

Full Length Research Paper

Effects of dietary intake of red palm oil on fatty acid composition and lipid profiles in male Wistar rats

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Little is known about the effects of the dietary intake of red palm oil (RPO) on fatty acid composition in the liver of rats. Male Wistar rats randomly divided into four groups were fed with different doses of red palm oil. The control group received no red palm oil; while the experimental groups were fed with 1 ml, 2 ml and 4 ml of red palm oil daily for seven weeks. In the liver of all the groups, palmitic acid (C16:0) followed by stearic acid (C18:0) were predominantly present among the saturated fatty acids. Oleic acid (C18:1c) and linoleic acid (C18:2) were largely present among the unsaturated fatty acids. There was no significant ($P<0.05$) increase in the levels of palmitic acid (C16:0) in all the groups while oleic acid (C18:1) significantly increased at 4 ml RPO when compared with the control ($p<0.05$). The total cholesterol (TC), triglycerides (TG) and very low-density lipoprotein (VLDL)-cholesterol levels were not significantly different in all the groups ($P<0.05$) when compared with the control group. Generally, there were no significant effects of RPO on levels of serum cholesterol, and triglycerides as well as accumulation of saturated fatty acids in the liver of the experimental rats.

Key words: Lipid profiles, fatty acid, red palm oil, rats.

INTRODUCTION

Red palm oil (RPO) has a deep orange-red colour and is extracted from the mesocarp of fruits of palm oil trees (*Elaeis guineensis*). All over the world, 90% of the RPO produced is used for edible purposes (Idris and Samsuddin, 1993; Edem, 2002). Red palm oil contains a variety of antioxidant vitamins necessary for maintaining good health (Bayorh, 2005). It is a good source of vitamin A (carotenes) (Sundram et al., 2003; Arora et al., 2006; Oguntibeju et al., 2010; Aboua et al., 2011) and vitamin E (tocopherols and tocotrienols) (Sundram et al., 2003; Arora et al., 2006; Muharis et al., 2010) and these are capable of scavenging free radicals thus preventing the damaging effects of oxidation in tissues. The

characteristic colour of RPO is as a result of the abundance of carotenoids (500 - 700 mg/L) in the crude oil (Edem and Akpanabiatu, 2006; Edem, 2009). The combined effect of carotenoids, tocopherols, tocotrienols and 50% of the unsaturated fatty acids gives palm oil a higher oxidative stability as compared to other vegetable oils (Arora et al., 2006). Red palm oil supplies fatty acids that are important for proper growth and development. Fatty acids play a vital role in metabolism because they are the building blocks of fat in the body and in food. They are a source of energy for the cell and form the structural basis of the cell. Red palm oil contains 50% saturated, 40% monounsaturated and 10% polyunsaturated fatty acids (Rukmini, 1994; Edem, 2002). From the nutritional point of view, the major concern for RPO has to do with their degree of saturation and the effect they have on blood lipids (Hayes and Khosla, 2007). Palmitic and stearic acids are saturated fatty acids which account for 45 and 5% of total fatty acids in red palm oil respectively (Hayes and Khosla, 2007; Dauqan

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Abbreviations: RPO, Red palm oil; TC, total cholesterol; TG, triglycerides; VLDL, very low-density lipoprotein.

Table 1. Nutritional composition of carotino red palm oil.

Serving size: 1 tablespoon 14 g	Per 100 ml	Per 14g serving
Energy	3400 KJ	510 KJ
Total fat	92 g	14 g
Monounsaturates	43 g	6.5 g
Polyunsaturates	12 g	1.9 g
Saturates	37 g	5.6 g
Trans fat	0 g	0 g
Cholesterol and Sodium	0 mg	0 mg
Protein, carbohydrate and dietary fibre	0 g	0 g
Natural carotenes	46 mg	7.0 mg
Beta carotene	22 mg	3.3 mg
Alpha carotene	17 mg	2.6 mg
Other carotenes	7.3 mg	1.1 mg
Natural vitamin E:	74 mg	11 mg
19.5% Tocopherols		
80.5% Tocotrienols		
Co- Enzyme Q10	4.0 mg	0.6 mg

