

Potential of red palm olein as an ingredient in designing functional food: effects on maintaining body weight and lipid profile of Wistar rats

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Article history:

Received: 27 November 2022

Revised: 21 April 2026

Accepted: 17 May 2026

Published: 27 May 2026

Keywords:

Red palm olein,
Wistar rat,
Atherogenic,
Lipid profile

DOI:

[https://doi.org/10.26656/fr.2017.10\(3\).583](https://doi.org/10.26656/fr.2017.10(3).583)

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Abstract

Red palm oil olein fraction (RPOn) is a derivative of palm oil products. RPOn has a good nutritional value such as carotene and tocopherol, which act like vitamins and antioxidants. The RPOn intervention shows antiatherogenic, antihypertensive, anticancer, and antidiabetic activity. This study aimed to determine the effect of red palm oil olein fraction (RPOn) intervention in standard and atherogenic (*ad libitum*) diets on the plasma lipid profile using Wistar rats as a model. The two types of diet conducted in this experiment comprised of a standard diet (by using Comfeed Finisher BR2) and atherogenic diet (prepared from two boiled duck egg yolks, 70 mL of pork oil, 1 g of cholic acid, and 300 g of complete cow feed). Wistar rats feeding on the two diet types were treated orally with and without RPOn (0.18 mL per 100 g of body weight) using a gastric probe. The treatments were replicated five times for each feeding type for five weeks. The rats' body weight was measured, and blood samples were collected at baseline (0 weeks) and after 1, 2, 3, 4, and 5 weeks. An ANOVA was conducted to analyze differences in body weight, total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) in rats treated with and without RPOn. The findings demonstrate the ability of RPOn to maintain body weight and lipid profile, indicating its potential as a functional food due to its richness in β -carotene and α -tocopherol. No significant differences ($p > 0.05$) were observed in body weight and total cholesterol between rats fed diets with and without RPOn after the fourth and fifth weeks of the experiment, respectively. A similar trend was observed for HDL, LDL, and triglyceride levels.

1. Introduction

Red palm oil (RPO) is derived from simple, refined crude palm oil (CPO), which is extracted from the mesocarp of palm (*Elaeis guineensis*) fruit. In Africa, which is the origin of the palm tree, RPO is usually produced by small-scale industries. The RPO is one of the primary fat sources and is known for its nutritional and healing potential.

The RPO has a balanced composition of saturated and unsaturated fatty acids in processed and unprocessed forms (Sundram *et al.*, 2003). It is rich in antioxidants and considered necessary in the diet for pregnant and lactating women (Khanna *et al.*, 2003; Jegede *et al.*, 2015; Sommerburg *et al.*, 2015; Emmanuel *et al.*, 2021). It also acts as an antiatherogenic, antihypertensive, anticancer, and antidiabetic, preventing vitamin A

deficiency (Sommerburg *et al.*, 2015; Emmanuel *et al.*, 2021). Antiatherogenic is the anti-narrowing of blood vessels. Several reports show that antioxidant compounds can increase the atherogenic index (Muruganandan *et al.*, 2005).

Besides containing α -carotene, β -carotene, and lycopene, the RPO contains at least 20 other carotenoids such as vitamin E, vitamin K, squalene, phytosterols, flavonoids, phenolic acids, and glycolipids. RPO is the richest source of vitamin E in the form of tocotrienols. These tocotrienols have an antioxidant activity 60 times that of regular vitamin E. The combination of vitamin E, carotene, and other antioxidants makes palm oil a super-antioxidant edible oil (Tomeo *et al.*, 1995; Nagendran *et al.*, 2000; Mba *et al.*, 2015).

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Refining palm oil into cooking oil (RBDPOil) goes through several processes, including degumming, neutralizing, bleaching, and deodorizing. First, the process is to get clear palm oil, then RBDPOil containing a liquid fraction (olein) and a solid fraction (stearin) is separated by fractionation into a liquid fraction (RBDPOlein) known as palm cooking oil and a solid fraction (RBDP Stearin).

