SHORT COMMUNICATION

Journal of Oil Palm Research Vol. 24 December 2012 p. 1562-1565

PALM OIL DIETS DO NOT RAISE TOTAL/HDL-CHOLESTEROL COMPARED TO A PARTIALLY HYDROGENATED VEGETABLE OIL DIET IN RATS

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ABSTRACT

A suitable replacement fat for a trans *fat rich partially hydrogenated vegetable oil (PHVO) has to be chosen and examined carefully before being introduced for any food applications. The aim of the replacement is to avoid putting the subgroups in Southeast Asia at risk of having high* trans *fatty acid content in their diets. A direct comparison between the effects of PHVO and unhydrogenated vegetable oils on blood lipids has not been evaluated extensively. Therefore, the objective of this study was to compare the effects of different unhydrogenated vegetable oils, using high oleic palm olein (HOPOo), palm oil (PO), and palm stearin (POs), on serum lipid levels with those of PHVO. Male* Sprague Dawley *rats (n=14 per group) were randomly allocated an isoenergetic meal, providing 34.0% of the total energy from fat, from one of the formulated diets for eight weeks. HOPO, PO and POs were used as sources of unhydrogenated PO in the diets while a PHVO obtained from soyabean oil was used as a positive control. Our study demonstrated that HOPOo, PO and POs did not raise total/high density lipoprotein cholesterol (HDL-c) compared with PHVO. Food intake was monitored during the feeding intervention. All the unhydrogenated palm oil diets exerted similar incremental body weights compared to PHVO. The present study confirmed that the three unhydrogenated PO diets did not adversely affect blood lipids compared with PHVO in rats.*

Keywords: high oleic palm olein, palm oil, palm stearin, total/HDL-cholesterol.

Date received: 9 August 2011; **Sent for revision:** 10 May 2012; **Received in final form:** 27 August 2012; **Accepted:** 29 August 2012.

INTRODUCTION

A metaanalysis of 27-trials by Mensink and Katan (1992) demonstrated that *trans* fatty acids in PHVO increase the risk of developing heart disease by altering the lipoprotein profile. Several studies (Hayes and Khosla, 2007; Mozaffarian *et al*., 2009; 2006) also demonstrated that *trans* fatty acids decreased serum HDL-c, increasing both total/HDL-c ratio and CHD events as compared with saturated fatty acids (C12:0 – C16:0), *cis*-MUFA and PUFA. This evidence of the unfavourable effects of *trans* fatty acids has resulted in PHVO being removed from many food products such as margarines, packaged snacks and fast foods. However, unhydrogenated vegetable oils such as palm oil (PO) and its fractions, palm stearin (POs), or blends, high oleic palm olein (HOPOo), exhibit a wide range of functional physical properties similar to partially hydrogenated vegetable oil (PHVO). PO is widely used in Southeast Asia for a wide range of food preparations because of its versatility (Hayes and Khosla, 2007). PO is able to provide a wide range of oil fractions with different properties to suit the diverse requirements of industry. POs is a solid fraction obtained from the fractionation of PO. POs is used as a natural hard stock for making

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trans-free fat. In addition, HOPOo is a new blend of vegetable oil obtained from mixing PO and a soft oil that is rich in monounsaturated (MUFA). HOPOo is fully liquid at ambient temperature and can also withstand a lower temperature and remain clear at 0°C for at least 5 hr. HOPOo is also able to extend and improve the oxidative stability of the soft oil. Therefore, it is important to investigate the effects of these palm based-unhydrogenated vegetable oils as possible replacement for PHVO before introducing it for use in food products.

MATERIALS AND METHODS

Animals and Diets

A total of 56 male *Sprague Dawley* rats (weighing 80-100 g each) were obtained from the Animal House, Universiti Kebangsaan Malaysia (Bangi, Selangor Malaysia). The animals were maintained on a 12-hr light-dark cycle at 25°C. Two animals were housed in one cage. Tap water and standard laboratory rat chow (Glen Forrest, Australia) were provided *ad libitium* before the dietary manipulation. Feeding intervention was started when the body weight had increased to 200-250 g.

