Chronic Intake of Red Palm Olein and Palm Olein Produce Beneficial Effects on Plasma Lipid Profile in Rats

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Abstract: Palm olein (PO) and red palm olein (RPO) are rich in tocopherols and tocotrienols. In addition, RPO also contains a high content of carotene. This study was to determine the effect of chronic intake of diets containing palm oils, varying in their vitamin E and carotene contents, on lipid profile in rats. Weaning male Wistar rats were fed either 18% RPO, 18% PO or 18% vitamin E-stripped palm olein (SPO) for 12 weeks. Plasma total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL) and low density lipoprotein cholesterol (LDL) were measured at weeks 4, 8 and 12. Feeding the different types of palm oil did not affect TC and HDL from week 4 through week 12, but there were reductions in TG in all dietary groups at week 12 compared to week 4 but differences between groups were not observed. The RPO group had lower LDL at week 12 (vs weeks 4 and 8) but LDL was not reduced in the PO and SPO groups. TC/HDL was reduced in the RPO group at week 12 compared to both weeks 4 and 8, but the PO group only reduced this ratio at week 12 compared to week 4. This finding suggests that chronic feeding of diets high in palm oils did not cause any detrimental effects on blood lipid profile. In addition, red palm olein which is rich in antioxidants in the forms of vitamin E and carotene, showed better effect in terms of reduction in LDL and TC/HDL.

Key words: Palm oil, tocotrienol, tocopherol, blood lipid profile

Introduction
Palm oil is obtained from a tropical plant, Elaeis guineensis and commonly used as a cooking oil in Malaysia, as well as in many parts of the world (Ong and Goh, 2000). It has an equal proportion of unsaturated and saturated fatty acids content. Palmitic (44%, saturated) and oleic (39%, monounsaturated) acids are two major fatty acids present in the oil. It also contains stearic (5%, saturated), linoleic (10%, polyunsaturated), linolenic (0.3%, polyunsaturated), lauric (0.1%, saturated) and myristic (0.1%, saturated) acids (Cottrell, 1991). High intake of palmitic acid in the diet was reported to increase plasma total cholesterol and low density lipoprotein cholesterol (Denke and Grundy, 1992), whereas oleic acid has a neutral effect on plasma cholesterol (Grundy, 1994). On the other hand, polyunsaturated fatty acids (PUFA) such as linoleic and linolenic acids, have hypocholesterolemic properties (Purushothama et al., 1994). Despite its high content of palmitic acid, many studies so far have documented that palm oil intake showed comparable effect on lipid profile to groundnut oil (high in oleic and linoleic acids) (Ghafoorunissa et al., 1995) and high oleic sunflower oil (Choudhury et al., 1997). The refined, bleached and deodorized (RBD) palm oil, palm olein, is rich in natural antioxidants ie tocopherols and tocotrienols (tocols). However, the unbleached palm oil, so-called red palm olein is also abundant of α- and β-carotenes, in addition to the higher content of the tocols (Ong and Goh, 2000). The tocols and carotenes have been shown to exhibit good antioxidant properties (Kamisah et al., 2000; Panasenko et al., 2000) and enhance immune response (Gu et al., 1997; Wood et al., 1999) in many studies. The tocols have also been reported to have antitumor properties (Nesaretnam et al., 1998) and to be antiplatelet possibly by increasing the ratio of the prostacyclin/thromboxane (Nolan et al., 1995). Unlike tocopherol, tocotrienol is claimed as a potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, a rate-limiting enzyme in cholesterol biosynthesis and thus, lowers serum cholesterol (Raederstorff et al., 2002). Both crude and refined palm oils were shown to reduce total and low density lipoprotein cholesterol, as well as the ratio of total to high density lipoprotein cholesterol (Niyongabo et al., 1999; Zhang et al., 1997a). Supplementation of both α-tocopherol and β-carotene showed a beneficial effect on tissue cholesterol content and development of atherosclerotic lesions in rabbits fed an atherogenic diet for 8 weeks (Sulli et al., 1998).
study by Kritchevsky et al. (2000) demonstrated that red palm olein was significantly less atherogenic than the RBD palm oil, supporting the hypothesis that carotenoids and vitamin E in the palm oil might protect against atherosclerosis, even though both edible oils had similar effects on serum and liver lipids. Therefore, this study was designed to investigate the effect of chronic intake of palm oil-containing diets, which vary in their vitamin E and carotene contents on lipid profile in rats, to determine whether the cholesterol-lowering effect of the oils is contributed by their antioxidant contents.

