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## Research Article

# Consumption of Different Forms of Palm Oil Formulated Diets Has No Effect On Learning and Memory in Mice

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**Abstract:** The consumption of different forms of palm oil has been reported to have an enormous systemic effect; this study was aimed at evaluating the effects of different forms of palm oil; Fresh and thermoxidized palm oil on learning and memory in CD1 mice. Twenty seven (27) mice were used for this study. There were arbitrarily assigned into three (3) groups, Control, Fresh palm oil (FPO) fed and Thermoxized palm oil (TPO) fed groups, n=9. The control group received normal rat feed. The FPO-fed and TPO-fed groups received fresh palm oil diet and thermoxidized palm oil diets respectively. All animals had access to water ad libitum. The feeding lasted for 14 weeks. At the termination of the feeding period, learning and memory was assessed using the Morrice water maze (MWM). The results obtained showed that there was no significant difference in the swim latencies in both acquisition and reversal training, also, no significant difference was observed in the quadrant duration during the probe trial, and the visible platform task. This is indicative of equal learning ability for all the animals across all the experimental groups. Long term consumption of FPO and TPO has no significant effect on visuospatial learning and memory.
**Keywords:** Fresh palm oil, Thermoxidized palm oil, Learning and memory, Morris water maze.

## INTRODUCTION

Red oil commonly known as palm oil is a conventional form of oil that has been a part of the human diet for decade of years. For generations, red palm oil has been revered as both a nutritious food and a valuable medicine. It is obtained from the fruit of the oil palm (Elaesis guineensis). Red palm oil is not only rich in essential fatty acids for proper growth and development, but it is packed with a variety of vitamins, antioxidants, and other phytonutrients such as carotenes (beta-carotene) and lycopene important for good health [1]. Red oil is consumed either in its fresh form or at various forms of oxidation. It is said to be fresh when it is not subjected to any form of frying at very high temperature. When red palm oil is subjected to prolonged heating, it is oxidized. The use of such oil has been implicated with a host of pathophysiology ranging from decrease biliary secretion and altered biliary electrolytes [2], distortion in villus morphology, with an attendant malabsorption of fluid and glucose [3], altered intestinal motility [4], growth retardation [5], increase faecal electrolytes [6], alteration in haematological parameters and serum lipid profile [7,8], atherosclerosis [9], alter serum electrolyte balance [10], deactivation of key enzymes required to enhance the process of metabolism, leading to essential fatty acid deficiency, nucleic acid deficiency, growth retardation, fatty liver, thrombosis and micronutrients malnutrition [11, 5, 12], as well as encouraging gastric ulceration [13, 14], among others. Considering the multiple systemic effects associated with the consumption of thermoxidized palm oil, reports of its effects on visuo-spatial learning and memory is inconclusive, this study therefore evaluates the effect of consumption of different forms of palm oil in visuospatial learning and memory in CD1 mice.

## METHODOLOGY

### Experimental Animals

Twenty seven (27) mice of both sexes weighing between 18g-24g were used for this study. The mice were obtained from the department of pharmacology, University of Calabar Nigeria.

### Animal Treatment and Protocol

The animals were allowed one week to acclimatize, after which they were divided randomly into three (3) groups of (9) mice each. Mice in the control group were fed with normal rat feed and water only, while those in the test groups were fed with FPO-diet and TPO-diet for a period of 14 weeks.

**Purchase of palm oil and characterization of fresh and thermoxidized palm oil**

Fifteen litres of fresh palm oil was obtained from Watt Market, Calabar, Nigeria. The oil was certified fresh because of its low oxidation value using the method of Rossel [15]. The palm oil was divided into two equal parts; a part was kept fresh by preserving it in a container covered with black cellophane and stored in a dark cupboard in order to avoid further oxidation while the remaining portion of the oil was thermoxidized. The fresh palm oil and thermoxidized palm oil diets were later formulated using the fresh palm oil and thermoxidized palm oil respectively.

