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THE PHYSIOCHEMICAL ANALYSIS OF THERMOXIDIZED SOYABEAN-OIL AND ITS EFFECT ON GROWTH IN RATS

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ABSTRACT

Physiochemical characteristics of Fresh and thermoxidized soyabean –oil were studied. Thermoxidized soyabean-oil was found to possess higher percentage of free fatty acids and peroxide value when compared with the fresh soyabean-oil. The rats fed with diet containing 5% and 15% level of thermoxidized soyabean-oil did not show any sign of retarded growth, but the weight gain was lower than those fed with Fresh soyabean-oil. The reason could possibly be as a result of mild accumulation of peroxide as well as gross accumulation of decomposition products of lipid peroxidation which are known to be toxic.

Key words: Free Fatty acids, peroxide, thermoxidation, peroxidation.

INTRODUCTION

Lipids are susceptible to oxidation both from internal and external sources (Frankel, 1980). If oxidation is not controlled, peroxidation results. Oxidation reaction of lipids has deteriorative effect owing to the fact that it proceeds via free radical generation. Free radicals are harmful, highly reactive species containing one or more unpaired electrons (Halliwell and Gutteride, 1984). They cause a chain of conjugation, polymerization and fragmentation reactions (Frankel, 1980). soyabean-oil is widely used and consumed by average Nigerians and much of the oil are exposed to heats during processing and cooking. Over the years, there has been concern about changes taking place in heated fats/oil and the effect of its derivatives on individual consuming them (Chow, 1989). Earlier reports of feeding studies using experimental animals were complicated by the possibility that some of the effects observed resulted from vitamin deficiencies brought by oxidation of nutrients in the diet (Longani and Davis, 1980). It was later discovered that other constituents of oxidized fats and related products may be carcinogenic or mutagenic (Chow, 1989). Hence, the purpose of the research is to ascertain the possible level of damages done to soyabean-oil during deep-frying under the domestic cooking conditions and the resultant effect on growth of experimental albino rats.

MATERIALS AND METHOD

Soyabean-oil (Loius Carter PLC KMC 97) was obtained from Fas-supermarket in Ilorin, Nigeria. The soyabean-oil was three weeks old from the registered date of manufacture to the date it was used. Malealbino rats (*Ratlus norvegicus*), of twenty-five days of age with average weight of 45 grammes were obtained from the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

Treatment of soyabean-oil

The Fresh and thermoxidized soyabean-oil were characteristically studied using Hofmann and Green method. Two litres of Fresh soyabean-oil were measured and kept in a stainless steel fry pan. It was deep-fried in an open air temperature of $200^0C \pm 20^0C$. The

deep-frying was done for two hours daily for ten days and left to cool in an open space each day. The thermoxidized soyabean-oil was used in formulation of diet for feeding experiment at 5% and 15% level.

Table 1: Composition of Diets (g/kg)

	A (FRESH)	B (OXIDIZED)	C (FRESH)	D (OXIDIZED)
Casein (defatted)	250	250	250	250
DL – Methionine	4	4	4	4
DL – Lysine	4	4	4	4
Corn-starch	412	412	250	250
Cellulose	130	130	255	255
Sucrose	100	100	37	37
Soya bean-oil	50	50	150	150
Minerial - mix	40	40	40	40
Vitamins - mix	10	10	10	10

Vitamin/Mineral premix provides per kilogram of diets Vitamin A. 300IU, Vit. D. 1000IU, Vit. K_3 2mg, riboflavin 4mg pantothenic acids 9mg, nicotinic acid 10mg, cobalamin 0.08mg, Folic acid 1.2mg. biotin 0.25mg, choline chloride 1.5mg, Iron 25mg Mangenese 80mg, Zinc 50mg, Copper 2mg, Iodine 1.2mg, Cobbait 0.2mg selenium 0.1mg.

Table 2: Proximate Analysis of Formulated Diets (g/100g)

	A (Fresh)	B (Oxidized)	C (Fresh)	D (Oxidized)
Lipids	4.7 ± 0.3	4.4 ± 0.6	13.8 ± 1.4	13.4 ± 1.3
Protein (N x 6.25)	29.5 ± 2.2	29.2 ± 3.4	29.4 ± 2.1	29.3 ± 2.2
Crude Fibre	7.1 ± 0.4	5.4 ± 0.7	4.2 ± 0.7	3.6 ± 0.3
Ash (%)	9.2 ± 0.1	9.2 ± 0.1	9.0 ± 0.5	9.0 ± 0.5
Moisture (%)	2.05 ± 0.7	3.2 ± 0.2	2.4 ± 0.8	3.6 ± 0.4
Carbohydrate	47.5 ± 0.5	47.6 ± 0.3	42.3 ± 4.4	41.2 ± 4.9

Each value is the mean of three determinations ± S. D.

DIET COMPOSITION

The diet was composed using Pearson square method of feed formulation. Each diet was kept in reagent bottles and kept in a cool dry place all through the research period.

Management of Rats

Thirty-two male albino rats (*Ratlus norvegicus*) were used for the experiment. They were divided into four groups, each containing eight animals and were maintained respectively on the following diets.

