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Changes in Oxidized Groundnut Oil and its Effect on Na^+/k^+ - Atpase in Rat Tissues

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Abstract: Groundnut oil was thermally oxidized at the temperature range of 180-200°C in open air for a period of 10 days at 4 hours per day. The extent of deterioration of thermoxidized groundnut oil were investigated spectroscopically, using infrared, ultra violet and atomic absorption spectroscopy and compared with fresh oil. The themoxidised oil samples differed in composition to the fresh oil, which served as control. Weanling rats were fed diet containing thermally oxidized groundnut oil while control rat were fed diet containing unheated fresh groundnut oil at 15% dietary level. The activity of Na^+K^+ ATPase were studied in the brain, liver, kidney, lungs and heart of experimental animals. The brain and the kidney had a relatively higher enzyme activity when compared to other tissues. Na^+K^+ ATPase activities of the liver, lungs and heart were not significantly affected (P> 0.05) while the ingestion of thermoxidised oil led to significant reduction (p< 005) of Na^+K^+ ATPase activity in the brain and kidney. It is considered that (a) thermoxidized oil causes reduced synthesis and structural changes in membrane phospholipids and (b) a probable impairment of kidney active transport and cerebral transmission of nerve impulse might have occurred due to reduced Na^+K^+ ATPase synthesis.

Key words: Thermal oxidation, groundnut oil, spectroscopy, Na^+K^+ ATPase, membrane function

Introduction

Most foods are subjected to processing before being consumed to improve their palatability and in some cases enhance their digestion (Sanders, 1993). Various food processing techniques have been found to leave deleterious effects on the processed foods and fats and oils are no exception (Gurr and James, 1975; Kubows, 1992, Ononogbu, 2002). Although fat and oil serve as the principals and inexpensive source of essential fatty acids and vitamins, during processing, they are subjected to oxidative degradation (Alexander, 1978; Frankel, 1980; Kubows, 1992; Ologan, 2002).

In the economically developing nations of the world, the intermittent use of reprocessed thermoxidised oil is common and uninhibited. Moreover, the semi-refined oils, which are predisposed to auto-oxidative deterioration, even without thermal processing, are the most cheaply and readily available. The compounds formed as a result of thermal oxidation are of special interest, since deep fried fat is continuously or repeatedly used at elevated temperatures in the presence of air and moisture. The peroxides and hydro peroxides do not survive the heating process while the non-volatile products that remain in the oil are absorbed into the food and subsequently ingested (Thomson and Aust, 1983). Degradative products that accumulate have been shown to be potentially toxic (Izaki *et al*., 1984; Okiy, 1988; Isong *et al*., 1996; Odutuga *et al*., 1997; Odutuga *et al*., 1999; Jimoh and Odutuga, 2002).

Most of the studies that have been carried out have involved the use of highly abused oxidized oils whose mode of oxidation cannot be compared to normal culinary practices (Andrew *et al*., 1960; Fujimoto *et al*., 1984; Mac Gregor *et al*., 1988). In this study therefore, groundnut oil was thermally oxidized in a way to simulate normal culinary practice, characterized and its effect on the activity of Na^+K^+ATPases in selected rat tissues was investigated.

Materials and Methods

Groundnut oil was obtained from Ipata market, Ilorin Nigeria. All chemical and solvents are of analytical grace (BDH and Aldrich).

Treatment of groundnut oil: Groundnut oil was divided into three portions and treated as follows:

(a) No thermal treatment and served as control.

(b) One litre groundnut oil was poured into a stainless steel pot and used intermittently to fry yam chips at a temperature range of 180-200°C in open air 4 hourly for 10days. The oil sample was left overnight to cool and was replenished with fresh oil 10 hourly This portion of groundnut oil was poured into a stainless steel pot and used to fry yam chips at a temperature range of 180-200°C in open air for a period of 4hrs daily for 10days. The sample was left overnight and not replenished throughout the period of use.

These treatments (b) and (c) simulated the process of repeated use of frying oil.

Spectroscopical analysis: A change in quality and the extent of deterioration of the oil samples were observed spectroscopically. The room temperature infrared, electronic and atomic absorption data were determined as reported previously (Obaleye and Orjiekwe, 1992; Obaleye and Orjiekwe, 1995). Infrared spectra were obtained neat while electronic and atomic absorption spectra were run in petroleum ether. The measured frequencies in infrared were accurate to $0.1cm^{-1}$. In the spectroscopy, all spectra data obtained between 400 and 200nm were corrected for background by solvent subtraction.