Materials and Methods

Animals, diets and study design: Weaning male Wistar rats (50-70 g) (Laboratory Animal Source Unit, Faculty of Medicine, Universiti Kebangsaan Malaysia) were either fed 15% red palm olein (RPO), palm olein (PO) or vitamin E-stripped palm olein (SPO) diet for 12 weeks. The rats were given free access to food and water. They were housed individually in polyethylene cages with mesh wire bottom. The rats were fasted overnight before blood was withdrawn via periorbital vein under ether anaesthesia, at weeks 4, 6 and 12 for plasma lipid profile determination. The experimental and animal handling procedures were approved by the Research Committee of Faculty of Medicine, Universiti Kebangsaan Malaysia.

Diets were prepared manually in the laboratory and mixed thoroughly using a mixer, and thereafter were pelleted. The diets were then left to dryness at room temperature overnight and stored at -20°C. Red palm olein was obtained from Malaysian Palm Oil Promotion Council (MPOPC) and palm olein (Cap Buruh brand) was obtained commercially. Individual components of the diet was purchased either from United States Biochemicals (Cleveland, OH, USA) or Sigma Chemical Co. (St. Louis, MO, USA). The composition of the diets was tabulated in Table 1. The source of vitamin E and carotene in the diets was solely from the added palm oils.

Plasma lipid profile analysis: Plasma total cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDL) were determined by enzymatic technique using commercial kits (Boehringer Mannheim GmbH, Germany). Plasma HDL was analysed after sodium phosphotungstate-Mg++ precipitation of apoB and apoE containing lipoproteins, after which the HDL content of the supernatant was measured by the same cholesterol enzymatic kit. Plasma low density lipoprotein cholesterol (LDL) was calculated from TC, HDL and TG values using the Friedwald equation (Friedwald et al., 1972).

Analysis of vitamin E: Vitamin E in the oils, diet and liver were extracted as previously described (Podda et al., 1996) with some modifications. Briefly, 100 mg sample were homogenized in a tube containing 50 μl ethanolic butyraldehyde hydroxytoluene (10 mg/ml) and 1 ml distilled water. One ml of sodium dodecyl sulfate (0.1 M) was then added into the homogenates. After addition of 1 ml ethanol, the homogenates were extracted with 3 ml hexane. An appropriate aliquot was dried down using vacuum concentrator (Heto Lab Equipment, Denmark) and reconstituted in hexane. The vitamin E in hexane lipid extract (20 μl sample) was analysed using an analytical high performance liquid chromatography (HPLC; Gilson 714). The stationary phase was a 250 mm Spherisorb 5 silica normal phase column, internal diameter 4.6 mm and particle size 5 μm, protected by a guard column (2 mm x 4.6 id mm). The mobile phase was hexane : isopropanol (99:1) at a flow rate of 1.5 ml/min. The column effluent was monitored with a fluorescence detector (Spectra System FL2000), set at 295 nm (excitation wavelength) and 330 nm (emission wavelength).

Statistical analysis: Results are expressed as mean with their standard error. Statistical analysis was performed by one way ANOVA followed by Tukey’s multiple comparison test. Values of P<0.05 were

<table>
<thead>
<tr>
<th>Component</th>
<th>SPO</th>
<th>PO</th>
<th>RPO</th>
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<tbody>
<tr>
<td>Sucrose</td>
<td>350</td>
<td>350</td>
<td>350</td>
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<tr>
<td>Vitamin-free casein</td>
<td>200</td>
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<tr>
<td>Corn starch</td>
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<td>Cellulose</td>
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<td>Vitamin E-free vitamin</td>
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<td>Vitamin E-stripped corn</td>
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<tr>
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<td>Choline bitartrate</td>
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<td>Palm olein</td>
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<td>180</td>
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<tr>
<td>Red palm olein</td>
<td>-</td>
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<td>180</td>
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SPO, stripped palm olein; PO, palm olein; RPO, red palm olein.