(a) Control diet containing fresh soyabean-oil at 5% level

(b) Experimental diet containing oxidized soyabean oil at 5% level

(c) Control diet containing fresh soyabean-oil at 15% level

(d) Experimental diet containing oxidized soyabean-oil at 15% level

The diets and water were given *ad. Libitum*. The animals were fed with these diets for six weeks. All rats were fed their respective diets daily and weighed until the end of the experiment. Analysis of variance was used to compare results at $P < 0.05$.

RESULTS AND DISCUSSION

Physicochemical properties of the soyabean-oil determined include; acid value, per cent free fatty acid, iodine value, density, specific gravity and viscosity of both fresh and oxidized

soyabean-oil.

Table 1 shows the composition of formulated diet for feeding analysis at 5% and 15% level of soyabean-oil. The chemical characteristics of the test samples of soyabean-oil are as shown in Table 3. The acid value, a measure of the free fatty acid resulting from the hydrolysis and peroxidation of the soyabean-oil was found to be 25% lower in fresh soyabean-oil when compared to thermoxidized soyabean-oil. This is statistically significant at $P < 0.05$. The peroxide value is an indicator of deterioration of soyabean-oil. As oxidation takes place, the double bonds in the unsaturated fatty acids are attached forming peroxides. The thermoxidized soyabean-oil has higher peroxide value when compared to the fresh soyabean-oil. This was attributed to the fact that peroxidation might have occurred and in turn affected the degree of unsaturation of the soyabean-oil.

The iodine, saponification and ester values are indices of the extent of damage done to the soyabean-oil due to thermal effect. The values for thermoxidized soyabean-oil were found to be lower than the fresh soyabean-oil indicating that damages were done to the oil as a result of thermal effect. However, fresh soyabean-oil probably had longer chain fatty acid than the thermoxidized especially during cooking (Simic and karal, 1980). The Lipid peroxidation chain reaction (rancidity) yields a high peroxide value such as fatty acid hydroperoxides which are known to be carcinogenic and mutagenic. The value of the density, specific gravity and viscosity of the thermoxidized soyabean-oil were found to be higher when compared to the fresh sample, although the values were not significantly different ($P > 0.05$).

Table 2, shows the proximate analysis of composed diets fed to the experimental rats on dry weight basis. The Lipids for groups A and B were found to be 4.7% and 4.4% whereas for groups C and D had 13.8% and 13.4%, respectively. The values were not statistically different at 5% alpha level.

Table 3: Physicochemical Analysis of Soya bean-oil sample

	Fresh oil	Thermozidized oil
Density (g/cm^3)	0.82 ± 0.03	0.83 ± 0.02
Specific Gravity (g/cm^3)	0.91 ± 0.03	0.92 ± 0.02
Viscosity (C. G. S.)	0.42 ± 0.02	0.48 ± 0.05
Acid value	1.12 ± 0.01	2.52 ± 0.20
Iodine value	$35.00^a \pm 0.01$	$24.00^b \pm 0.10$
Ester Value	$41.68^a \pm 4.96$	$37.48^b \pm 8.92$
% Free Fatty acid	$2.24^a \pm 0.01$	$5.04^b \pm 0.40$
Saponification Value	$42.80^a \pm 0.96$	$30.00^b \pm 0.92$
Peroxide Value	$20.00^a \pm 0.07$	$35.00^b \pm 0.01$

Each Value is mean of five determinations ± S. D. a, b
Row Values with different superscripts are significantly different ($P < 0.05$).

The rats were closely monitored and observed to be morphologically normal at the beginning of the experimental period. At the end of the experiment, the rats fed on thermoxidized soyabean-oil showed unhealthy appearance. The fur appeared grayish and scattered with little or no unusual behavioural activities. This was suspected to be as a result of nutrient reduction induced by the thermal destruction of essential vitamins and fatty acids in the soyabean-oil or by autoxidation of vitamins in the diet fed after the oxidized soyabean-oil has been mixed in the composed food (Kirk, 1984).

Irritation of the mucosa by peroxyl products of the thermoxidized soyabean-oil is capable of interference with nutrients

