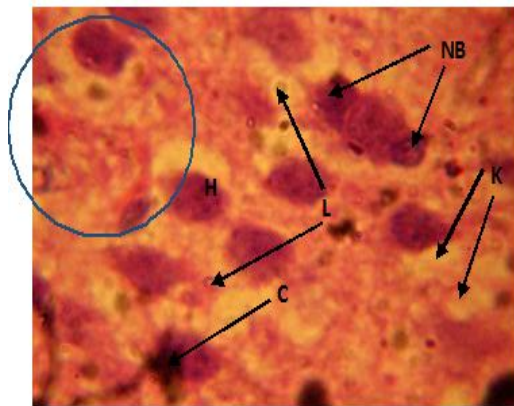


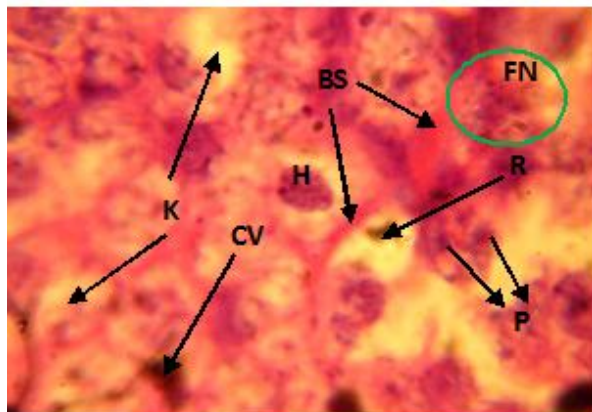
PLATE 1: GC-LIVER (Mag: x100)



The liver of the General Control (GC) group showed normal histoarchitecture, divided into lobules, the center of which is the central vein (CV) from which anatomizing and branching cords of hepatic cells radiate; and at the periphery are the portal triads. This section of the liver also shows hepatic sinusoids (S) surrounded by a discontinuous layer of flattened endothelial cells with a little amount of cytoplasm and flattened darkly stained nuclei (N). The lumens of these sinusoids were seen to be bulged into by the large and prominent-nucleid kuppfer cells (K) (this bulging is identified in the blue circle).

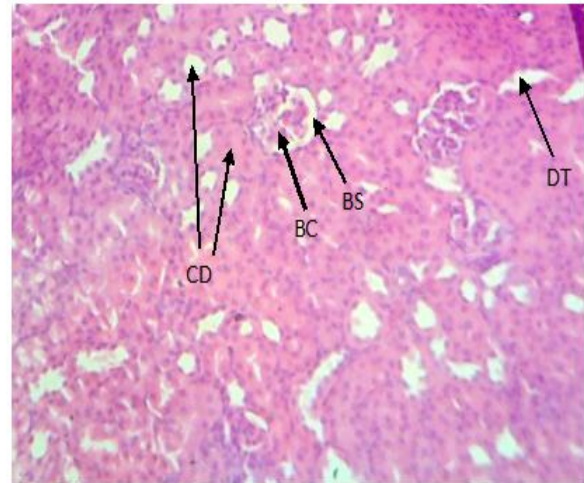


**PLATE 3 (Mag: x100): TPSO-LIVER**  
Plate 3 of the TPSO group manifested abnormal redness in the sinusoids (circled in blue colour), karyolysis in the nuclei of the hepatocytes of this group; Liver sections obtained from this group revealed necrobiotic (NB) changes in the hepatocytes, vacuolation in the cytoplasm, proliferation of lymphocytes (L) and some degenerated areas. Kupffer cells (K) and hepatocytes (H) were also observed in this section.

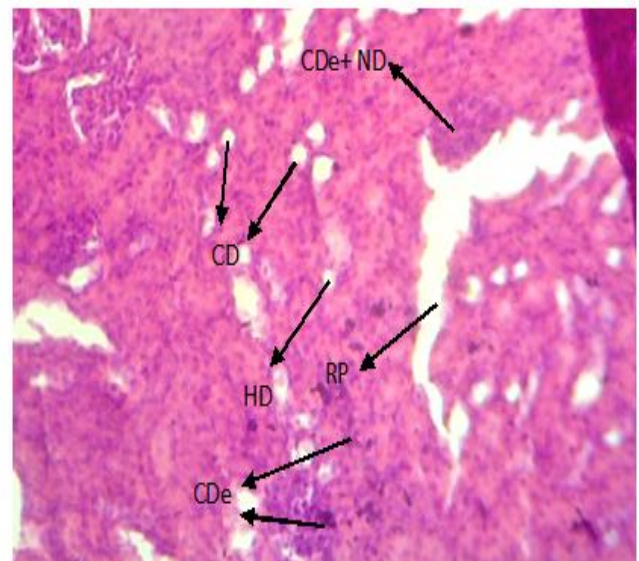


**PLATE 4 (Mag: x100): TBSO-LIVER:** Plate 4 of the present study showed liver cross-section with congestion in blood vessels in the sinusoids (BS), increase of inflammatory cells (Proliferation=P), haemorrhages (R), focal necrosis (FN=Green

circle) and vacuolation in the hepatocytes cytoplasm. The liver of this group exhibited an increase in both endothelial and Kupffer cells (K), while most of the hepatocyte nuclei were mostly karyolytic or pyknotic. Blood sinusoids were also observed (BS).



