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Chemical Parameters of Palm Oil (*Elaeis guineensis*) and its Effects on the Cardiovascular System of Adult Male Rats (*Rattus norvegicus*)

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Abstract | **Original Research Article**

Palm oil is the most widely consumed vegetable oil in the world. Due to its high content of saturated fatty acids (SFA), particularly palmitic acid, it is considered by some authors to be potentially harmful to health. This study aimed to evaluate the impact of consuming palm oil (crude and refined) on the cardiovascular system of rats (*Rattus Norvegicus*). The study involved chemical analysis of crude and refined palm oil, followed by a nutritional experiment using rats fed diets containing crude palm oil, refined palm oil, and sunflower oil. A histological study was conducted to observe the rats' arteries. Results showed that the chemical parameters of refined palm oil were superior to those of sunflower oil. Palm oil consumption had a notable impact on animal growth, with rats fed palm oil diets showing greater weight gain than those fed sunflower oil. Moreover, the presence of palm oils in the diets did not significantly affect the relative weights of harvested organs. Finally, histological analysis of the arteries revealed that sunflower oil posed a higher cardiovascular risk than palm oil.

Keywords: palm oil, sunflower oil, cardiovascular health, chemical parameters, histology, arteries.

INTRODUCTION

Palm oil is the most widely used edible vegetable oil globally. It is extracted from the pulp of oil palm fruits (*Elaeis guineensis*) and has a reddish-orange color due to its richness in beta-carotene (Sundram *et al.,* 2003; Imoisi *et al.,* 2015). It ranks first among vegetable oils worldwide, with production increasing from 15.2 million tons in 1995 to 54 million tons in 2014 (Mondé *et al.,* 2019). In Côte d'Ivoire, palm oil is the most consumed edible oil, with national production estimated at 370,000 tons/year (FAO, 2014). It is mainly consumed in two forms: crude palm oil, also known as "red oil," and refined palm oil, which is more common and undergoes processes such as bleaching and deodorization. Like all fats, palm oil is composed of nearly 100% lipids in the form of glycerides, containing approximately 50% saturated and 50% unsaturated fatty acids. It is found in many food products, contributing texture, aroma, and neutral taste: baked goods, candies, cakes, cheese analogs, chips, chocolates, biscuits, fried foods, frozen meals (pancakes, pies, pizzas, potatoes), instant meals, etc.

However, due to its high saturated fat content, palm oil is subject to controversy and prejudice regarding health and environmental concerns. Health-wise, it is accused of being atherogenic and thus promoting cardiovascular diseases (Mondé *et al.,* 2019). A report by the World Health Organization (WHO, 2003) indicated convincing evidence implicating myristic and palmitic acids in increasing cardiovascular disease (CVD) risk. Béké (2015) found that at the Abidjan Cardiology Institute, patients with hypertension and ischemic heart disease were advised to avoid palm oil in 56% and 43.13% of cases, respectively. Nevertheless, several studies have highlighted the benefits of palm oil consumption (Mondé *et al.,* 2009 ; Imoisi *et al.,* 2015) and its diverse uses (Gogbe *et al.,* 2016). Additionally, some patients were unaware of the oil's virtues, despite its use dating back at least 5,000 years, as evidenced by archaeological findings in Egypt (Kenneth *et al.,* 2007). The general objective of this study is to assess the effects of palm oil consumption on the cardiovascular system of rats.

MATERIALS AND METHODS

MATERIALS

Animal Material

The animal material consisted of male rats (Rattus norvegicus, Muridae) weighing between 165 and 339 g. The animals were housed in polypropylene cages placed on racks at the Laboratory of Animal Physiology, Faculty of Biosciences, FÉLIX HOUPHOUËT-BOIGNY University (Cocody-Abidjan). They were maintained under standard breeding conditions with controlled ambient temperature (22 ± 2 °C), a 12 h/12 h light/dark cycle, and ad libitum access to water and food.

DIETARY MATERIAL

Various ingredients were used to formulate the rats' diets: crude palm oil sourced from Jacqueville (southern Côte d'Ivoire); iodized salt, white cane sugar, corn flour, sunflower oil, and refined palm oil purchased from a supermarket in Cocody (Abidjan); powdered dried fish obtained from a local market in Adjamé (Abidjan). A vitamin complex (Vitaflash) was also purchased from a pharmacy in Cocody.