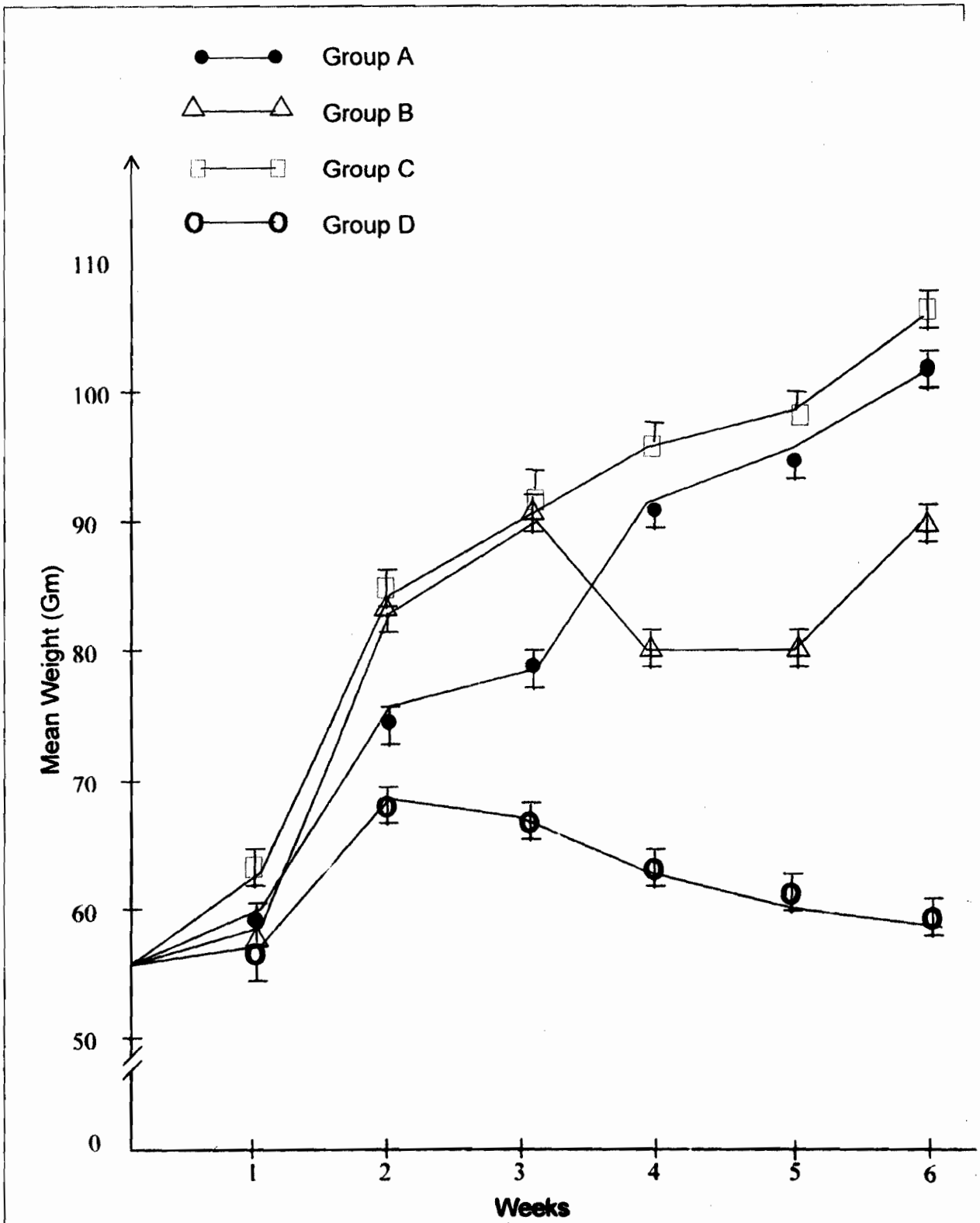

Fig. 1. Growth curve of rats on different diets
Each point is the mean of 8 albino rats ± S.D

absorption in the diet. This is in agreement with the works of Kirk (1984), Chow (1989) and Miller and Long (1990) where it was shown that growth retardation and other harmful effects may result from high intake of long chain polymers which may cause the fat to be less absorbable and thus interfere with the absorption of the other fat soluble nutrients in the diet. Figure 1 shows the growth behaviour of the rats fed with the various diets. Rats fed with fresh soyabean-oil had unhindered growth. This might establish the role of soyabean-oil in the diet as indicated by Oke (1978)

The role includes improved palatability, better absorption of other nutrients, higher calorific value of soyabean-oil, provision of essential oil and the provision of essential fatty acids. Those rats fed 5% and 15% thermoxidized soyabean-oil suffered some depressant effects on growth when compared to the rats on fresh soyabean-oil. The effect of thermoxidized soyabean-oil on the experimental rats are secondary to the direct toxic actions of lipid oxidation products themselves. When the thermozidized soyabean-oil was fed in large quantity (15% level) to these groups, the taste and odour characteristic of the diet deteriorated with a subsequent decrease in food intake.

In lipid thermoxidation, some amino acids have been reportedly destroyed as a result of lipid peroxide-protein interaction (Longani and Davis, 1980) These amino acids include arginine, serine, glutamic acid, methionine, tyrosine, phenylalanine and thronine. In such instances, the effect of thermoxidation on food items would be reduction in nutritional level, which might have been accounted for by the stunted growth recoded in the group of experimental rats fed the diet containing the thermoxidized soyabean-oil. Growth depression is one of the consequences of ingested thermoxidized soyabean-oil.

CONCLUSION

There are several contradictory researches on the long-term effect of consuming thermoxidized fats produced under conditions that mimic commercial and domestic cooking conditions. It is inferred that in the present study, the observed growth retardation of rats fed the thermoxidized soyabean-oil was probably as a result of the presence of peroxides and other toxic materials emanating from the oil. These toxic substances might have exerted their adverse effect on the rats through interaction with proteins and destruction of essential nutrients necessary for normal and healthy growth.

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