**Thermoxidation of palm oil**

The thermoxidation of palm oil was carried out following the methods reported by [16, 17, 18] and used by Obembe *et al.;* [13, 14]. Fresh palm oil was heated in a stainless steel pot over a heating mantel piece at a temperature of 190$^{o}$C for five consecutive times of 20 minutes in each round with 5 minutes cooling interval between two rounds.

**Characteristics of fresh and thermoxidized palm oils**

The consistency of the oil fed to the animals was ascertained by measuring the acid value, iodine value and peroxide value of the fresh and thermoxidized palm oils before formulating the two forms of test diets.

**Determination of acid value**

Acid value is defined as the milligrams of potassium hydroxide (KOH) required neutralizing the fatty acids in a gram of fat [19]. This value indicates the degree of rancidity.

**Experimental procedure:**

Two grams of oil was dissolved in 20ml of neutral alcohol in a measuring flask and the solution titrated with 0.1ml sodium hydroxide in triplicate using 3 drops of phenolphthalein as indicator. The volume of alkaline was noted and the acid value calculated.

Acid value (MgNaOH/gFat)

$$= \frac{40 \times M \times V}{W}$$

Where

40 = Molecular weight of NaOH
M = Molecular weight of alkaline
V = Volume of alkaline used in titration
W = Weight of oil sample.

**Determination of iodine value**

This is defined as the number of grams of iodine absorbed by 100g of fat. It is a measure of unsaturations [20]. A greater degree of unsaturation represents more iodine value. It is also a measure of the quality of oil.

**Experimental procedure**

A drop of oil sample of between 0.1 - 1 g was spread as a thin film on a weighed microscope slide and the weight of the sample estimated. The microscope slide with the oil sample was exposed to bromine vapour by placing it on a beaker containing some drops of bromine in a fume cupboard. The beaker was then fumed for an hour. After one hour, the microscope slide was removed from the fume cupboard and excess bromine shaken off by warming it gently on a heating mantle at 50$^{o}$C to 60$^{o}$C. The microscope slide was then weighed to determine the amount of bromine absorbed.

**Calculation**

Iodine value g/Br/100g oil

$$= \frac{158.8\ W_2 - W_1}{W_1}$$

Where

$W_1$ = Initial weight of bromine
$W_2$ = Weight of brominated oil sample

**Determination of peroxide value**

This is defined as the quantity of peroxide oxygen present in the sample and is expressed in mMol active oxygen per 2kg of the sample. It is a measure of the incipient rancidity.

**Experimental procedure**

The peroxide value was determined using the method of Paquot [21]. 5g of sample (Fresh or thermoxidized palm oil) was accurately weighed into 250ml flask and 30ml of acetic acid-chloroform (3.2v/v) added. The flask was agitated until the sample dissolved and 0.5ml of saturated potassium iodide added using measuring pipette. The solution was allowed to stand and occasionally shaken for one minute. 30ml of distilled water was then added to 0.1ml of sodium thiosulphate ($Na_2S_2O_3$) gradually with constant vigorous shaking. The titration was continued until yellow colour starts disappearing. 0.5ml of 1% starch indicator was added and the titration continued with much shaking near the end point to liberate all the iodide from the chloroform layer. Thiosulphate solution was added drop by drop until blue colour disappeared. The determination was carried out in triplicate and the peroxide value computed. The blank determination was carried out as above with oil sample.

**Calculation**

Peroxide value (mg/kg of fat)

$$= \frac{(a-b) \times m \times 10}{W}$$

Where

m = Molality of $Na_2S_2O_3$
a = volume of test $Na_2S_2O_3$
b = volumes of blank $Na_2S_2O_3$
w = weight of oil sample

**Formulation of experimental diets**

The experimental diets of fresh and thermoxidized palm oil were formulated by adding 15g of the oil to 85g of rat feed in each case according to the method of Umoh *et al.;* [22]. This formulation was based on the fact that palm oil forms 15% of the average Nigerian diet. The rat feed was purchased from Top feed Nigeria Limited, Bukurru-Jos Nigeria. The constituents of the feed include 21% (minimum) protein, 3.3% (minimum) for fat, 6% (maximum) fiber, 0.8% calcium and 0.8% phosphorus. The control group was fed on rat chow only.