Technical Equipment

Technical Equipment Used for Animal Experimentation

The technical equipment included a precision balance (1/100), Denver brand (Germany), used for weighing the rats and the various dietary formulations. Polyethylene cages with wire mesh lids were equipped with water bottles for rat hydration.

Technical Equipment Used for the Analysis of Chemical Parameters of Oils

The equipment consisted of a magnetic stirrer with heating plate (IKA, RET basic, Reference 0003810000, France) for solution agitation, a stand for securing burettes, a magnetic stirring bar, an analytical balance (DENVER SI-403, Artikelhinweise, Germany) for weighing chemical reagents, and a rotary evaporator (RotaVapor, Stuart brand, oil bath RE300OB) for solution evaporation. The glassware included Erlenmeyer flasks, pipettes, burettes, volumetric flasks, jars, micropipettes, as well as various consumables (stoppers, Whatman paper, aluminum foil).

Technical Equipment Used for Diet Preparation

The technical equipment primarily included a gas cylinder for cooking, a saucepan for preparing the formulations, and a spatula used to mix the preparations during cooking.

Technical Equipment Used for Histological Sectioning

The equipment included a *Technicom* automated processor for dehydration and impregnation of organ samples, an embedding station and molds for paraffin embedding, as well as a *Technicom* mini-refrigerator for rapid paraffin solidification. A microtome was used to perform organ sectioning, and a water bath was employed to relax the obtained sections. The sections were mounted on slides and covered with cover slips.

For sample processing, toluene was used to dissolve the paraffin, followed by rehydration using alcohols of increasing concentrations. Staining was performed with eosin for the cytoplasm and hematoxylin for the nuclei. The slides and cover slips were fixed using *EUKIT* mounting medium, and the sections were observed under a light microscope.

METHODS

Animal Allocation and Housing

The animal experiment was conducted over a one-month period during June and July 2022. Fifteen animals were used, divided into three groups of five rats per cage, with an average body weight of 205 g. The cages were lined with wood shavings and covered with wire mesh. They were placed on metal stands in the animal facility of the Laboratory of Animal Physiology.

Preparation of Rat Diets

Basic Data

The diets were formulated to be isoenergetic (4230 kcal/kg DM) and isoproteic (100 g/kg DM) (Adrian *et al.,* 1991). The proportions of carbohydrates and lipids were calculated to meet the required caloric intake, considering the energy contributions of 4 kcal per gram for carbohydrates and proteins, and 9 kcal per gram for lipids. This formulation method was designed to meet the nutritional requirements of the animals (Table I) (Adrian *et al.,* 1991).

Table I: Daily Macronutrient Requirements for Rats

Macronutrients	Required Intake (% per day)
Protein	9-18
Carbohydrates	53.5-70
Lipids	3-10

Diet Composition

The composition of the different diets is presented in **Table II**. A total of three diets were formulated. All diets contained fixed proportions of corn starch and white sugar as carbohydrate sources, powdered dried fish (herring) as the protein source, as well as a vitamin complex and iodized salt as mineral sources. The only difference between the diets was the lipid source, i.e., the type of oil incorporated. The diets were categorized as follows:

- Crude Palm Oil Diet **(RHPB)**
- Refined Palm Oil Diet **(RHPR)**

- Sunflower Oil Diet **(RHT)**

Table II: Composition of Rat Diets (in grams)

Ingrédients	RHPB	RHPR	RHT
Vitamin	5	5	5
Iodized Salt	1	1	1
Crude Palm Oil	50	0	0
Refined Palm Oil	0	50	0
Sunflower Oil	0	0	50
Dried Herring Powder	140	140	140
Corn Flour	553.74	553.74	553.74
Table Sugar	250	250	250
Water	1L	1L	1L
Energy Content (kcal/kg DM)	4230	4230	4230

Diet Preparation Procedure

The preparation of the diets involved dissolving 250 g of sugar, 1 g of iodized salt, 5 g of vitamin complex (Vitaflash), and 553.74 g of corn starch in 1 liter of water. The mixture was then thoroughly homogenized using a spatula and heated over low flame. While stirring continuously, 140.26 g of powdered fish and 50 ml of the oil corresponding to each diet category were added. The mixture was cooked for 10 minutes.