Cooking oil security is very important as the world's demand increases over time, especially after February 2022, when Ukraine, one of the largest cooking oil producers, was at war. Indonesia experienced increasing cooking oil prices because cooking oil was unavailable in the local market. Red palm oil olein fraction (RPOn) is an alternative to solving the problem.

This fractionation technique is based on differences in each fraction's melting point and triglyceride composition (Mba *et al.*, 2015). To obtain RPOn, modifications to the purification process, process steps, and low temperatures without chemicals have been carried out. First, RPO was obtained from CPO by the water degumming method, followed by the fractionation and deodorization process to obtain RPOn (Falade *et al.*, 2015; Khaskheli and Chou, 2020).

This study offers the novelty of looking at the possibility of RPOn production and the potential of RPOn as a functional food in maintaining body weight and lipid profile in the body system in a regular diet, as well as against an atherogenic diet.

2. Materials and methods

2.1 Materials, animals, and ethics statement

Crude palm oil (CPO) and rats (*Rattus norvegicus*) were provided by PT Wilmar Nabati Indonesia at Gresik, East Java, and the Bioscience Laboratory at Brawijaya University, respectively. RPOn was self-prepared by the CPO at the laboratory. The standard diet (Comfeed finisher BR2 with 19% crude protein and 5% crude fat content) was provided by PT JAPFA Comfeed, Indonesia, while the atherogenic diet was self-prepared from two boiled duck egg yolks, 70 mL of pork oil, 1 g of cholic acid, and 300 g of complete cow feed. The lipid content of the atherogenic diet was 24.59%. The duck eggs were purchased from a traditional local market in Malang City. Pork oil and cholic acid were provided by the local market in Malang and Sigma-Aldrich, respectively. The complete cow feed (Susu PAP) was provided by PT JAPFA Comfeed Indonesia, which contains min 14% crude protein, max 7% fat, max 10% ash, 0.6-0.8% calcium, max 20% aNDF, and min 70% TDN. Egg yolk is rich in fat, consisting of 65.50% triglycerides, 5.20% cholesterol, and 28.30%

phospholipids, or contains about 270 mg of cholesterol/egg.

All experimental protocols involving animals have been approved by the Animal Care and Use Committee of Universitas Brawijaya (No: 1178-KEP-UB, 2019). Six-week-old Wistar white rats with body weights ranging from 100 to 160 g were used. All rats had *ad libitum* access to food and drinking water during the experiment, whereas red palm olein (RPOn) was administered orally using a gastric probe at a dose of 0.18 mL per 100 g of rats' body weight per day (at 08:00-11:00 h).

2.2 Experimental design and data analysis

The rat's cage was prepared using a wood shaving base. Feeding and drinking were conducted *ad libitum*. Rats were adapted for one week before the study. The effect of RPOn intervention was studied in two groups, using standard and atherogenic diets, each replicated five times. The effect of RPOn intervention in each type of feed on body weight and lipid profile was analyzed by ANOVA and continued by the LSD test.

2.3 Determination of free fatty acid, peroxide value, moisture content, α -tocopherol, and β -carotene

The FFA of the oil sample was determined using the American Oil Chemists' Society (AOCS) Official Method Ca 5a-40 (AOCS, 2017). Moisture content was determined using the AOCS Official Method Ca 2c-25 (AOCS, 2009). Peroxide value (SNI 3741:2013) α -tocopherol was estimated by dissolving the oil samples in ethanol, and absorbance was measured at 446 nm. The α -tocopherol content of the oil samples is expressed as mg α -tocopherol/kg of oil (ppm). Carotenoid was estimated by dissolving the oil samples in hexane, and absorbance was measured at 291 nm. The carotenoid content of oil samples is expressed as mg β -carotene/kg of oil (ppm)

2.4 Preparation of red palm olein

Red palm olein (RPOn) was made by degumming CPO using hot water at 60°C, then fractionated by letting it sit at room temperature for 24 h, and then separated with filter paper (450 μ m). The olein fraction was then deodorized at a vacuum pressure of 50 mmHg at 90°C for 3 h using a self-designed instrument (available as a data supplement). No chemicals were used in the RPOn preparation.