Animals and diet: Thirty six female albino rat (*Rattus norvegicus*) with mean weight of 40.5±2.22 obtained from the Animal Breeding Unit, Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. They were divided into 3 groups of 12 animals each and were maintained respectively on:

(a) Control diet containing fresh groundnut oil - Group A (fresh)
(b) Diet containing oil replenished 10 hourly after use - Group B (replenished).
(c) Diet containing oil used for frying but not replenished all through the period of use - Group C (not replenished)

The diets were isoprotenous and isocaloric the composition of the diet is shown in Table 1. The appropriate diets and water were given *ad libitum* for 12 weeks. The animals were kept in plastic metabolic cages at room temperature.

Table 1: Composition of Experimental Diets (g/Kg)

Component	Group A	Group B	Group C
Soymeal	500	500	500
Lipid (oil)	150	150	150
Sucrose	100	100	100
Methionine	10	10	10
*Vitamin / mineral mix	30	30	30
Com Starch	200	200	200
Lysine	10	10	10

A - Diet of animals fed with fresh groundnut oil. B - Diet of animals fed with replenished groundnut oil. C - Diet of animals fed with not-replenished groundnut oil. * Mineral mix contained (g/kg diet): $CaCO_3$ (15.258); $CoCl_2.6H_2O$(0.001); $ZnCl_2$ (0.001); $CuSO_4.5H_2O$ (0.019); $FeSO_4.7H_2O$ (1.078); $MgSO_4$ (2.929); $MnSO_4.2H_2O$ (0.178); KI (0.032); KH_2PO_4 (15.559) and NaCl (5.573). The vitamin mix contained (g/kg diet): Thiamine (0.02); Riboflavin (0.03); Pyridoxine (0.01); p-Aminobenzoic acid (0.20); Myo-inositol (2.00); Biotin (0.001); Menadione (0.01); Ergocalciferol (0.4); Choline-HCl (2.0) and Cellulose (3.31).

At the end of the experimental period, the animals were sacrificed while still under anaesthesia by cervical dislocation. They were quickly dissected and the tissues of interest brain, liver, kidney, lungs and heart were removed into ice cold 0.25M sucrose solution. Each tissue was then homogenized separately in ice-cold 0.25M sucrose buffer solution. The homogenates were kept frozen overnight before enzyme assay to allow unbroken cells to lyse (Ngaha, 1982).

Enzyme and protein measurement: Inorganic phosphate was determined using the methods described by Fiske and Subbarow (Fiske and Subbarow, 1925).

Protein concentration was measured by the Biuret method (Plummer, 1978). All measurements were done using Spectronic 20 spectrophotometer. All results were subjected to an analysis of factorial experiments and the mean were separated using Duncan's multiple range test.

Results

Ultraviolet spectroscopy: The electronic spectra data of the three oil samples are shown in Table 2. In complete analogy to the fresh oil sample, both replenished and not-replenished oil samples show a substantial red shift in the electronic absorption band at 224.3nm whereas both the two other samples exhibited peak at ~238nm. There are variations in the absorbance values of these three oil samples. The n →Λ* transition is observed at ~260nm for the fresh sample while the two treated oil samples exhibit this transition around 272nm.

There are additional peaks for both the treated oil samples at around λ max ~ 280nm. This band however cannot be resolved for fresh oil.

Infrared spectroscopy: The major infrared bands and their assignments are shown in Table 3.

In analogy to the fresh oil sample which shows very strong and sharp band at $1724cm^{-1}$ due to (C=O) both the replenished and not –replenished groundnut oil samples undergo a red shift to 1715 cm^{-1} and 1720 cm^{-1} (which represents carbonyl functions, ester linkages) probably from fatty acyl glycerol bonding characteristics) respectively, for v (C=O) due to their thermal oxidation. On the other hand, the very weak band near $1369cm^{-1}$ in fresh with subsequent red shift ~ $1350cm^{-1}$ in both the other processed oils, apparently belong to the vibration of the 0-H of the carboxylic group. Bands in the finger print region, which undergo changes upon oxidation, are at 1430,1369,1220,1140 and $695cm^{-1}$ (fresh).

Atomic absorption spectroscopy

Metal analysis: Table 4 shows the metal constituents of the oil samples. The variance in composition of the three oil sample is also confirmed from the difference in traces of metal constituents of the oil samples. The percentage of heavy metals is higher in both the replenished and not replenished oils when compared with the fresh one. On the other hand there is a decrease in the percentage of alkali metal (Na) for both the affected oil samples.

