



Effect of Fresh and Stored Palm Oil on Oxidative Stress and Inflammatory Markers in Wistar Rats



Ojeaburu S.I.^{1*} & Asiriwa N.U.²

¹Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

²Biochemistry Division, Nigerian Institute for Oil Palm Research (NIFOR), P.M.B. 1030, Benin City, Edo State, Nigeria.

*Corresponding Author Email: samuel.ojeaburu@uniben.edu.

ABSTRACT

Palm oil is the most widely consumed vegetable oil globally, valued for its balanced composition of saturated and unsaturated fatty acids and its richness in phytonutrients such as carotenoids, tocotrienols, and phytosterols. This study examined the effects of varying free fatty acid (FFA) levels in palm oil on oxidative stress and inflammatory responses in Wistar rats. Thirty-six rats were divided into six groups of six rats each: Group I received a normal diet, while Group II served as the experimental control and was given fresh palm oil (0.4% FFA). Groups III–VI were administered palm oil with increasing FFA levels (4.8%, 8.4%, 21.9%, and 42.7%) obtained from oil stored for sixteen months. After four weeks of oral administration (480 mg/kg body weight), oxidative stress markers—superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), malondialdehyde (MDA)—and inflammatory cytokines (IL-6 and IL-10) were analyzed using standard methods. Fresh palm oil did not significantly alter antioxidant enzyme activities or MDA levels, while oils with elevated FFA content markedly reduced SOD, CAT, and GPx activities. IL-6 levels were significantly lower ($p < 0.05$) in rats fed both fresh and stored oils compared with controls, but IL-10 and IL-6/IL-10 ratios showed no significant changes ($p > 0.05$). Rats receiving highly oxidized oils (21.9% and 42.7% FFA) exhibited the greatest cytokine reduction. These findings suggest that its FFA content influences the nutritional and health effects of palm oil; thus, proper processing and storage are essential to preserve its quality.

Keywords:

Palm oil, Free fatty acid, Oxidative stress markers, Inflammatory factors.

INTRODUCTION

Palm oil, derived from the fruit of the *Elaeis guineensis* tree, is one of the most widely consumed vegetable oils worldwide, contributing significantly to global dietary fat intake. It is a complex lipid mixture composed predominantly of triglycerides, with varying amounts of saturated and unsaturated fatty acids (Asiriwa *et al.*, 2025). While its economic and culinary benefits are undeniable, concerns regarding its potential health implications, especially when oxidized, have intensified, particularly in relation to oxidative stress, lipid peroxidation, and inflammatory responses. Palm oil is naturally rich in antioxidants such as carotenoids, tocopherols, and tocotrienols, which contribute to its oxidative stability and potential health benefits (Sundram *et al.*, 2003). Crude palm oil contains high levels of β -carotene, a precursor of vitamin A, which plays a crucial role in cellular protection against oxidative damage (Nagendran *et al.*, 2000).

The presence of tocotrienols, a unique form of vitamin E, has been shown to exhibit neuroprotective and cardioprotective properties, primarily through their ability to reduce oxidative stress and inflammation (Sen *et al.*, 2006). However, despite these beneficial compounds, the health effects of palm oil remain a subject of debate due to variations in its processing, storage, and dietary intake.

Some studies have highlighted that dietary intake of fresh and oxidized palm oil can differentially affect antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). These enzymes play a critical role in neutralizing reactive oxygen species (ROS), thus mitigating oxidative stress-induced damage. The modulation of these antioxidant defense mechanisms by different FFA levels in palm oil remains a subject of ongoing investigation.

Lipid peroxidation - a biochemical process in which reactive oxygen species (ROS) induce oxidative damage to lipids, leading to cellular dysfunction and tissue damage, can arise due to prolonged storage and exposure of palm oil to high temperatures, which increases its free fatty acid (FFA) levels, and then, subsequently, enhance lipid peroxidation (Ebong *et al.*, 1999). Malondialdehyde (MDA), a by-product of lipid peroxidation, is widely used as a biomarker for oxidative stress (Ayala *et al.*, 2014). Inflammation plays a key role in the pathogenesis of numerous chronic diseases, including cardiovascular diseases, metabolic disorders, and neurodegeneration. The consumption of oxidized palm oil has been associated with an increase in pro-inflammatory cytokines such as interleukin-6 (IL-6) and a reduction in anti-inflammatory cytokines like interleukin-10 (IL-10), which are crucial in regulating immune responses (Odia *et al.*, 2015). An imbalance in these cytokines can lead to chronic inflammation, which may contribute to disease progression.