Feeding and Growth Measurement of Rats

Rats were grouped in batches of five according to diet and cage, and fed daily at 2:00 p.m. for a period of 30 days. The diets were served in paste form to minimize waste. Water was provided ad libitum and renewed every two days. Both the distributed feed and the refusals were weighed daily. Feed intake was calculated as the difference between the amount distributed and the amount refused. Animals were weighed at the beginning of the experiment and then every two days, with the final weighing conducted at the end of the trial. Weight gain was determined by subtracting the initial weight from the final weight.

Organ Collection Procedure

At the end of the experiment, the rats were fasted starting at 4:00 p.m. On the following morning, between 8:00 and 10:00 a.m., they were sacrificed by decapitation after anesthesia with ethyl urethane. Following sacrifice, a longitudinal laparotomy was performed to collect the heart, liver, spleen, both kidneys, abdominal fat, and blood vessels (carotid artery, aorta, and pulmonary artery). The organs were weighed, except for the blood vessels, which were preserved in containers filled with 10% diluted formalin.

METHODS FOR HISTOLOGICAL SECTIONING

Dehydration and Impregnation

Dehydration and impregnation of the samples were carried out using an automated tissue processor. The processor included baths of formalin, graded ethanol, toluene, and liquid paraffin. Formalin baths were used to fix the samples, followed by progressive dehydration through ascending ethanol concentrations. The process continued with clarification using toluene and was completed by impregnation with liquid paraffin.

Embedding in Paraffin

Embedding was performed using an embedding station composed of three units: two heated compartments for embedding and one cooling unit for solidifying the molds. This step involved orienting the sample within a mold, then adding liquid paraffin while ensuring the correct sectioning plane was maintained. The molds were subsequently placed on the cooling station to harden the paraffin, thereby forming tissue blocks.

Sectioning of Blocks

Samples were sectioned using a microtome. This is a critical step in slide preparation, as it determines the quality of microscopic observation. Transverse sections were cut into thin slices of 2 to 3 micrometers. This process yielded paraffin ribbons containing tissue sections. The sections were then spread on a water bath and mounted onto glass slides, which were subsequently placed in an oven prior to staining.

Staining of Sections

The hematoxylin-eosin staining technique (hematoxylin stains nuclei violet; eosin stains cytoplasm pink) enhances contrast to facilitate the identification and differentiation of biological structures. Prior to staining, the samples were deparaffinized in a toluene bath and rehydrated through a series of descending ethanol concentrations, allowing polar dyes to penetrate the tissues. This ensured effective interaction between the dyes and the cellular components.

Method for Physicochemical Parameter Analysis

Iodine value, saponification index, peroxide value, and acid value were determined according to the method described by AOAC (1997).

Statistical Treatment and Data Analysis

Results are presented in tables and figures. Figures were generated using GraphPad software version 7.00, which was also used for statistical analyses. One-way analysis of variance (ANOVA) followed by

Newman-Keuls post hoc test (at a 5% significance level) was applied to calculate means and standard deviations. Superscript letters (a, b) following the means in tables and figures indicate statistical differences. Means followed by different letters on the same row are significantly different.

RESULTS AND DISCUSSION

RESULTS

Chemical Parameters of the Oils

The acid value, peroxide value, saponification value, and iodine value were the four key indices used to assess the physicochemical characteristics of the different oils (refined palm oil – HPR, sunflower oil – HT, and crude palm oil – HPB). Table III presents the values of these chemical indices measured in samples of refined palm oil, crude palm oil, and sunflower oil.

The three oils showed markedly different acid values, with crude palm oil exhibiting the highest value (7.58 mg KOH/g), refined palm oil showing a very low value (0.56 mg KOH/g), and sunflower oil showing none (0 mg KOH/g).

Regarding the peroxide value, the results ranged from 6 to 16 meq O_2/kg. Sunflower oil and crude palm oil had the highest values 16 meq O_2/kg for sunflower oil (HT) and 10 meq O_2/kg for crude palm oil (HPB). The lowest value was recorded for refined palm oil (HPR) at 6 meq O_2/kg.

Identical saponification values (210.37 mg KOH/g) were obtained for both crude and refined palm oils, which were higher than the value recorded for sunflower oil (189.33 mg KOH/g).

However, the iodine values varied between crude and refined palm oils, with crude palm oil having the higher value. The iodine values ranged from 31.92 g I_2/100 g to 138 g I_2/100 g. The highest value was observed in sunflower oil, while the lowest was found in refined palm oil.