**Assessment of learning and memory**

The Morris water maze was used to assess visuospatial learning as described by Vorhees and William [23] and used by Bisong *et al.;* [24], Ajiwhen & Bisong [25] and Okon *et al.;* [26].

**Statistical Analysis:**

Data obtained were expressed as Mean ± SEM, Analysis of data was done following the one way analysis of variance (ANOVA) and Post hoc Neuman keul test with the help of the statistical package (SPSS 15.1) and were considered significant at $p<0.05$.

**RESULTS**

**Swim Latencies during Acquisition Training**

The mean swim latencies on the Day 1 of acquisition training for control, FPO-diet fed and TPO-diet fed groups was 11.55 ± 2.25; 14.43 ± 2.85 and 8.57 ± 1.69 seconds respectively. On day 2, the mean swim latencies was 6.65 ± 0.66; 8.91 ± 1.58 and 10.56 ± 1.96 seconds for control, FPO-diet fed and TPO-diet fed groups respectively. For day 3 of the acquisition training the swim latencies was 9.55 ± 1.73; 9.99 ± 1.63 and 10.61 ± 2.53 seconds control, FPO-diet fed and TPO-diet fed groups respectively. There was no significant difference in the swim latencies among all the experimental groups during acquisition training (Figure 1).

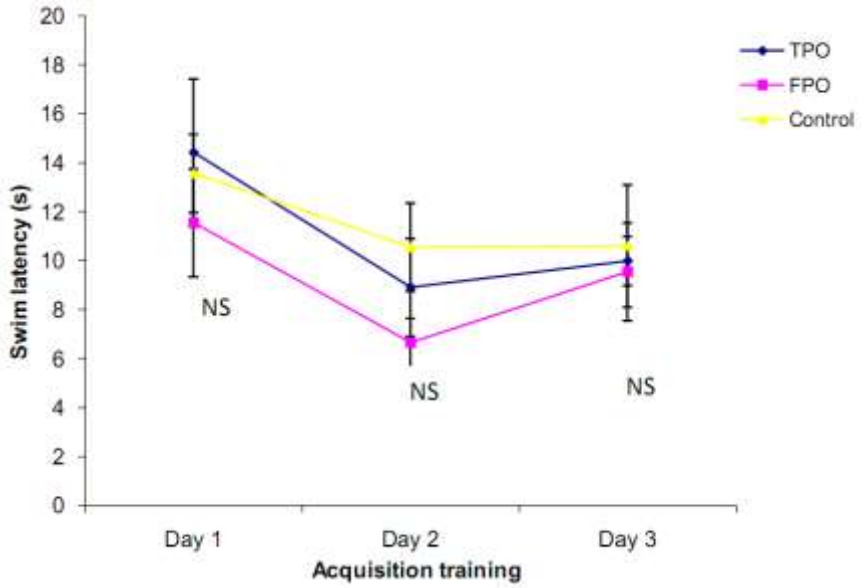


**Fig. 1: Swim latencies during acquisition training in the Morris water maze by mice fed fresh, thermoxidised and control diets; n = 9.**

**Reversal training**

The mean swim latencies on the Day 1 of the reversal training for control, FPO-diet fed and TPO-diet fed groups was 13.03 ± 2.35; 8.57 ± 1.32 and 16.30 ± 8.06 seconds respectively. On day 2, the mean swim latencies was 6.82 ± 1.40; 7.52 ± 1.84 and 4.65 ± 0.91 seconds for control, FPO-diet fed and TPO-diet fed groups respectively. For day 3 of the acquisition training the swim latencies was 3.38 ± 0.71; 4.90 ± 0.85 and 6.29 ± 2.05 seconds, for control, FPO-diet fed and TPO-diet fed groups respectively. There was no significant difference in the swim latencies among all the experimental groups during acquisition training (Figure 2).