Source: Table adapted from the nutritional label of the Carotino Palm Fruit Oil from Malaysia.

et al., 2011). More than 95% of palm oil consists of mixtures of triglycerides, each esterified with three fatty acids (Akinola et al., 2010). The various types of dietary lipids have shown to affect lipid metabolism differently (Ajayi and Ajayi, 2009). Wu et al. (2011) reported that dietary lipids directly affect fatty acids composition in animal tissues. The aim of this study was to investigate the levels of fatty acids and lipid profiles in rats following the dietary intake of red palm oil at different doses.

MATERIALS AND METHODS

Experimental animals and management

Male Wistar rats (195- 240 g) were obtained from Stellenbosch University, Tygerberg, South Africa and used throughout the study. The study was conducted after obtaining Ethical Committee Clearance from Cape Peninsula University of Technology. The rats were housed in a well controlled environment set at 22°C±2 with 50±5% humidity and a 12 h light cycle. They were randomly placed in four groups. Group 1 (n=5) received no supplementation and served as the control while groups 2 (n=6), 3 (n=6) and 4 (n=6) received 1 ml, 2 ml and 4 ml red palm oil (RPO) respectively. Each group of rats was allowed free access to water and standard rat chow (SRC) for seven weeks. Carotino palm fruit oil from Malaysia at different doses (1ml, 2ml and 4ml) was added to the standard rat chow daily diet of the experimental animals for seven weeks. The nutritional composition of the red palm oil is shown in Table 1. At the end of the seven weeks, all the animals were sacrificed using euthanasia after overnight fasting. Blood samples were collected from the abdominal aorta and then centrifuged to obtain the serum used for lipid analysis while the liver was removed for fatty acid determination.

Fatty acid determination

Fatty acids determination was carried out by the modified method of

Association of Official Analytical Chemists (AOAC) (2005). The liver samples were placed on the vortex to achieve homogeneity. Liver samples ranging from 0.4 to 1 g were weighed into 70 ml digestion tubes and 100 mg pyrogallol acid was added followed by 2 ml of undecanoic acid (internal standard) solution, 2 ml of ethanol and 10 ml of 32% hydrochloric acid. The tubes were then placed in the water bath at 75°C with gently shaking for 40 min. The fatty acids were extracted by adding 25 ml of diethyl ether and 25 ml of petroleum ether. The organic phase was dried and the residue was derivatized using 2 ml of 2% sulphuric acid in methanol and 1 ml of toluene at 100°C for 45 min. After cooling to room temperature, 5 ml distilled water and 1 ml of hexane were added and the hexane solution was then dried with anhydrous sodium sulphate and transferred into a vial for gas chromatographic analyses.

Lipid profile determination

Triglycerides, total cholesterol and high density lipoprotein (HDL)-cholesterol were evaluated with kits using a clinical chemistry analyzer (EasyRa medical) according to the manufacturer's instructions. Very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL)-cholesterol were calculated according to Friedewald's formula (Friedewald et al., 1972). VLDL-cholesterol = TG/5 and LDL-cholesterol = TC - VLDL-cholesterol - HDL-cholesterol.

Statistical analysis

Data were expressed as the means ± standard deviations (S.D). Significant differences between mean values of different groups were determined by one-way analysis of variance (ANOVA) with Turkey's test using GraphPad Prism 5. Differences were considered significant at p<0.05.

RESULTS AND DISCUSSION

Table 2 indicates the percentage of body weights gain in

Table 2. Body weights gain in the rats fed with different doses of red palm oil (RPO).

RPO Dosage (ml)	Initial weight(g)	Final weight(g)	% Body weight gain (%)
0	225 ±11.79	352 ±18.43	56 ±4.02
1	222 ±10.52	360 ±29.93	62 ±6.48
2	212 ±11.31	359 ±21.26	69 ±4.37*
4	214 ±17.25	387 ±26.62	80 ±6.04*

(*) Indicates significant difference from control group at $p < 0.05$.