**PLATE 5 (Mag: x100): GC-KIDNEY**  
The general control of the kidney showed normal renal histological structures, showing Bowman's capsule (BC) and Bowman's space (BS), Distal tubule (DT), Collecting duct (CD), amongst others.



**PLATE 6 (Mag: x100): FSO-Kidney**  
The renal pelvis (RP) and collecting ducts (CD) were clearly showcased in this kidney, although there was a small degree of cellular degradation (CDe) and nuclei disappearance (ND) in the tubular epithelium. Hyaline droplets (HD) were also observed.

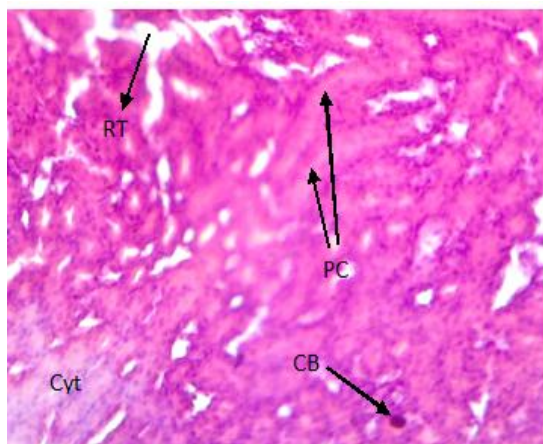


PLATE 7 (Mag: x100): TPSO-KIDNEY: Congestion of renal blood vessels and red blood cells bleeding (CB) and presence of protein cast (PC) in the lumen of some renal tubules. Necrosis in the cells of the kidney was also observed. Normal renal tubules (RT) and collecting ducts (CD) were observed in the kidney of this group. Lightly-stained cytoplasm (Cyt) was observed in this section.

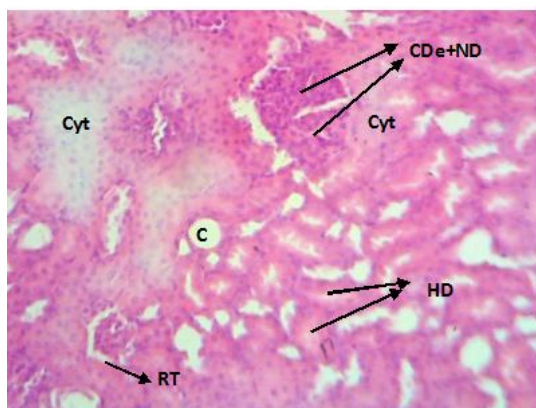


PLATE 8 (Mag: x100): TBSO-KIDNEY: There was necrosis with disappearance of nuclei; hyaline droplets (HD) and fatty droplets were observed and lightly-staining cytoplasm (cyt) with no presence of nucleus. Cellular degradation (CDe) and nuclei disappearance (ND). Degradation of epithelial cells lining the tubules (RT) and mild cystic dilatation (C) in corticomodullary junction was also observed.

## DISCUSSION

Table 3 reveals the physicochemical properties of the oxidized oils compared with fresh oil. There was increase in the acid value (AV) of the thermoxidized soya bean oil compared to the control. A high AV is not accepted in any commercial product because of the strong off-flavor caused by the degradation products (volatile and non-volatile compounds) of the free fatty acids

during deep-frying. Acid test checks for the level of acidity of oil by means of neutralizing the carboxylic groups of the free fatty acids. Thus, the increase is a sign that more free fatty acids were produced. Acid value of 0.00 to 3.00 mgKOH/g is recommended for oil to find application in cooking (Bello *et al.*, 2011). The acid values measured in this study were 0.15mgKOH for fresh soybean oil, 2.91mgKOH for thermally oxidized soybean oil with potato, 4.11mgKOH for thermally oxidized soybean oil with beans. These values obtained are in agreement with the study of Diop *et al.*, (2014) where the values range from 0.39 to 1.08mgKOH after single round of frying. Low levels of AV for the fresh oils suggest good condition and edibility of the oils (Akbar, *et al.*, 2009; Okpuzor *et al.*, 2009), as acid value of oil suitable for consumption should not exceed 4mgKOH (Tan *et al.*, 2002). High acid value indicates high level of free fatty acids in the oil. High level of free fatty acids in the body system can cause both insulin resistance and inflammation in the major insulin target tissues (skeletal muscle, liver and endothelial cells) and thus are an important link between obesity, insulin resistance, inflammation and the development of type 2 diabetes mellitus, hypertension, dyslipidemia, disorders of coagulation and ASVD (Guenther, 2008).