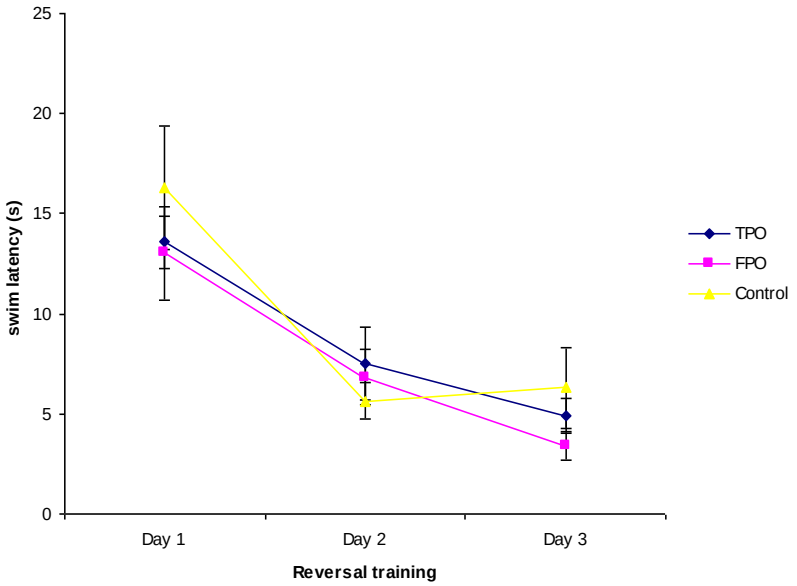


**Fig. 2: Swim latencies during reversal training in the Morris water maze by mice fed fresh, thermoxidised and control diets; n=9**

**Probe trial**

The mean swim latencies during the probe trial was 20.29 ± 5.32; 14.91 ± 0.09 and 14.98 ± 0.05 seconds for control, FPO-diet fed and TPO-diet fed groups respectively. No significant difference was observed in the swim latencies during the probe trial test (figure 3).

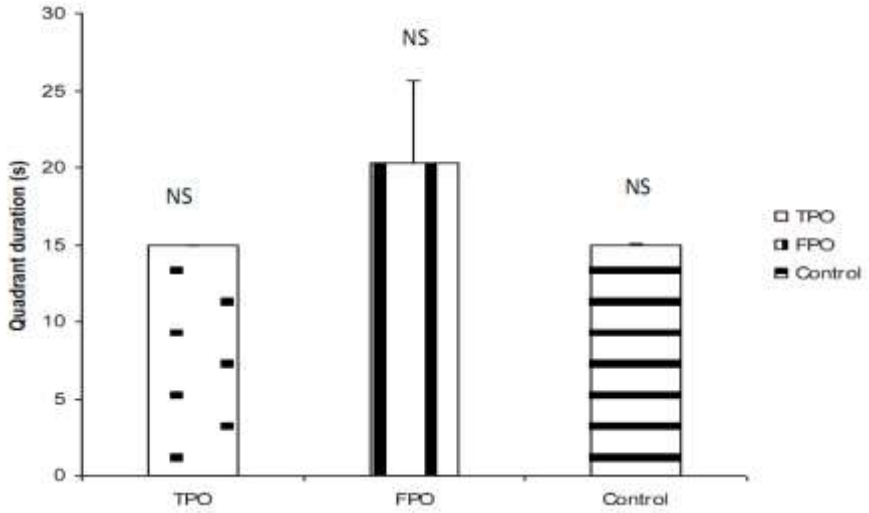


**Fig. 3: North east quadrant during the probe trial in the Morris water maze by mice fed fresh, thermoxidised and control diets; n = 9.**

**Visible platform task**

The swim latencies during the visible platform task was 9.47 ± 1.99; 6.78 ±1.65 and 8.46 ± 2.85 seconds for control, FPO-diet fed and TPO-diet fed groups respectively. There was no significant difference in swim latencies among the groups (Figure 4).