Table 3. Total fatty acids (g/100g) in the liver of rats fed with different doses of RPO.

RPO Dosage (ml)	Total fatty acid
0	1.136 ±0.0950
1	1.176 ±0.1383
2	1.131 ±0.1806
4	1.245 ±0.1025

(*) Indicates significant difference from control group at $p < 0.05$.

Table 4. Levels of saturated fatty acids (g/100g) in the liver of rats fed with different doses of RPO.

RPO Dosage (ml)	C14	C15	C16	C17	C18	C24
0	0.003±0.0004	0.004±0.0005	0.298±0.0209	0.010±0.0011	0.254±0.0118	0.008±0.0007
1	0.004±0.0003	0.003*±0.0006	0.313±0.0350	0.007*±0.0005	0.243±0.0242	0.008±0.0005
2	0.003±0.0006	0.002*±0.0000	0.300±0.0441	0.005*±0.0004	0.228±0.0129	0.007*±0.0005
4	0.003±0.0005	0.002*±0.0005	0.330±0.0219	0.004*±0.0005	0.222*±0.0175	0.006*±0.0006

(*) Indicates significant difference compared with control group at $p < 0.05$.

the rats fed with different doses of RPO. There was a significant increase in the body weights gain in both 2 ml and 4 ml RPO fed groups when compared with the control group. Table 3 indicates the total fatty acids in the liver of rats fed with different doses of RPO. There was no significant difference in the total fatty acids in all palm oil fed groups when compared with the control group.

The levels of saturated fatty acids in the liver of rats fed with different doses of red palm oil are indicated in Table 4. The two most abundant saturated fatty acids in the liver of all the groups were palmitic acid (C16) and stearic acid (C18). The values of palmitic acid were not significantly different in all RPO fed groups when compared with the control. Stearic acid was significantly lower for the 4 ml RPO group only when compared to the control. Other saturated fatty acids present were myristic acid (C14), pentadecylic acid (C15), margaric acid (C17) and lignoceric acid (C24). No significant differences were noted for myristic acid for any of RPO fed groups. Pentadecylic acid (C15) and margaric acid (C17) significantly decreased in the all palm oil fed groups while C24 significantly decreased at 2 ml and 4 ml RPO when compared with the control group.

The levels of unsaturated fatty acids in the liver of rats fed with different doses of red palm oil are indicated in Table 5. The two most abundant liver unsaturated fatty acids in all the groups were oleic acid (C18:1c) and linoleic acid (C18:2). There was significant increase in the values of C18:1 at 4 ml RPO and the values of C18:2 at 2 ml and 4 ml RPO significantly decreased when compared with the control. Other unsaturated fatty acids present in minute amounts were elaidic acid (C18:1t), linolenic acid (C18:3) and docosahexaenoic acid (DHA) (C22:6). There was significant decrease in C18:1t and C18:3 levels in all experimental groups when compared with the control group. The level of C22:6 were significantly reduced for the 4 ml RPO fed group when compared with the control group.

The serum lipid profiles of rats at different doses of palm oil are indicated in Table 6. There were no significant differences in the serum total cholesterol, triglycerides and VLDL-cholesterol when compared with the control group. There was a significant difference in the level of HDL-cholesterol at 1 ml RPO fed group and a significant decrease in LDL-cholesterol at 4 ml RPO fed group when compared with the control group.

Table 5. Levels of unsaturated fatty acids (g/100g) in the liver of rats fed with different doses of palm oil.

RPO Dosage (ml)	C18:1t	C18:1c	C18:2	C18:3	C22:6
0	0.020±0.0018	0.162 ±0.0279	0.302 ±0.0392	0.009 ±0.0024	0.065 ±0.0073
1	0.015*±0.0016	0.249 ±0.0847	0.265 ±0.0289	0.006 ±0.0017*	0.064 ±0.0038
2	0.011* ±0.0008	0.278 ±0.0898	0.224 ±0.0340*	0.004 ±0.0011*	0.060 ±0.0040
4 (n=5)	0.009* ±0.0005	0.386 ±0.0470*	0.229 ±0.0193*	0.003 ±0.0005*	0.052 ±0.0043*

(*) Indicates significant difference compared with control group at $p < 0.05$.