Table III: Physicochemical Parameters of the Oils

Physicochemical Parameters	Sunflower Oil	Refined Palm Oil	Crude Palm Oil	Codex Alimentarius Standard
Acid Value (mg KOH/g)	0	0.56	7.58	<4
Peroxide Value (meq O_2/kg)	16	6	10	<10
Saponification Value (mg KOH/g oil)	189.33	210.37	210.10	187-195
Iodine Value (g I_2/100 g)	138.07	31.92	49.53	65-72

Effect of Different Diets on Rat Growth

The growth curves of rats fed with crude palm oil, refined palm oil, and sunflower oil diets are reported in Figure 1. During the first five days of experimentation, the body weight of rats fed with the three diets without exception decreased. However, after the fifth day, these body weights progressively increased, with greater growth in rats fed the Refined Palm Oil Diet (RHPR) and lower growth in rats fed the Sunflower Oil Diet (RHT). But rats fed with the Crude Palm Oil Diet showed the greatest body weight gain in the last week of experimentation. Indeed, a progressive and regular increase in body weight was observed in rats fed with the Crude Palm Oil Diet, while the body weight of rats fed with refined palm oil decreased at the end of the experiment. The growth of rats fed with sunflower oil did not undergo major changes; it remained almost constant from the second experimental week. The body weight of rats fed the Sunflower Oil Diet hardly increased. It showed a rather low progression, rising from 202 g to 202.6 g; that is, an average weight gain of 0.6 g or the equivalent of 0.0206 g/day over 29 days. As for the growth of rats fed the Refined Palm Oil Diet (RHPR), their body weight showed a fairly visible increase, rising from 203.4 g to 211.8 g; that is, an average weight gain of 8.4 g or the equivalent of 0.289 g/day over 29 days. Similarly, the body weight of rats fed the Crude Palm Oil Diet (RHPB) increased regularly and rose from 203.8 g to 221.4 g, corresponding to an average weight gain of 17.6 g or the equivalent of 0.6 g/day over 29 days. The differences observed after consumption of the diets containing palm oils are not significant, but the differences observed between the diets containing palm oils and the diet containing sunflower oil are significant.

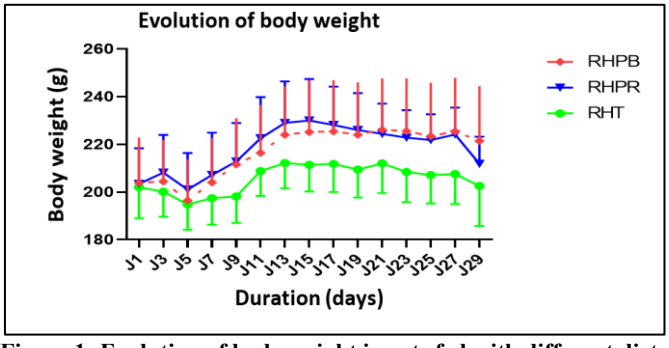

Figure 1: Evolution of body weight in rats fed with different diets

Effect of diets on relative organ weights

Relative organ weights are reported in Table IV. Statistical analysis of the obtained values indicates that the differences observed in the relative weights of the organs (liver, kidneys, spleen, and heart) are not significant ($p > 0.05$). For the liver, the relative weight values are approximately the same across all diets, as is the case for the relative weights of the heart, kidneys, and spleen. However, regarding abdominal fat, the highest values were recorded following consumption of diets containing palm oil, specifically crude palm oil and refined palm oil (2.147 ± 1.052 and 1.357 ± 0.233, respectively).

Table IV: Variation in relative organ weights and abdominal fat in rats

Organs	Dietary Treatments		*Pvalues*	
	RHPB	RHPR	RHT	
Liver	3,641± 0,375[a]	3,076±0,427[a]	3,591±1,320[a]	0,4
Heart	0,324±0,007[a]	0,325±0,042[a]	0,349±0,060[a]	0,1
Abdominal fat	2,147±1,052[a]	1,357±0,233[a]	0,573±0,310[a]	0,09
Kidney	0,567±0,075[a]	0,569±0,054[a]	0,617±0,229[a]	0,4
Spleen	0,223±0,066[a]	0,246±0,094[a]	0,441±0,362[a]	0,07

Values in the same row bearing the same letter are not significantly different.