2.5 Body weight measurement, blood analysis, and liver histopathology

Body weight and blood samples were taken weekly for four weeks of the experiment from each experimental group. Total cholesterol levels were measured from

blood samples taken from the lateral tail vein using Nesco glucose, cholesterol, and uric acid (GCU) with a detection limit of 100-400 mg/dL. Meanwhile, at week 5, blood samples from the heart were taken to measure TC, LDL, HDL and TG using the enzymatic colorimetric method (Corso *et al.*, 2016) using a UV-Vis spectrophotometer. The absorbance was measured at 505 nm. The histopathological preparations of rat liver tissue were made in the fifth week, utilizing the standard hematoxylin and eosin (HandE) staining method. The observation was done using a microscope with 400× magnification.

3. Results and discussion

3.1 Characteristics of red palm olein

RPO_n produced using the simple purification method had a high nutritional value (Table 1). It contained approximately one-tenth of the β-carotene and

α-tocopherol contents found in RPO. RPO contains about 500 ppm of carotene, of which 90% is present as α- and β-carotene. Its vitamin E content is around 800 ppm, 70% of which is in the form of tocotrienols (mainly α-, β-, and δ-tocotrienols) (Nagendran *et al.*, 2000; Rao, 2000; Andreu-Sevilla *et al.*, 2009). According to May (1994), the Tenera, Pisifera, and Dura varieties contain 56.02, 54.39, and 56.02 ppm β-carotene, respectively. These results indicate that the oil palm contents used in this study were not different from those reported in other studies.

During the experimental period, the growth of rats based on body weight showed the same pattern in each treatment, which tended to increase (Table 2). These results indicated that the condition of the rats during the experiment was quite good, where the rations consumed could be used by the body for growth. According to Rebecca *et al.* (2014), high-fat diets significantly

Table 1. The Chemical content of red palm olein (RPO_n) used in this study.

Component	RPO _n *	May (1994)	Hasibuan and Ijah (2018)	Widarta <i>et al.</i> (2012)	Nagendran <i>et al.</i> (2000)	Abdullah <i>et al.</i> (2018)
Water content (%)	0.20±0.02			0.58		
FFA as palmitate (%)	0.15±0.02		0.71	0.13	0.04	
Carotene (ppm)		673.00	263.00	464.96	513.00	
β-Carotene (ppm)	57.54±0.04	56.02				
α-Tocopherol (ppm)	100.87±0.61				166.00	
Peroxide value (mE/kg)	0.87±0.11			2.20	0.10	
DPPH (IC ₅₀) (ppm)	7861.9					12050

Values are presented as mean±SD of 5 replications. The data was compared to the reported chemical content of RPO_n.

Table 2. Effect of red palm olein (RPO_n) on body weight of Wistar rats.

Treatment	Week				
	0	1	2	3	4
a. Rat body weight (g) at each week					
Standard diet					
SDWO	110.80±4.66	119.20±4.15	124.0±3.94	130.40±22.28 ^b	140.00±20.85 ^b
SDW	111.20±13.32	120.80±13.92	131.60±14.41	141.40±14.99 ^{ab}	159.00±10.78 ^{ab}
Atherogenic diet					
ADWO	132.14±26.38	139.40±26.12	147.80±23.79	156.80±14.48 ^a	164.60±17.41 ^a
ADW	132.55±14.43	139.60±17.56	149.40±19.22	160.80±4.22 ^a	171.60±6.74 ^a
b. Average of rat body weight and gain during the four weeks of experiments					
	Weight of Rat (g), (mean±SD)*		Weight gain of rat (g), (mean±SD)**		
Standard diet					
SDWO	124.88±9.90		7.30±2.13 (6.59%)		
SDW	132.80±16.57		11.95±3.80 (10.75%)		
Atherogenic diet					
ADWO	148.15±11.65		8.11±0.75 (6.14%)		
ADW	150.79±14.09		9.76±1.92 (7.36%)		