Modified AIN-93G diets were formulated with 158 g of test fat/kg diet. The test fats were HOPOo, PO (from Malaysian Palm Oil Board, Malaysia), POs (Nisshin Oillio, Japan) and PHVO (Nisshin Oillio, Japan). Every kilogrammes of the diets contained 247 g casein, 474 g dextrose, 53 g cellulose, 11 g vitamin mix, 53 g salt mix and 3 g methionine. Each diet was designed to provide 34.0% of its energy from fat, 23.5% energy from protein and 42.5% energy from carbohydrate. The diets were pelletised and baked at 60°C. The fatty acid compositions of the diets were analysed and are shown in *Table 1*. The pellets were stored at 4°C and provided fresh to the rats daily.

After seven days of conditioning in the animal facility, the 56 rats were randomly assigned to four groups and fed with a particular test diet for eight weeks. Pellets were weighed and put into food cups for the animals according to the test fat group, allowing for feeding *ad libitium*. All rats had free access to food and water during the feeding intervention. Food consumption and body weight were recorded weekly. The percentage of fat intake and incremental body weight were calculated according to Equations (1) and (2), respectively:

$$\% \text{ fat intake} = \frac{15.8\% \times \text{accumulated food intake} \times 1000 \text{ g}}{\text{final body weight} \times 56 \text{ day}} \quad (1)$$

$$\text{Incremental body weight (\%)} = \left[\frac{(\text{terminal weight (g)} - \text{initial weight (g)})}{\text{initial weight (g)}}\right] \times 100 \quad (2)$$

The rats were killed in the fasting state according to the guidelines approved by the Animal Care and Use Committee, University of Malaya (ACUC). Blood was collected by cardiac puncture and allowed to clot at room temperature over 1 hr; serum was then collected by centrifugation at 1000X g. Serum was stored at -80°C prior to analysis. All procedures involving the animals have been approved under ACUC, University of Malaya. The project protocol number was registered as FIS/07/04/2008/TKT(R).

Fatty Acid Analysis

Fatty acid compositions of the test fats were measured by gas-liquid chromatography (GLC) on a SP-2560 column (100 m × 0.23 mm × 0.2 mm) (Agilent Technologies, USA) using a flame ionisation detector (FID) on an Autosystem (Perkin Elmer, USA). Carrier gas (helium) was set at 40 psi while the injector temperature was set at 250°C. Oven temperature was set as 240°C for 58 min without ramping. Fatty acids were determined in the form of methyl esters as described earlier (Voon *et al*., 2011). A 37-fatty acid methyl ester mixture (Sigma-Aldrich, US) was used as the external standard. Results were expressed in % of energy (*Table 1*).

Serum TAG, Total and HDL-c Analyses

Serum TC and TAG were assessed using enzymatic assays (cholesterol CHOD-PAP and TAG GPO-PAP kits). HDL-c was measured by enzymatic colorimetric assays (HDL-C plus 3rd generation). All reagent kits were purchased from ROCHE, and all assays were carried out using a Hitachi 902 autoanalyser (Roche Diagnostics GmbH, Germany).

TABLE 1. FATTY ACID COMPOSITION (percentage of total energy) OF THE EXPERIMENTAL DIETS

Fatty acid	Diet[a]			
	HOPOo	PO	POs	PHVO
C14:0	0.2	0.2	0.5	n.d.
C16:0	7.3	10.3	21.1	5.8
C18:0	0.9	1.1	2.0	3.1
C18:1t	n.d.	n.d.	n.d.	16.0
C18:1	18.1	13.6	8.5	7.1
C18:2	5.7	8.2	1.8	0.7
C18:3	1.2	0.1	n.d.	n.d.

Note: [a]% of total energy of the experimental diets.
n.d. – not detected.
HOPOo – high oleic palm olein.
PO – palm oil.
POs – palm stearin.
PHVO – partially hydrogenated vegetable oil.

TABLE 2. PERCENTAGE OF FAT INTAKE AND THE EFFECTS OF EXPERIMENTAL DIETS ON WEIGHT GAIN AND SERUM LIPIDS IN YOUNG MALE RATS (n = 15) AFTER A EIGHT-WEEK FEEDING INTERVENTION