An interesting finding by Asiriwuwa *et al.* (2025) is that the health impact of palm oil, to a large extent, depends on its FFA content, which is often overlooked in general discussions about the health effects of palm oil. FFA levels is indicative of oil quality, with higher levels suggesting poor quality due to hydrolytic rancidity. Given the controversy over the health effects of palm oil, specifically the benefits from antioxidants versus the risks from saturated fats, this study aims to clarify how FFA levels influence these outcomes, thereby filling a knowledge gap.

MATERIALS AND METHODS

Preparation and collection of the palm oil samples
Freshly milled palm oil sample (A), and stored palm oil samples (B-E) that was stored for sixteen months at room temperature, and of free fatty acid (FFA) levels of 0.4 %, 4.8 %, 8.4 %, 21.9 % and 42.7 % respectively was obtained from Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Edo State.

Determination of free fatty acids (FFA) levels

The FFA in the palm oil samples was determined according to the AOCS official method Ca 5-40 by titration. Results were expressed as a percentage of palmitic acid.

Animal grouping and administration of palm oil samples

Adult albino rats (Wistar strain) of weights 130-150g of female sex were selected for this study. The animals were obtained from the Animal House of the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo state, Nigeria. Thirty-six (36) Wistar rats were divided into six groups of six rats per

group and treated as follows: Group I (normal control) given feed and water only. Group II (Experimental Control), given freshly milled crude palm oil containing 0.4 % FFA. Groups III-VI were given stored palm oil of varied FFA levels (4.8 %, 8.4 %, 21.9 % and 42.7 %), respectively. A dosage of 480mg/kg bwt was administered to the animals orally for four weeks.

The animals were kept in clean cages in a 12-hour light/dark cycle room with daily litter change. The animals were acclimatized for two weeks before the experiment commenced and were fed with Growers mash and water ad libitum. The weights of the rats were monitored throughout the duration of the experiment. During the study, rats were maintained under standard conditions. The ethical conditions and experimental protocols governing the use and conduct of experiments with live animals were strictly observed as approved by the university of Benin's ethical committee on the use of laboratory animals for experimental purposes (FLS/REC/2025/001), which is in accordance as recommended by the National Research Council (US) Committee Guide for the Care and Use of Laboratory Animals (2011). The animals were euthanized in mild anaesthesia at the end of the treatment period of four weeks after an overnight fast. A portion of the blood was collected by cardiac puncture into plain sample bottles for biochemical analyses.

Antioxidants and lipid peroxidation index estimation

Superoxide dismutase (SOD) was determined according to the methods of Misra and Fridovich, (1972).

Catalase (CAT) activity was estimated by the method described by Cohen *et al.*, (1970).

Glutathione peroxidase (GPx) was determined according to the method of Nyman, (1959).

Malondialdehyde was determined using the thiobarbituric acid assay as described by Buege and Aust, (1978).

Inflammatory factors

Interleukin-6 was determined in the serum of rat as described in ELISA Kit manual. Catalog No: E-EL-R0015 96T.

Rat IL-10(Interleukin 10) in serum was determined using ELISA Kit manual. Catalog No: E-EL-R0016 96T.

Data Analyses

Statistical analysis of data was carried out using the statistical package for social science (SPSS) version 21.0. Results were expressed as mean $\pm$ SEM of six replicates. The levels of homogeneity amongst groups were tested using one-way analysis of variance (ANOVA) with $p < 0.05$ considered significant. Duncan's multiple range test was used to separate homogenous groups.

RESULTS AND DISCUSSION

Effect of palm oil on antioxidant enzyme activity and malondialdehyde (MDA) levels in rats.

Administration of fresh palm oil (group II) to rats did not alter the serum levels of SOD, CAT, GPx, and

MDA compared to the normal control rats, whereas rats that received stored palm oil samples (groups III-VI) had reduced levels of SOD and GPx and increased levels of CAT and MDA, respectively. The results are presented in Table 1 below:

Table 1: Antioxidant enzymes and malondialdehyde levels of rats

Groups	SOD (u/mL)	CAT (u/mL)	GPx (u/mL)	MDA (mol/L)
1 (Normal Control)	1.35±0.02 ^b	0.0031±0.0001 ^a	1.71±0.01 ^c	9.37±0.41 ^a
II (0.4% FFA)	1.32±0.44 ^b	0.0034±0.0001 ^a	1.61±0.00 ^c	9.49±0.87 ^a
III (4.8% FFA)	1.22±0.02 ^b	0.0140±0.0001 ^d	0.35±0.00 ^a	8.17±0.00 ^a
IV (8.4 % FFA)	0.68±0.01 ^a	0.0076±0.0001 ^b	0.47±0.00 ^b	9.85±2.17 ^a
V (21.9% FFA)	1.35±0.02 ^b	0.0141±0.0001 ^d	0.71±0.00 ^b	10.06±0.09 ^a
VI (42.7% FFA)	0.68±0.01 ^a	0.0259±0.0001 ^e	0.61±0.00 ^c	7.69±0.83 ^a

Values are in mean ± SEM (n = 4). Data with the same superscript are not significantly different ($p > 0.05$), while data with different superscripts are significantly different ($p < 0.05$).

Effect of palm oil on inflammatory factors

There was a significant reduction ($p < 0.05$) in the IL-6 levels in the serum of rats fed fresh and stored palm oil samples compared to the normal control group. There was no significant difference in the IL-10 level and IL-6/IL-10 (Table 2)

Table 2: Inflammatory factors of rats

Groups	IL-6(pg/mL)	IL-10 (pg/mL)	IL-6/ILK-10 (pg/mL)
1 (Normal Control)	237.19±69.37 ^b	6.19±0.79 ^b	42.64±17.07 ^a
II (0.4 % FFA)	179.11±54.15 ^b	6.30±1.21 ^b	34.50±16.11 ^a
III (4.8 % FFA)	170.78±47.40 ^b	7.56±0.67 ^b	24.11±8.52 ^a
IV (8.4 % FFA)	90.13±38.18 ^a	4.56±0.34 ^a	19.38±6.44 ^a
V (21.9 % FFA)	93.36±21.10 ^a	5.34±0.39 ^b	17.08±2.71 ^a
VI (42.7 % FFA)	74.00±2.39 ^a	4.20±0.08 ^a	17.65±0.93 ^a

Values are in mean ± SEM (n = 4). Data with the same superscript are not significantly different ($p > 0.05$), while data with different superscripts are significantly different ($p < 0.05$).

The findings of this study highlight the complex relationship between palm oil consumption, free fatty acid (FFA) levels, and their effects on oxidative stress, antioxidant enzymes, and inflammatory markers in Wistar rats. The results demonstrate that fresh palm oil (0.4% FFA) had beneficial effects, corroborating previous studies that emphasize the beneficial antioxidant properties of crude palm oil due to its high content of carotenoids, tocopherols, and tocotrienols (Sundram *et al.*, 2003; Nagendran *et al.*, 2000). However, stored palm oil with elevated FFA levels (8.4 % to 42.7 %) exhibited some alterations in antioxidant enzyme activities (SOD, GPx, CAT), and increased the lipid peroxidation (MDA levels), suggesting oxidative stress. This aligns with Ebon *et al.* (1999), who reported that prolonged storage and

high FFA content in palm oil can promote lipid peroxidation and cellular damage.

The inflammatory response showed a significant reduction in IL-6 levels of palm oil-fed rats (179.11±54.15 pg/mL - 74.00±2.39 pg/mL) compared to normal control (237.19±69.37 pg/mL). IL-6 is a pro-inflammatory cytokine. The reduction in IL-6 levels is desirable and further confirms the health benefits of palm oil. The IL-10 levels were fairly maintained across the groups, even at high FFA levels. This study contradicts some reports linking oxidized lipids to increased pro-inflammatory cytokines (Ayala *et al.*, 2014). However, the non-significant reduction in the IL-6/IL-10 ratio suggests an inflammatory balance. A higher IL-6/IL-10 ratio indicates a pro-inflammatory state, while a lower ratio reflects an anti-inflammatory or immune-regulated state. Dietary fats, including palm oil, can influence this balance through their effects on lipid metabolism and oxidative stress pathways (May *et al.*, 2020).

This research underscores the importance of FFA levels in determining palm oil's health effects. While fresh palm oil is beneficial, oxidized palm oil may pose risks, supporting the need for proper storage and processing to mitigate oxidative damage.

CONCLUSION

This study demonstrates that the health impacts of palm oil are closely linked to its free fatty acid (FFA) content and oxidative stability. Fresh and stored palm oils with low FFA levels exhibited beneficial effects on antioxidant enzyme activity, lipid peroxidation, and inflammatory responses, consistent with the oil's established antioxidant potential. These results underscore the importance of strict quality control during palm oil processing and storage to prevent FFA buildup and oxidative deterioration, thereby preserving its nutritional and health-promoting properties.

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