Effects of Diets on Blood Vessel Structure

Figures 7 to 12 show the histological sections of blood vessels. The results indicate that the consumption of all diets had noticeable effects on vascular structure. Fatty deposits were observed in the arterial walls of animals from all diet groups. The diet containing sunflower oil (RHT), in addition to lipid streaks, showed a perivascular inflammatory infiltrate. In contrast, only fatty deposits were observed in the arteries of rats fed the RHPR diet, as well as in those fed the RHPB diet, where lipid streaks were present in the arterial walls.

These findings are summarized in Table V, which presents, by diet group, the percentage of animals exhibiting the observed histological features.

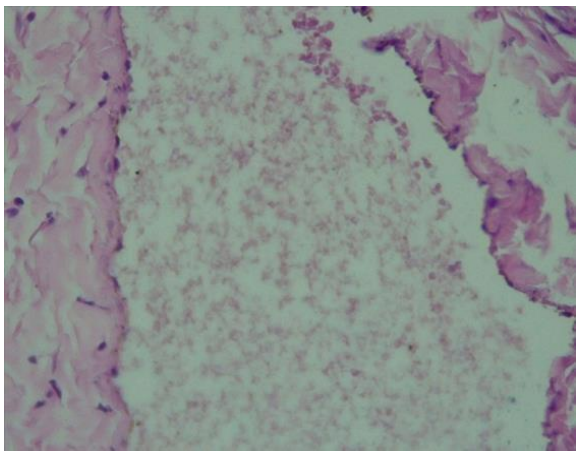

Figure 7: Microscopic section of a pulmonary artery with normal histological appearance in rat. (Staining: Hematoxylin and Eosin; Magnification: ×100)

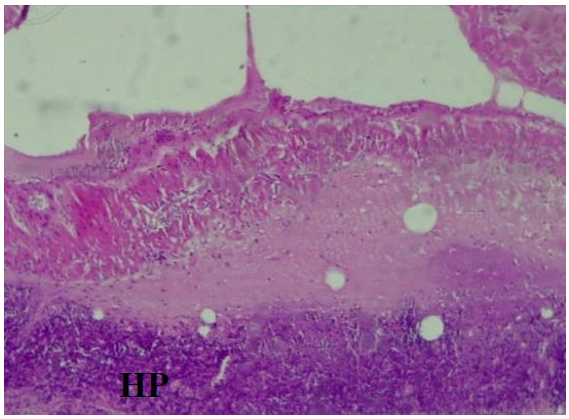

Figure 8: Microscopic section of pulmonary artery from rats fed the RHT diet, showing perivascular inflammatory infiltrate (PII). (Staining: Hematoxylin and Eosin; Magnification: ×100)

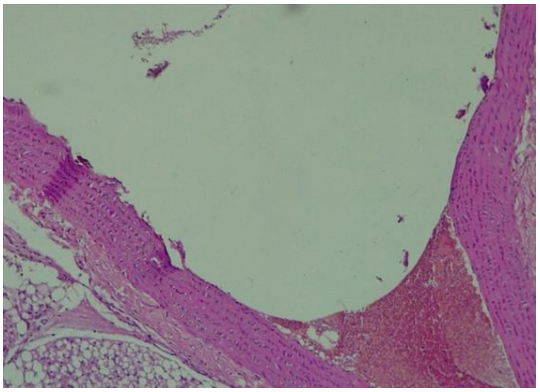

Figure 9: ***Microscopic section of aortic artery tissue with normal histological appearance in rats.*****(Staining: Hematoxylin and Eosin; Magnification: ×100)**

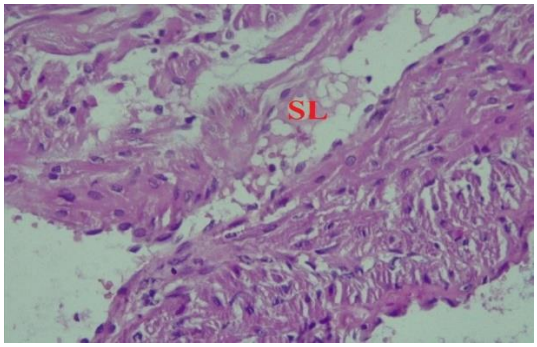

Figure 10: Microscopic section of aortic artery from rats fed the RHT diet, showing lipid streaks (LS). (Staining: Hematoxylin and Eosin; Magnification: ×100)