Table 2: Electronic spectral data (nm) of the oil samples in the region 400-200nm

Fresh oil		Replenished oil		Not-replenished oil	
λ max	Absorbance	λ max	Absorbance	λ max	Absorbance
224.3	1.897	238.9	1.674	238.7	1.639
260.2	0.650	272.1	0.581	272.0	0.545
		281.0	0.484	280.1	0.451

Table 3: Prominent infrared absorption bands (cm^{-1}) observed in fresh, replenished and not-replenished groundnut oil

Fresh oil	Replenish-ed oil	Not-replenished	Tentative assignment	Remark
3422vw	3440vw	3400w	0-H stretch ; carboxylic group	A shift
2980w,sh	2942vs, sp	2960w,sh	C-H stretch (COOH, OH, Mono-and diacyl-glycerides and hydro-peroxide group)	A shift
2900 vs, sp	2879 vs, sp	2894 vs, sp	C-H asym. stretch (CH_2, for unsaturated aldehydes)	Bathochromic shift
2823 s,sp	2804s,sp	2820s,sp	CH_3, sym. stretch (for carboxylic group, ester linkages) probably from fatty acyl glycerol bonding characteristics, anhydrides, aldehydes, ketones, acid peroxides, aldehydes, ketones, acid peroxides in descending orders.	Bathochromic shift
1724 vs,sh	1715vs,sp	1720vs,sp	C=O stretch, carboxylic group, (ester linkages probably from fatty acyl glycerol bonding characteristics anhydrides, aldehydes, ketones, acid peroxides in descending orders	Barthochromic shift
1430m	1442m	1432m	C-H bend, CH_3 group (alkanes, aldehydes, alcohol, aldehyde	Hypsochromic shift
1369w	1350vw	1350w	O-H bend	Barthochromic shift
1220w	1222w	1221w	C-O stretch; C-OH carboxylic group	Hypsochromic shift
1140s	1143s	1130s	C-O stretch (carbonyl compounds alcohols)	A shift
695w	705w	697m	C-OH carboxylic group	Hypsochromic shift

Abbreviations: w-weak; vw-very weak; b-broad; m-medium; s-strong; sp-sharp; sh-shoulder.

Effect of diet on Na^+K^+ ATPase activity: Na^+K^+ ATPase activity of the various rat tissues is shown in Table 4. The ingestion of replenished groundnut oil caused a significant $P<0.05$ reduction in the activity of the enzyme from the brain and lungs, the reduction being 32.0% and 32.5% respectively. The liver, kidney and heart Na^+K^+ ATPase activities were however not significantly affected. On the other hand, the ingestion of not-replenished groundnut oil containing diet led to a significant ($P<0.05$) reduction in Na^+K^+ ATPase activity in the brain, kidney and heart. It was noted that although a 32.0% reduction was recorded in the brain enzyme activity in animals fed replenished oil, the reduction was 71.56% in animals fed not- replenished groundnut oil.

Discussion

The present investigations demonstrated that the thermally oxidized groundnut oil samples were different in composition to the fresh (control, unheated) oil and also had many different spectral features. In the UV analysis, the peak ~260-272nm corresponds to secondary or end products formed by subsequent degradation of alkyl or acyl chains (Odutuga *et al.*, 1997). This absorption appears weak in the fresh oil because of partial autoxidation of hydrocarbon chains exposed to atmospheric oxygen. It has been previously noted that secondary products characteristics of lower hydrocarbons such as carbonyl compounds were detected by an abrupt change in intensity of the 270nm peak (Lamba *et al.*, 1991).

In the various oil complexes during IR analysis, the greater shifts in the v (C=0) and v+δ (O-H) bands coupled with slight changes in associated v (O-H) band are strong evidences of thermal effect on both the replenished and not replenished groundnut oil. There is very high increase in intensity in oil sample C compared with others which showed highest oxidation and highest number of conjugated bands formed. (Rouxhet *et al.*, 1950; Kemp, 1979 and Williams and Fleming, 1980).

Bands on the finger print region, which undergo changes upon oxidation, are at 1430,1369, 1220, 1140, and 695cm^{-1} (fresh). Changes observed here for the treated samples confirmed the difference or oxidation induced change in the physical state of the treated samples. It also shows the reduction in the *van der* walls force / interactions of the oxidized products.