Table 1: Composition of the experimental diets (g/kg diet)
considered statistically significant. All statistical analyses were performed using GraphPad Prism 2.1 software (1997; GraphPad Software Inc., San Diego, CA, USA).

Results

Toccols levels in the oils and diets: Five vitamin E isomers were detected in the palm oils i.e. α-, γ- and δ-tocopherols, as well as α-, γ- and δ-tocotrienols (Table 2). Overall, red palm olein had the highest content of vitamin E, whilst stripped palm olein was shown to contain very little vitamin E. α-Tocopherol was the major component of vitamin E in both red palm olein and palm olein, followed by α-, γ- and δ-tocotrienols. The tocotrienols represented 64% and 71% of the total vitamin E in the red palm olein and palm olein respectively.

Table 3 shows vitamin E content in the synthetic diet that had been prepared. As expected, red palm olein diet (RPO) had the highest content of vitamin E, followed by palm olein diet (PO), whereas almost completely absent in stripped palm olein (SPO).

Plasma lipid profile: The results of plasma lipid profile determined at three time intervals (weeks 4, 8 and 12) in rats that were fed different types of palm oil is shown in Fig. 1. Total cholesterol (TC) and high density lipoprotein cholesterol (HDL) levels were unaffected in plasma rats in all three groups throughout the study. However, plasma triglyceride (TG) was decreased in all treatment groups at week 12, as compared to week 4, but no differences were seen between the groups.

Feeding palm olein (PO) and stripped palm olein (SPO) diets did not influence rat plasma low density lipoprotein cholesterol (LDL) from week 4 through week 12. Nevertheless, feeding 16% red palm olein for 12 weeks did reduce this lipoprotein level compared to weeks 4 and 8, but was not significantly different from the LDL levels of PO and SPO (at week 12).

Both dietary PO and RPO reduced TC/HDL after 12 weeks of treatment compared to the ratio calculated at week 4. However, reduction by dietary RPO was significantly different compared to both weeks 4 and 8, compared to dietary PO (Fig. 2).

Hepatic vitamin E content: Only three vitamin E isomers i.e. α-tocopherol, α- and γ-tocotrienols were detected in the liver after 12 weeks of treatment (Fig. 3). All treatments resulted in significant increases in hepatic vitamin E (α-tocopherol, α- and γ-tocotrienols) content. More than 90% of the vitamin E detected was in the form of α-tocopherol, whilst the rest (10%) was α- and γ-tocotrienols.

Discussion

Vitamin E analysis done on the tested oils confirmed that red palm olein (RPO) contained a higher amount of vitamin E than palm olein (PO). α-Tocopherol was the
major isomer detected for tocopherol in the palm oils (PO and RPO). In the diets prepared, both PO and RPO diets showed similar patterns of vitamin E content to the oils, with the RPO diet having the highest level of total vitamin E as compared to both PO and SPO diets, with the SPO diet containing little vitamin E. Body weight and weight gain showed that they were similar for all diet groups (data not shown), indicating that diets were adequate and food consumption was similar. Chronic feeding of high fat (20% w/w) palm oil for 12 weeks did not affect both plasma total cholesterol (TC) and high density lipoprotein cholesterol (HDL). There is possibility that a significant decrease in TC may be seen with a longer treatment duration. All the tested oils had a similar fatty acid composition but varied in their antioxidant levels. Higher contents of both vitamin E and carotenoids in RPO did not have any further influence on TC and HDL. However, in contrast, Sulli et al. (1998) showed both α-tocopherol and β-carotene supplementation reduced plasma cholesterol in hypercholesterolemic rabbits after 8 weeks. This may be due to the higher dosage of α-tocopherol (5000 mg/kg diet) and β-carotene (25 mg/kg body weight, intravenously) used in the study, as compared to our study (113 mg vitamin E/kg diet). β-Carotene level was not measured in this study, but Malaysian red palm oil has been reported to contain about 500 mg/kg carotenoids, with approximately 250 mg/kg β-carotene and 200 mg/kg α-carotene (Kritchovsky et al. 2000). Thus, the RPO diet may contain about 80 mg carotenoids per kg diet.