Fat intake, g kg^{-1} per day	HOPOo	PO	POs	PHVO
	6.9 ± 1.5	**5.9 ± 0.4**	**6.3 ± 0.6**	**6.3 ± 0.4**
Weight gain, g	151.4 ± 10.1[ab]	159.5 ± 7.1[a]	122.9 ± 8.9[b]	137.5 ± 4.4[ab]
Weight gain, %	62.3 ± 5.5	63.2 ± 4.0	49.5 ± 4.4	56.0 ± 3.2
TC, mg dl^{-1}	60.7 ± 2.9	58.7 ± 3.1	63.0 ± 2.8	57.5 ± 4.0
HDL-c, mg dl^{-1}	51.5 ± 2.4[a]	47.2 ± 2.3[a]	48.6 ± 2.3[a]	36.0 ± 1.4[b]
TC/HDl-c	1.2 ± 0.0[a]	1.2 ± 0.0[a]	1.3 ± 0.0[a]	1.6 ± 0.1[b]
TAG, mg dl^{-1}	58.0 ± 6.3[a]	68.5 ± 10.1[a]	79.9 ± 10.8[ab]	104.7 ± 10.4[b]

Note: Values are expressed as means ± SEM. All data were analysed by ANOVA. Values within a row bearing unlike superscript letters [a,b,c] were significantly different from each other, $p < 0.05$.
HOPOo – high oleic palm olein.
PO – palm oil.
POs – palm stearin.
PHVO – partially hydrogenated vegetable oil.

Statistics

All results were expressed as means ± SEM of the 14 animals in a group fed on each diet. Statistical significance was determined using the one-way ANOVA, followed by Tukey's Multiple Comparison Test. The PASW Statistics 18 software was used for the statistical analyses. All results were accepted to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

The percentage of fat intake was monitored and was found to be similar across the groups (*Table* 2). Calculations were made based on the daily food intake records. Results demonstrated that POs diet significantly lower body weight gain (~36.6 g) compared with that of a PO diet in rats. However, no significant changes in the percentage of body weight were observed when comparing HOPOo, PO and POs diets to the PHVO diet. A difference of 13.7% between POs *vs*. PO diets was not statistically significant. This may be due to inter-individual variations which resulted in no differences in percentage weight gain relative to initial weight. Serum total cholesterol (TC) levels were not altered by the experimental diets. However, the HOPOo, PO and POs diets raised HDL-c significantly compared with PHVO ($p < 0.05$). In addition, HOPOo, PO and POs decreased TC/HDL-c significantly compared with PHVO. Further, HOPOo and PO also decreased serum triglycerides (TAG) levels compared with PHVO, while no change was observed for POs compared to the control.

In this study, the three unhydrogenated PO diets did not show hypercholesterolemic effects compared with the PHVO diet. The results of our study are in agreement with those of some other studies (Judd *et al.*, 2002; Mozaffarian *et al.*, 2009; Zock and Katan, 1992) which suggest that *trans* fat-rich diets decrease HDL-c levels, and subsequently cause a decrease in TC/HDL ratio. Unlike the *trans* fat in PHVO, unhydrogenated palm oil diets increase HDL-c, and hence decrease TC/HDL-c. Our findings are also in line with those of Hayes and Khosla (2007) which suggest that relative to oleic acid, palmitic acid in palm oil is not hypercholesterolemic in a cholesterol-free diet (Khosla and Hayes, 1993). The findings are supported further by those of Kritchevsky *et al*. (2001) which revealed that with the cholesterol-free diets there were virtually no differences in the effects between PO, corn oil and the mixtures of both on serum total or HDL cholesterol. Besides, Oluba *et al.*, 2008 also found that serum TC and TAG were significantly reduced in rats fed with palm oil compared with soyabean oil (5% of total energy) in a six-week feeding intervention.

Our findings on PHVO are also in agreement with that of Huang *et al.* (2009) who suggest that *trans* fat intake lowers both TC and HDL cholesterol levels. Thus, it is confirmed that the unhydrogenated PO diets used in our experiment show that there are no adverse effects on the lipid profile in this study, unlike the PHVO diet. Our findings also suggest that unhydrogenated PO fractions in their natural state may be preferred as functional alternatives in place of PHVO, due to their beneficial effects on blood lipids. When a *trans* fatty acid-rich fat is eliminated from foods, further studies need to be carried out to carefully examine the effects of PO diets with different saturated fatty acid (SFA) content to ensure that the type of palm fats chosen as a replacement fat is beneficial to health.

CONCLUSION

Our study was able to provide preliminary findings on the non-adverse effects of unhydrogenated vegetable oils obtained from PO on serum lipid profile, and considers these oils as possible functional alternatives to PHVO in food applications.

ACKNOWLEDGEMENT

The authors wish to thank the Director-General of MPOB for permission to publish the data and Dr Siew Wai Lin for supplying HOPOo for the study. This study was sponsored by MPOB. The first two authors contributed equally to the study.

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