Values are presented as mean±SD of 5 replications. Values with different superscripts in the same column are statistically significantly different (p<0.05), as analyzed by ANOVA and LSD test. *Data were calculated from 0-4 weeks of the experiment. **Data were the mean of the gain of each week. SDWO: Standard diet without RPO_n, SDW: Standard diet with RPO_n, ADWO: Atherogenic diet without RPO_n, ADW: Atherogenic diet with RPO_n.

increase rats' body weight. Weight gain was also caused by the intake of fat and other nutrients in the rat feed.

Until the second week of the experiment, the rats' body weights on the two different diets were not significantly different ($p > 0.05$) (Table 2, columns 2–4). Body weights began to diverge after the third week of the experiment (Table 2, columns 5–6); however, no significant differences were observed among the body weights of rats receiving RPOn treatment by diet type (standard and atherogenic diets). RPOn treatment did not significantly affect the average body weight or weekly weight gain of the rats (Table 2, column 7). These findings indicate that the administration of RPOn did not significantly contribute to increased body weight in rats, suggesting that RPOn can be used as an ingredient in functional foods due to its ability to maintain body weight and its antioxidant properties.

Data in Table 2 (column 8) show that a standard diet with RPOn increased the rats' body weight in the fourth week by 6.59%, whereas a diet without RPOn increased body weight by 10.75%. In contrast, under the atherogenic diet, RPOn appeared effective in maintaining weight gain at 7.36%, while a diet without RPOn increased body weight by 6.14%. These findings highlight the importance of RPOn consumption, particularly given that most modern diets are generally high in fat (Mozaffarian *et al.*, 2014; Hooper *et al.*, 2015). In this study, the fat content of the atherogenic diet was five times greater than that of the standard feed.

Based on a review by a group of researchers, there is insufficient evidence to demonstrate the impact of palm oil intake on changes in body weight or body mass index (BMI) (Muhamad *et al.*, 2018). Rats with different thyroid statuses showed no significant differences in body weight (Rauchová *et al.*, 2018), regardless of the presence of RPOn in their diets. In contrast, Widjaja *et al.* (2019) reported that supplementation with palm oil in rats can increase BMI, abdominal circumference, and fat mass. Effect of red palm olein on lipid profile

Table 3 presents the effect of RPOn treatment on the total cholesterol of rats having a standard and an atherogenic diet during this experiment (four weeks). Starting at the second week of the experiment, the cholesterol level of rats having a standard diet with RPOn (SDW) was significantly higher ($p < 0.05$) than the rats having a standard diet without RPOn (SDWO). However, the average and the increase in cholesterol levels each week were not significantly different ($p > 0.05$). This finding showed that RPOn is a candidate for functional food ingredients in an antiatherogenic diet.

Consumption of RPOn can raise cholesterol (to

9.44% at week 4) compared to a standard diet, which decreases by 1.05%. However, the RPOn was shown to suppress the increase in total cholesterol while fed the atherogenic diet. The total cholesterol in rats fed an atherogenic diet without the RPOn increased by 55.99% in the fourth week, while those fed an atherogenic diet with the RPOn only increased by 39.41%. This fact makes the consumption of RPOn again crucial because most diets today are generally rich in fat (Mozaffarian *et al.*, 2014; Hooper *et al.*, 2015).