Table 4: Metal analysis of the oil sample

Metals Analyzed	Na (ppm)	Mn (ppm)	Fe (ppm)	Zn (ppm)	Mg (%)	Ca (%)	K (%)
Fresh oil	29.61	0.00	0.00	2.10	0.001	0.017	0.007
Replenished oil	17.27	0.00	0.00	2.38	0.001	0.007	0.007
Not –replenished oil	7.83	0.02	3.98	2.13	0.002	0.023	0.026

(Rouxhet *et al.*, 1950; Williams and Fleming, 1980; Kemp, 1979).

The fact that the different functional groups were identified in the fresh oil confirms the fact that most of the oils retailed in the market even without thermal treatment has already started deteriorating. This may because of exposure to environmental factors such as sunlight and air, it could also be due to the fact that the oils are not usually refined after extraction (Leo, 1983; Nnadozie *et al.*, 1990).

The concentration of the various functional groups obtained by calculating the relative areas occupied by such peaks show the accumulation of anhydrides, aldehydes, ketones, acid peroxides and alcohols in the oil that were subjected to thermal treatments.

Increase in heavy metal content and other representative metals in the thermoxidized oil sample as recorded in this study is likely to increase the toxicity effect of the affected samples. The pro-oxidant materials in oil are the trace amounts of these metals (Lamba *et al.*, 1991).

The activity of Na^+K^+ ATPase in the brain and kidney were found to be relatively higher when compared to the other tissues. This is due to the fact that these organs are highly membranous and are also involved in active transport processes than the others. Lehninger *et al.* (1993) reported that the activities of the ATPases are usually highest in tissues where it constitutes the main mechanism for producing physiologic work.

Na^+K^+ ATPase is involved in active transport across the plasma membrane within virtually all cell types; the sodium concentration is relatively low while that of potassium is high. Most animal cells maintain intracellular K^+ at relatively high and constant concentration between 120mM and 160mM, whereas the intracellular Na^+ concentration is usually less than 10mm (Wills, 1985). The cell requires a high intracellular level of K^+ for correct conformation and function of proteins / enzymes, a defect in the activity of Na^+K^+ ATPase will affect various metabolic processes. The reduction in the activity of Na^+K^+ ATPase in these organs might therefore affect the transmission of nerve impulse, a function to which it is directly involved in the brain (Wills, 1985). In the kidney, it is involved in the re-absorption of substances such as sugars, amino acids and electrolytes back to the blood. An impairment in the function of this energy dependent pump in the kidney could lead to loss of sugars, amino acids and electrolytes in the wine.

The decrease in the activity of Na^+K^+ ATPase in the brain and kidney observed in the present study might be a consequence of (a) incorporation of deoxidized fatty acids into membraine phospholipids and (b) increased lipid peroxidation in the membrane.

Table 5: Na^+K^+ ATPase activity of tissues of rats maintained on diets containing fresh and oxidized groundnut oil

Tissues	Group A	Group B	Group C
Brain	11.90 ± 1.85^a	8.09 ± 0.23^b	$3.46+1.09^c$
Liver	0.81 ± 0.23^a	1.38 ± 0.47^a	$0.73+ 0.15^a$
Kidney	10.75 ± 2.4^a	10.40 ± 2.01^a	$3.62+1.03^b$
Lungs	2.37 ± 0.66^a	1.60 ± 0.29^b	$3.03+1.05^{ab}$
Hear	2.11 ± 0.63^a	1.98 ± 0.55^b	$1.32+0.45^b$

Enzyme activities are expressed as specific activity in moles Pi / tir / mg protein. The results are the values for 5 determinations (± SEM). a,b,c values along the same row with different superscripts are statistically significant

The substantial red shift in electronic absorption (peak at 238nm) exhibited by the heated oil samples would indicate the presence of a conjugated double bond band (also known as k band) in the fatty acid molecule (Lamba *et al.*, 1991) Incorporation of this altered fatty acid molecule into membrane phospholipids may likely lead to loss of essentially of the phospholipids and affect lipid membrane structure and function relationship in biological systems (Odutuga, 1977; Odutuga *et al.*, 1997).

Odutuga and Ajayi (1998) reported reduced alkaline phosphatase (a membrane bound enzyme) synthesis as well as loss of this enzyme in essential fatty acid and zinc deficiencies due to changes in the organization of membrane phospholipid matrix. The reduction in Na^+K^+ ATPase activity observed in the present investigation, therefore is considered to be a result of the ingestion of peroxidized groundnut oil affecting the phospholipids matrix and changing the structure and function of brain or kidney cells and membranes (Odutuga, 1977) and probably impairing the proper coupling of oxidative phosphorylation.

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