Table 6. The lipid profiles in the serum of the rats at different doses of palm oil.

RPO Dosage (ml)	Total Cholesterol (mmol/L)	Triglycerides (mmol/L)	HDL-Cholesterol (mmol/L)	VLDL-Cholesterol (mmol/L)	LDL-Cholesterol (mmol/L)
0	1.70± 0.07	0.57± 0.14	0.48 ±0.05	0.11 ±0.03	1.11 ±0.06
1	1.92 ±0.26	1.07 ±0.49	0.60 ±0.05*	0.21 ±0.10	1.10 ±0.15
2	1.65 ±0.09	0.53 ±0.12	0.54 ±0.04	0.11±0.02	1.01 ±0.08
4	1.62 ±0.11	0.79 ±0.28	0.55 ±0.07	0.16 ±0.06	0.91 ±0.11*

(*) means significantly different compared to with control at $p < 0.05$. HDL, High density lipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein.

Total cholesterol is made up of LDL cholesterol, HDL cholesterol, and VLDL cholesterol and increased levels of LDL increase the risk of heart disease and stroke while high levels of HDL reduce the risk of cardiovascular disease (Birtcher and Ballantyne, 2004). Hayes and Khosla (2007) also reported that circulating cholesterol is linked to heart disease and can serve as a relevant index of our nutritional well-being that is sensitive to fat intake and composition. The vital lipids whose increase are implicated in the hindrance of blood supply to the heart, brain, liver or kidney and could cause coronary heart diseases, stroke or kidney failure are cholesterol and triacyl-glycerols (Owolabi et al., 2010). Yuan et al. (2007) reported that high levels of triglycerides could contribute independently to increased risk of cardiovascular disease and severe hypertriglyceridemia is also associated with an increased risk of acute pancreatitis. Oguntibeju et al. (2009) reported that the link between dietary fats and cardiovascular disease has created an increasing interest in dietary red palm oil research. The intake of saturated fatty acids increased total cholesterol, LDL and HDL while polyunsaturated fatty acids in fats decreased these values (Hayes and Khosla, 2007).

Dauqan et al. (2011) showed a significant decrease in cholesterol in animals fed with red palm olein. Red palm oil supplementation has been reported to have beneficial or neutral effects on serum total cholesterol despite its high saturated fat content (Kruger et al., 2007). Our results indicate that RPO does not significantly increase cholesterol and triglycerides levels in RPO fed rats after seven week feeding period. Ajayi and Ajayi (2009) reported that both polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) could have an effect on lipoprotein metabolism with hypocholes-

terolemic effect. Palm oil contains only 0.2% lauric acid (Kochikuzhyil et al., 2010) and a high quantity of palmitic acid as well as considerable amounts of oleic and linoleic acids (Edem, 2002). Lauric and palmitic acids are hypercholesterolemic when compared with oleic acid while lauric acid increased cholesterol levels more than palmitic acid (Temme et al., 1996). Similarly, Sundram et al. (1994) reported that the dietary combination of lauric and myristic fatty acids increased serum cholesterol than palmitic acid in healthy normocholesterolemic men fed with low cholesterol diet. Red palm oil contains equivalent amounts of saturated and unsaturated fatty acids (Oguntibeju et al., 2010). Our results showed no abnormal retention of saturated fatty acids in the liver of the rats. Palmitic acid which is known to be largely present among the saturated fatty acids in RPO did not increase significantly in the liver of the RPO fed groups when compared with the control group.

In conclusion, the dietary intake of RPO did not result in accumulation of saturated fatty acids in the liver. Also, it did not significantly alter the serum levels of both cholesterol and triglycerides levels and it could have the potential to reduce the levels of bad cholesterols and triglycerides especially in diseased conditions. Hence, further investigations are recommended as RPO could help to lower the risk of atherosclerosis and other related diseases.

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