Many reports have been published about the effect of palm oil on cholesterol levels. Osim et al. (1996) claimed that chronic consumption of a diet containing 15% palm oil for 18 weeks increased total cholesterol but no differences were seen in low and high density lipoprotein cholesterol compared to the control group. However, many other studies have demonstrated that when palm oil was used to replace a major part of other fats in a traditional diet, it did not increase serum cholesterol or affect HDL (Zhang et al., 1997a; Zhang et al., 1997b).

Red palm oil (14% diet) was reported not to elevate TC in rabbits fed low cholesterol diet (0.1%), but increased the TC level when the animals were fed a higher percentage of cholesterol (0.2%), compared to refined,
bleached and deodorized (RBD) palm oil. In the study, red palm oil-fed rabbits also had a significantly lower severity of thoracic aorta atherosclerosis as compared to RBD-palm oil group (Kitchevsky et al., 2000). It was also demonstrated that RPO (20% w/w) raised serum TC and HDL in rabbits determined at weeks 8 and 12 of treatments, but the ratio of TC/HDL in this group was comparable with the control group after 12 weeks of study (Kamisah and Nafeeza, 1997).

Palmitic acid (16:0) which is abundant in palm oil, has been claimed in many studies to contribute to hypercholesterolemic effect of the fat (Denke and Grundy, 1992; Cuesta et al., 1998). However, in our study, the results obtained suggest that palmitic acid in the oil did not cause an increase in plasma TC. The atherogenic potential of the palmitic acid is attributed to the presence of the acid at sn-2 position. It has been reported that palm oil contains more than 40% palmitic acid, but the actual amount of the fatty acid at the sn-2 position is only 2.6% (Kitchevsky et al., 2000). Fats containing palmitic acid at the sn-2 position are absorbed more completely than other fats (Tomarelli et al., 1988) and triglycerides containing a saturated fatty acid at sn-2 are cleared more slowly from the circulation (Lien et al., 1997). In the palm oil, palmitic acid is predominantly located at the sn-1 and sn-3 positions (Small, 1991), locations that are believed to be less hypercholesterolemic (Renaud et al., 1995). This may explain the hypocholesterolemic effect of this oil. A few studies have reported that palmitic acid only becomes hypercholesterolemic in the presence of dietary cholesterol (Khosla and Hayes, 1993; Wijendran et al., 2003) when LDL receptors are suppressed (Renaud et al., 1995). Along with cholesterol intake, palmitic acid elevates plasma TC but does not affect plasma TG, HDL and TC/HDL, relative to 18:0-rich diet (Wijendran et al., 2003). Furthermore, palm oil has only a minimal content of hypercholesterolemic intermediate chain fatty acids like myristic (14:0) and lauric (12:0) acids, and none of other atherogenic short chain fatty acids, caproic (6:0), caprilic (8:0) or capric (10:0) acids (Cottrell, 1991).

In contrast to TC and HDL, plasma triglyceride (TG) levels fell with the increasing duration of treatment in all groups, but significant reductions were only seen after 12 weeks of treatment as compared to week 4. Decreased plasma TG concentrations with palm oil diets are considered as beneficial since elevated TG levels constitute an independent risk factor for coronary heart disease in man (Yarnell et al., 2001). No differences were observed in TG levels in all treatment groups at week 12, suggesting that this TG-lowering effect was not contributed by the antioxidant vitamins that were present in the PO or RPO. The effects of palm oils (PO and RPO as well as SPO) on plasma TG in this study was in agreement with another study which reported that rats consumed palm oil (10% fat) for 3 weeks had lower serum and liver TG than corn oil-fed group, but had no effect on TC. They observed liver TG levels fell with increasing dietary palmitic acid (Kitchevsky et al., 2001).