Debnath *et al.* (2012) reported that peroxide value (PV) is a good indicator of lipid oxidation under normal conditions at the initial stages. Peroxides are the initial reaction products of lipid oxidation and responsible for primary oxidation. However, at high temperatures, the hydro-peroxide breaks down and subsequently forms secondary oxidation products during cooking. In this study, the peroxide value was observed to be higher in the test groups (TBSO, TPSO) compared to the FSO. This is in agreement with the findings of Priyanka *et al.*, (2006) which stated that cooking practices like shallow frying, deep frying and sautéing significantly increase the peroxide level in edible oil and fats. The initial peroxide value of FSO was 1.01mEqO<sub>2</sub>/kg. After frying potatoes (TPSO) and bean cake (TBSO), peroxide value increase drastically to 3.2mEqO<sub>2</sub>/kg and 3.80mEqO<sub>2</sub>/kg respectively. Oils with a high degree of unsaturation are most susceptible to autoxidation. Peroxide value, which is the concentration of peroxide in an oil or fat, is useful for assessing the extent to which spoilage has advanced.

Saponification value (SV) is an indicator of the molecular weights of triacylglycerol in oil and its determination is a reasonable means of characterizing fats (Babatunde and Bello, 2016). In this report, the SV of FSO was found to be equal to 177.09mgKOH. The value obtained is in line with the standard guidelines set by CODEX as well as



some other literatures (Nkafamiya *et al.*, 2010; Musa and Aberuagba, 2012; Wali *et al.*, 2015). The lower saponification value of FSO (177.09mgKOH) than the saponification value of TPSO (192.31 mgKOH) and TBSO (195.17 mgKOH) can be ascribed to possible chain fragmentation during the heat oxidation process since saponification value is inversely proportional to the average molecular mass or chain length of the fatty acids (Muhammed *et al.*, 2011; Atinafu and Bedemo, 2011).

### Histopathology

Histopathology is the microscopic study of diseased tissue and it basically compares diseased or experimentally altered tissues with matching sample from healthy or control counterparts. Microscopic evaluation of the liver and the kidney was carried out as presented in Plates 1 to 8.

The liver of the control rats exhibited normal histoarchitecture containing the central vein from where the hepatic cells radiate with the portal triads and the hepatic cell plate clearly visible. The hepatocytes (H) of the liver which have been reported to be polyhedral in shape (Rady and Amany, 2006) and hepatic cell plates (HCP) were clearly visible.

Although the histologic presentation of FSO showed normal parameters, there was slight sign of vacuolation, karyolysis (dissolution of the nucleus) and mild red cell extravasation with redness of the sinusoids.

It was observed that there was gross alteration in the histology of the test groups marked by abnormal redness in the sinusoids due to congestion in the blood vessels and haemorrhage; karyolysis, proliferation of lymphocytes (TPSO and TBSO); hydropic degeneration of hepatocyte, dilation of hepatic sinusoids and fatty infiltration (TPPO and TBPO).

Evaluation of the kidney revealed slight alteration in the kidney architecture of FSO compared with the control (GC). This is evidence by cellular degradation, swelling of tubular epithelium and degeneration. The kidneys of the test groups were characterized by necrosis, congestion of renal blood vessels, cystic dilation in corticomedullary junction and slightly stained cytoplasm which have been reported as a feature of damaged cells (Totani *et al.*, 2009).

These findings are supported by previous studies (Jimoh and Odutuga, 2004; Shastry *et al.*, 2011; Boniface *et al.*, 2014). Disruption of cellular membranes was reported as one of the consequences of ingestion of oxidized oils as a result of peroxidation of membrane phospholipid due to free radical attack. Totani *et al.* (2009) pointed out that acrylamide, acrolein and pyridine which are products of heated oil have high potential for carcinogenicity toxicity and could cause toxic

responses at the biomembrane level. Extreme accumulation of hyaline droplets has been suggested to damage the kidney epithelium gradually (Akagi *et al.*, 2002; Totani and Ojiri, 2007). However, Boniface *et al.* (2014) observed that excessive consumption of fresh palm oil could induce vacuolation of renal corpuscle and tubular necrosis in the kidney.

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