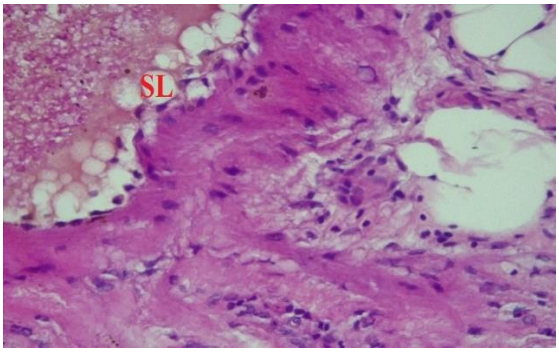

Figure 11: Microscopic section of aortic arteries from rats fed the RHPR diet, showing lipid streaks (LS). (Staining: Hematoxylin and Eosin; Magnification: ×100)

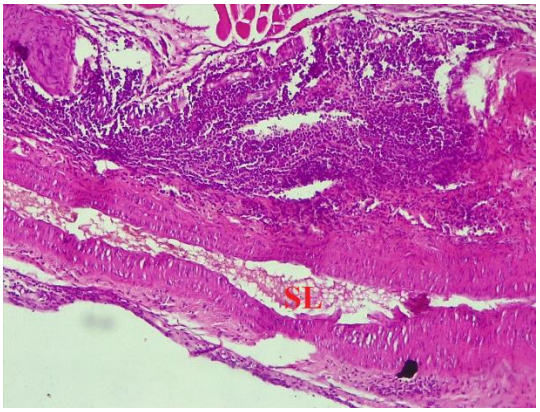

Figure 12: Microscopic section of arteries from rats fed the RHPB diet, showing lipid streaks (LS). (Staining: Hematoxylin and Eosin; Magnification: ×100)

Table V. Microscopic Observations of Rat Arteries According to Dietary Treatments

Dietary Treatment	Histological Features	
	Lipid Streaks (%)	Inflammatory Infiltrates (%)
RHT	33	33
RHPR	33	0
RHPB	33	0

DISCUSSION

The chemical parameters of the oils were the key indicators used in this study to assess the impact of palm oil. The amount of free fatty acids (FFA) in a fat is reflected by its acid value, which is a major quality factor. The acid values obtained in this study 0.56 mg KOH/g for refined palm oil and 0 mg KOH/g for sunflower oil comply with Codex Alimentarius standards (<4 mg KOH/g) and are consistent with previously reported values: 1.11 mg KOH/g for unheated refined palm oil (N'Guessan *et al.,* 2018) and 0.1 mg KOH/g for fresh sunflower oil (Rouaki, 2016). However, the acid value of crude palm oil (7.58 mg KOH/g) exceeds Codex standards and is significantly higher than that of refined palm oil, aligning with Bauer *et al*. (2010), who demonstrated that alkali treatment during chemical refining removes free fatty acids.

The peroxide value reflects the oxidative state of oils and helps predict future deterioration of organoleptic qualities, though it does not provide information on past oxidation (N'Guessan *et al.,* 2018). The peroxide value for crude palm oil (10 meq O_2/kg) was higher than that reported by N'Goran *et al*. (2017) in Soubré (3.7 meq O_2/kg). Similarly, refined palm oil (6 meq O_2/kg) exceeded the value reported by N'Guessan *et al*. (2018) (2.48 meq O_2/kg), and sunflower oil (16 meq O_2/kg) was also higher than the 1 meq O_2/kg reported by Rouaki (2016). These discrepancies may be due to oxidation occurring during processing, storage, or handling of raw materials (Frankel, 2005). Among the three oils tested, only the peroxide value of refined palm oil met Codex Alimentarius standards (<10 meq O_2/kg).

These findings highlight the high oxidative state of crude palm oil compared to refined palm oil, likely due to the refining process. According to Graille (2003), unsaturated fatty acids free or bound in triglycerides or phospholipids react with oxygen. Moreover, moderate consumption of oxidized n-3 PUFAs leads to elevated plasma concentrations of 4-HHE (4-hydroxy-hexenal), inflammatory markers, and activation of inflammatory pathways and endoplasmic reticulum stress (Awada, 2012). Thus, refined palm oil appears to be the most suitable for consumption based on its peroxide value.