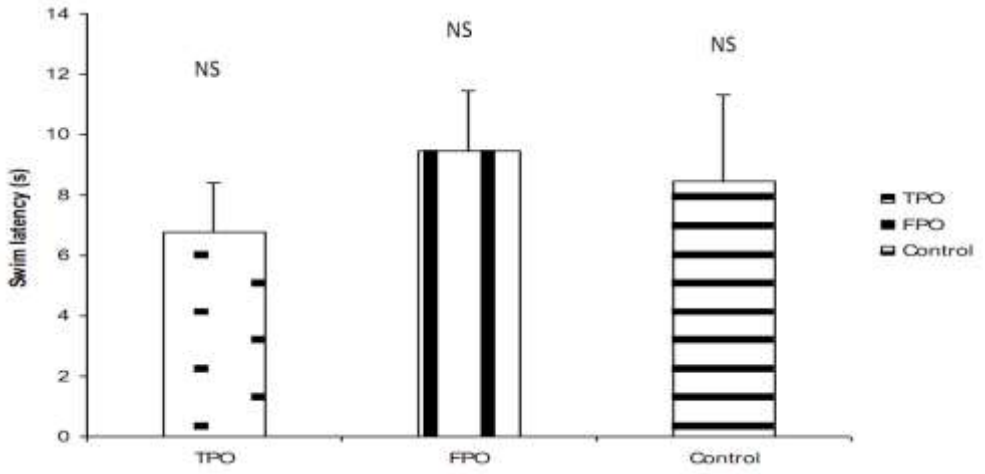


**Fig. 4: Swim latency during the visible platform task in the Morris water maze by mice fed fresh, thermoxidised and control diets; n = 9.**

## DISCUSSION

Learning is a process by which novel information is used to modify subsequent responses, while memory uses processed information to modify subsequent behavior. Although it is not clear if memory is attainable without learning, however it is imaginable that learning cannot occur if there is no memory. Also, since these processes are not directly observable, they cannot be measured directly and thus must be inferred from observed changes in behavior over time. Measures of cognitive function may also be influenced by sensory, motor, attention and motivational variables [27].

The Morris water maze (MWM) has been explored as a test for spatial learning in rodents [28]. It is one of the tests used to assess the effect of brain lesions and evaluate the properties of cognitive enhancers [29] and the effect of the maze parameter on strain differences in spatial learning [30]. The hidden platform version of the Morris water maze is a test of spatial memory which is sensitive to hippocampal damage while the visible platform of the maze is a non-hippocampal task which is disrupted by dorsal striatum lesions [31]. Thermoxidation of palm oil during deep and constant frying has been known to generate harmful reactive oxygen species (free radicals) which are linked to the etiology of neurodegenerative diseases including memory deterioration. In our study, feeding of experimental mice with diet formulated with different forms of palm oil for a period of ninety days resulted in no significant change in the learning curves during the acquisition and reversal training in the MWM in both the test groups and the control group indicating that mice in all the groups possess equal learning ability. No significant difference was observed in reversal training during the probe trial and visible platform task in animals of all the experimental groups also suggesting that all the animals in the experimental groups had equal learning capability. The North-East quadrant duration did not also differ among the groups. Therefore, feeding with fresh and thermoxidised palm oil diets resulted in no cognitive deficit.

## CONCLUSION:

Although consumption of different forms of palm oil has been implicated with the generation of a host of reactive oxygen species with a multifaceted systemic damage, it has no deleterious effect cognition function in mice.

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