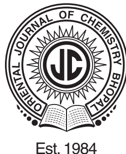
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Deterioration in Thermally Oxidized Mustard Oil: A Spectroscopic Investigation and Toxicological Impact on Selected Rat Tissues

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ABSTRACT

Mustard oil is used widely in food processing in many economically developing countries. Reuse of edible oils is very common in such countries. Although oils are inexpensive sources of fat and Vitamins, processing leaves a effect on processed food. Present study is so concerned with such type of common reuse of mustard oil in food processing and subsequent toxicological impact on selected rat tissues.

Key words: Thermal oxidation, Mustard oil, spectroscopy, Na +K+ ATPase, membrane function.

INTRODUCTION

Mass of population in India consumes a verity of edible oil in large amount (mustard oil, Ground nut oil, Soybean oil, etc.) edible oil are used to increase the palatability and to enhance the digestion of food. (Sander, 1993). For this purpose various food processing techniques using edible oils are used, which are found to leave deleterious impact on processed food. (Gurr and James, 1975; Kubows, 1992; ononogbu, 2002).

Although fat and oil servers the principal and inexpensive source of essential fatty acids and vitamins, during processing, they are subjected to oxidative degradation (Alexander, 1978; Frankeel, 1980; Kubows, 1992; Ologan, 2002).

In the economically developing nations of the world, the intermittent use of reprocessed thermoxidized 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 nonvolatile products that remain in the oil are absorbed into the food and subsequently ingested (Thomson are Aust, 1983).

Derivative 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*, 1997; 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, mustard oil was the thermally in a way to simulated normal culinary practice, characterized and its effect on the activity of Na^+K^+ ATPases in selected rat tissues was investigated.

MATERIALS AND METHODS

Mustard oil was obtained from General commodity market, Bikaner (India). All chemical and solvents are of analytical grade. (Ranbaxy, Renkem).

Treatment of mustard oil : Mustard oil was divided into three portions and treated as follows :

a. No thermal treatment and served as control.
b. One liter mustard oil was poured into a stainless steel pot and used intermittently to fry potato chips at a temperature range of 120^{0}C in open air 4 hourly for 10 days.
c. The oil sample was left overnight to cool and was replenished with fresh oil 10 hourly. This portion of mustard oil was poured into a stainless steel pot and used to fry potato chips at a temperature range of 180-200^{0}C in open air for a period of 4 hrs daily for 10 days. 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.

Spectroscopic analysis

Change in quality and the extent of deterioration of the oil samples were observed spectroscopically. At the room temperature infrared, electronic and atomic absorption data were determined. Infrared spectra were obtained neat while electronic and atomic absorption spectra were run in petroleum ether. The measured frequencies in infrared were accurated to 1.0 cm. In the U.V. spectroscopy, all spectra data obtained between 400 and 200nm were corrected for background by solvent subtraction.

Animals and diet

Animals and diet : Thirty six female Swiss albino rats (musmusculus) with mean weigh of 40.5± 2.22 obtained from the Animal Breeding Unit, (Dept. Of Zoology, Govt. Dungar College,MGS University, Bikaner, Rajasthan, India) they were divided into 3 groups of 12 animals each and were maintained respectively on :

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

The diets were iso portenious 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.

A-Diet of animals fed with fresh groundnut oil. B-Diet of animals fed with replenished groundnut oil. C-Diet of animlas 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); $FeSO_4$. $7H_2O$(1.078); $FeSO_4$ $2H_2O$ (0.178); $MgSO_4$ (2.929); KH_2PO_4 (15.559) and NaCl (5.573). the vitamin mix contained (g/kg diet) : Thimaine (0.02); Riboflavin (0.03); Pyridoxine (0.01); p-Aminobenzoic acid (0.20); Myo-inosito (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 anesthesia by cervical dislocation. They were quickly dissected and tissues of interest brain, liver, kidney, lungs and heart were removed into ice cold 0.25 M sucrose solution. Each tissue was then homogenized separately in ice-cold 0.25 M sucrose buffer solution. The homogenates were kept frozened overnight before enzyme assay to allow unbroken cells to lyses (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 biurate method (Plummer, 2002). All measurements were done using Systronic 1100 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 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* transition is observed at ~260nm for the fresh sample while the two treated oil samples exhibit this transition around 272nm.

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 mustard oil samples undergo a red shift to 1715 cm^{-1} and 1720cm^{-1} (which represents carbony1 functions, ester linkages probably form fatty acyl glycerol bonding characterstics) respectively, for v (C=O) due to their thermal oxidation. On the other hand, the very weak band near 1369cm-1 in both the other processed oils, apparently belong to the vibration of the O-H of their carboxylic group. Bands in finger print region, which undergo changes upon oxidation, are at 1430, 1369, 1220, 1140 and 659cm^{-1} (fresh).

Atomic absorption spectroscopy
Metal analysis

Table 4 shows the metal constituents of the oil samples. The variance in composition of the deference 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 decrease in the percentage of alkali metal (Na) for both the affected oil samples.

Effect of diet on Na $^+$K$^+$ ATPase activity

Na$^+$ K$^+$ ATPase activity of the various rat tissues is shown in Table 4. ingestion of replenished mustard 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

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
Common Starch	200	200	200
Lysine	10	10	10

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

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	Replenished oil	Not-replensished	Tentative assignment	Remarks
3422vw	3440 vw	3400w	O-H stretch; carboxylic group C-H stretch (COOH, OH, Mono-and diacyl-glycerides and hydro-peroxide group)	A shift
2980w,sh	2942 vs, sp	2960 w,sh		A shift
2900vs, sp	2879 vs,sp	2894 vs,sp	C-H asym, Stretch (CH_2, for unsaturated aldhydes)	Bathochromic Shift
2833s,sp	2804 s,sp	2820 s,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	1715 vs,sp	1720 vs,sp	C=O stretch, carboxylic group, (estelinkages probably from fatty actyl glycerol bonding characteristics anhydrides, aldehydes, ketones, acid peroxides in descending orders)	Bathochromic Shift
1430m	1442m	1432m	C-H bend, CH_3 group (alkanes, aldhydes, alcohol, aldehyde)	Hypsochromic Shift
1369w	1350w	1350w	O-H bend	Bathochromic 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

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

affected. On the other hand, the ingestion of not-replenished mustard 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 mustard oil.

DISCUSSION

The present investigations demonstrated that the thermally oxidized mustard oil and also had many different ~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=O) and v+o (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 Mustard 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, 1440 and 695 cm^{-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 Vander wall force/interactions of the oxidized products.

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 deterioration. 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; Nnadoze *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 the 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 liely 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 an 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 highand 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 reabsorption of substances such as sugars, amino acids and electrolytes back to the blood. An Impairment in the function of the energy dependent pump in the kidney could lead to loss of sugars, amino acids and electroytes in the urine.

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 membrane phospholipids and (b) increased lipid per oxidation in the membrane.

The substanitial 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 phosphotase (a membrane bound enzyme) synthesis as well as loss of this deficiency due to changes in the organization of membrane phospholipids 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 mustard 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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