An atherogenic diet is a high-fat diet that consistently increases plasma cholesterol levels. An atherogenic diet containing egg yolk and lard elevated rats' cholesterol levels and body weight (Barona and Fernandez, 2012; Widjaja *et al.*, 2019). The increase in cholesterol levels was attributed to the high cholesterol and saturated fatty acid content of the atherogenic feed. In contrast, rats fed an atherogenic diet with RPOn (ADW) showed different outcomes. RPOn supplementation significantly decreased the cholesterol levels of the rats (Table 3, columns 3–6). These findings indicate that RPOn mitigates the adverse effects of the atherogenic diet. The results are consistent with those of French *et al.* (2002), who reported that a high palmitic acid content in food does not significantly affect total cholesterol (Mancini *et al.*, 2015). In addition to palmitic acid, vitamin E (tocopherol) can regulate plasma cholesterol levels by inhibiting the activity of the HMG-CoA reductase enzyme, which controls cholesterol synthesis in the liver (Sundram *et al.*, 2003). This aligns with studies reporting that tocotrienols can inhibit cholesterol synthesis in vivo (Mukherjee and Mitra, 2009; EL-Hak *et al.*, 2019; Sufarnap *et al.*, 2021). Loganathan *et al.* (2017) documented various health-enhancing effects of RPO in both human and animal studies, including antiatherogenic, antihemorrhagic, antihypertensive, anticancer, and anti-infective properties. Emmanuel *et al.* (2021) further demonstrated that RPO treatment can mitigate the toxic effects of CCl_4

The finding that RPOn treatment could significantly maintain serum cholesterol levels was demonstrated by the assay of total cholesterol in rats after the fifth week of the experiment, using an enzymatic colorimetric method (Table 4). The total cholesterol levels of rats fed an atherogenic diet with RPOn treatment (ADW) were significantly lower ($p < 0.05$) than those of rats fed an atherogenic diet without RPOn (ADWO).

RPOn treatment in the standard diet of rats resulted in higher LDL levels compared to those without RPOn. However, under the atherogenic diet, LDL levels in blood serum did not differ significantly between rats with RPOn (ADW) and those without RPOn (ADWO). In addition, triglyceride levels were not significantly

Table 3. Effect of red palm olein (RPOn) intervention on total cholesterol levels (mg/dL) from week 0 to week 4 in rats having a standard and atherogenic diet.

Treatments	Week					Average
	0	1	2	3	4	
Standard diet						
SDWO	133.40±20.95	140.00±9.55 ^b	140.00±12.05 ^d	139.40±14.98 ^d	132.00±27.26 ^d	136.96±3.93
SDW	142.00±11.34	145.20±3.92 ^b	161.00±2.70 ^c	159.80±13.37 ^c	155.40±15.41 ^c	152.68±8.62
Atherogenic diet						
ADWO	148.60±16.10	158.20±1.58 ^a	264.40±1.58 ^a	258.00±1.34 ^a	231.80±13.27 ^a	212.20±55.15
ADW	128.40±20.98	147.40±4.92 ^b	182.60±3.94 ^b	199.80±3.70 ^b	179.0±2.88 ^b	167.44±28.88

a. Average of total cholesterol increase

	Increases per week	
	Total increases from week 0 to 4	
Standard diet		
SDWO	-0.35±5.72	-1.40 (-1.05%)
SDW	3.35±8.87	13.40 (9.44%)
Atherogenic diet		
ADWO	20.80±58.79	83.20 (55.99%)
ADW	12.65±23.72	50.60 (39.41%)

Values are presented as mean±SD. Values with different superscripts in the same column are statistically significantly different ($p < 0.05$), as analyzed by the LSD test. Cholesterol level was measured by rapid test for cholesterol analysis (GCU). SDWO: Standard diet without RPOn, SDW: Standard diet with RPOn, ADWO: Atherogenic diet without RPOn, ADW: Atherogenic diet with RPOn.

Table 4. Effects of five weeks of RPOn treatment on cholesterol and triglyceride levels of rats having a standard and atherogenic diet.