Palm oil reportedly increased plasma TC, LDL and HDL concentrations but decreased TG level (ie both beneficial and detrimental effects) in Scottish volunteers. However, these changes were small considering the high proportion of palm oil in the diet (26% of energy) and were similar to those in the ‘wild’ group (consumed habitual diet only) which would have contained no more than 3% of energy as palm oil (Mutilib et al., 1999). A study done by the same group discovered that palm oil-
enriched diets reduced pre-prandial plasma Lp(a) (Mutalib et al., 2002), one of the major independent risk factors for heart disease (Sandkamp et al., 1990). In the present study, dietary RPO lowered plasma LDL concentration significantly after 12 weeks feeding compared to weeks 4 and 8. In the PO-fed group, a decreasing trend in LDL concentration was seen with increasing feeding period, but the changes were not significant. A higher content of bioactive components (tocopherols, tocotrienols and carotenoids) in RPO may play an important role in reducing the lipoprotein level. Several studies have observed a significant reduction (Zhang et al., 1997a, Zhang et al., 1997b) or rise (Cuesta et al., 1998; Mutalib et al., 1999) in plasma LDL with palm oil dietary intake, while others reported no such effect (Bosch et al., 2002; van Jaarsveld and Benade, 2002). Though palm oil increased LDL concentrations, it afforded better protection of the LDL particles against lipoperoxidation (Cuesta et al., 1998) and significantly reduced the risk for developing early lesions in peripheral arteries and aortas (van Jaarsveld et al., 2002). 

In our study, reductions in the TC and HDL ratio, which is a more useful index of atherogenicity, of the dietary PO (vs week 4) and RPO (vs weeks 4 and 8) observed after 12 weeks of treatment, were suggested to be attributed to the high antioxidant contents of the oils. Higher levels of vitamin E and carotenoids in RPO afforded further reduction in the index. Similar effect of palm oil on the ratio was also reported by other investigators (Zhang et al., 1997b). Previous studies have shown that dietary palm oil had comparable effect on TC/HDL ratio in normo- and hypercholesterolemic subjects (Zhang et al., 1997a; Zhang et al., 1997b), as well as in aged women (Cuesta et al., 1998) to dietary peanut oil or oleic acid-rich sunflower, respectively.

α-Tocopherol was the major isomer detected in the liver after 12 weeks of dietary treatment. Although the PO and RPO diets had a quite similar proportion of tocotrienols to α-tocopherol, the concentrations of these individual tocotrienol isomers were not as high as of the α-tocopherol. One possible explanation for the lack of bioavailability of tocotrienol in vivo, may be due to poor absorption or rapid clearance from plasma (Fairus et al., 2004). The latter is likely due to a low affinity of tocotrienols to hepatic α-tocopherol transfer protein (Hosomi et al., 1997). Tocotrienol, especially the γ-tocotrienol possesses hypocholesterolemic activity (Raederstorff et al., 2002), which may be attributable to posttranscriptional suppression of HMG CoA reductase, and a concomitant upregulation of the LDL receptor (Parker et al., 1993). α-Tocopherol has been reported to attenuate the inhibitory effects of tocotrienols on HMG CoA reductase (Qureshi et al., 1996). However, this effect of the α-tocopherol was not observed in this study. The reduction in LDL level as well as in TC/HDL ratio were the greatest in the RPO group, even though the group had the highest hepatic concentration of α-tocopherol. This suggests that α-tocopherol also have a role in lowering the plasma cholesterol level.

In conclusion, the results of the study suggest that the hypocholesterolemic effects of the palm oils are attributable to their balanced fatty acid composition and high content of antioxidant vitamins. Therefore, the effects that palm olein and red palm olein have on blood lipids is different and that palm oil should not be categorized as a saturated vegetable oil in the same group as coconut oil.

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References
Kamisah et al.: Effect of palm oil on lipid profile


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