The iodine value measures the degree of unsaturation in fats, increasing with the number of double bonds in fatty acids. Oils with high iodine values contain more unsaturated fatty acids (N'Guessan *et al.,* 2018). In this study, refined palm oil (31.92 g I_2/100 g) and crude palm oil (49.53 g I_2/100 g) showed lower values than those reported by N'Guessan *et al*. (2018) (55.43 g I_2/100 g) and N'Goran *et al*. (2017) (64.9 g I_2/100 g). These differences may be due to oil degradation from extraction conditions, raw material quality, or inadequate storage (Hasimah *et al.,* 2016). The iodine value for sunflower oil (138.07 g I_2/100 g) was close to that reported by Rouaki (2016) (130 g I_2/100 g). None of the measured values in this study met Codex Alimentarius standards (65–72 g I_2/100 g), suggesting that palm oil refined or crude contains few unsaturated fatty acids, while sunflower oil is an excessive source.

The saponification value for refined palm oil (210.37 mg KOH/g) was higher than that reported by N'Guessan *et al*. (2018) (178.24 mg KOH/g). The same value was obtained for crude palm oil and was close to that found by N'Goran *et al*. (2017) in Daloa (211.8 mg KOH/g). The value for sunflower oil (189.33 mg KOH/g) matched Keeilli and Trache (2014) (189.01 mg KOH/g) and was the only one within Codex Alimentarius standards (187–195 mg KOH/g).

The nutritional experiment conducted on rats fed diets containing refined palm oil, crude palm oil, and sunflower oil showed that body weights of rats consuming palm oil (refined or crude) were significantly higher ($p<0.05$) than controls. However, no significant difference was observed between rats fed crude versus refined palm oil. This may be due to growth-promoting compounds present in palm oil but absent or less abundant in sunflower oil. Lecerf (2013) noted that palm oil is rich in "minor" compounds such as tocols (tocopherols and tocotrienols). Red palm oil is the richest dietary source of carotenoids, precursors of vitamin A. The organoleptic properties of palm oil may also explain its high consumption by rats.

Histological examination of blood vessels revealed that all diets affected vascular structure. All experimental groups (RHPR, RHPB, RHT) showed lipid streaks in equal proportions, representing the initial stage of atherosclerosis caused by lipid accumulation in arterial walls and intimal thickening (Sanchez, 2017). These findings align with studies by Hornstra (1988) and Kritchevsky *et al*. (2002), which showed that in rabbits made atherosclerotic, palm oil had similar effects to other oils on lesion count and induced the lowest degree of atherosclerosis alongside sunflower oil. The beneficial effects of red palm oil on atherosclerosis may be linked to high doses of carotenoids and tocotrienols. **Grynberg (2005)** also highlighted its benefits in managing hypertension and protecting the cardiovascular system.

The low atherosclerotic rate observed supports the good quality of crude red palm oil, which is rich in tocotrienols. Tocotrienols may help reduce plasma cholesterol and have anticancer effects in animals (Sundram *et al.,* 2003). Unrefined red palm oil contains around 11 carotenoids with variable profiles depending on species and is the richest source of β-carotene, a vitamin A precursor (Rice *et al.,* 2010). Carotenoids and tocotrienols exert antioxidant effects. Palm oil is also a significant source of phenolic compounds, especially phenolic acids with known antioxidant properties (Neo *et al.,* 2010). Numerous studies support the cardioprotective role of tocotrienols (Vasanthi *et al.,* 2012).

Additionally, histological sections revealed perivascular inflammatory infiltrates only in the pulmonary arteries of rats fed the sunflower oil diet (RHT). An infection appeared to have spread among these animals, leading to mortality in the RHT group, which showed symptoms prior to death. Similar symptoms were observed in rats fed palm oil diets but resolved after a few days, suggesting a potential immunomodulatory effect of palm oil.

CONCLUSION

At the end of this study, it can be concluded that the chemical parameters of refined palm oil are superior to those of sunflower oil. Palm oil promotes healthy weight gain without significantly increasing fat mass or the relative weight of organs. Moreover, microscopic sections of arteries confirmed these observations: the proportion of rats showing early stages of atherosclerosis was similar across all dietary groups.

In summary, there is no perfect oil, but our results demonstrate that the consumption of palm oil is no more harmful to the cardiovascular system than sunflower oil. Although the refining process removes certain major and potentially beneficial components from crude palm oil, it does not compromise the overall quality of the oil.

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