Treatment	Total cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Triglycerides (mg/dL)
Standard diet				
SDWO	50.00±5.05 ^c	25.00±3.39 ^b	11.00±3.74 ^b	70.00±10.61
SDW	56.00±2.12 ^c	22.00±2.92 ^b	16.50±2.78 ^a	61.00±8.09
Atherogenic diet				
ADWO	118.00±5.48 ^a	35.00±4.90 ^a	8.00±2.92 ^b	57.00±8.72
ADW	103.00 ^b ±7.38 ^b	23.00±4.06 ^b	12.10±3.68 ^{ab}	61.00±9.62

Values are presented as mean±SD. Values with different superscripts in the same column are statistically significantly different ($p < 0.05$) as analyzed by ANOVA and LSD test. All parameters were analyzed from blood serum using the enzymatic colorimetric method. SDWO: Standard diet without RPOn, SDW: Standard diet with RPOn, ADWO: Atherogenic diet without RPOn, ADW: Atherogenic diet with RPOn.

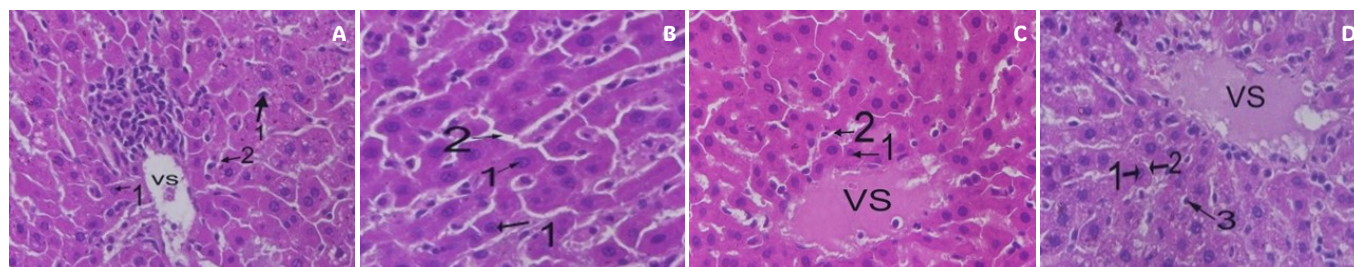


Figure 1. Histopathological description of the rat liver with Hematoxylin and Eosin staining. Magnification of 400×. (A) SDWO: Standard diet without RPOn, (B) SDW: Standard diet with RPOn, (C) ADWO: Atherogenic diet without RPOn, (D) ADW: Atherogenic diet with RPOn. 1: normal liver cells, 2: sinusoid, 3: liver cells are fatty, VS: vena centralis.

different ($p > 0.05$) between diets with or without RPOn. A study conducted in China comparing palm oil, soybean oil, peanut oil, and lard showed that palm oil could increase high-density lipoprotein (HDL) and reduce low-density lipoprotein (LDL) in the blood (Zhang *et al.*, 2003). The results obtained in this study differed from those reported by Karaji *et al.* (2006), who used Wistar rats fed a diet containing 12% palm oil for

60 days and observed significant differences (Alasia *et al.*, 2020; Edem and Akpanabiatu, 2006; Górnicka *et al.*, 2019). Figure 1 illustrates the potential of RPOn to maintain fatty liver levels in rats fed standard or atherogenic diets with and without RPOn.

There are many reports about the fact that consumption of RPOn does not improve cholesterol

levels, resulting in cardioprotective benefits. Moreover, tocopherols and tocotrienols can potentially act as anticancer, antidiabetic, anti-inflammatory, and antithrombotic (Sulaiman *et al.*, 2022). However, until now, the mechanism regarding RPO consumption and its health effects has been challenging.

4. Conclusion

RPO has potential as a functional food due to its *b*-carotene and *a*-tocopherol content, antioxidant capacity, and ability to maintain body weight and lipid levels in the Wistar rat model.

Conflict of interest

The authors declare no conflict of interest

Acknowledgments

The authors thank Prof. Dr. oec. troph. Ir. Krishna Purnawan Candra, M.S, Mulawarman University, advises the writing of this manuscript.

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