BLOOD PLASMA AND LIVER LIPIDS OF RATS FED PHYSICALLY REFINED AND RE-REFINED PALM OIL

Keywords: Physically-refined palm oil; chemically-refined palm oil; triglyceride; cholesterol.

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Physically-refined palm oil (PRPO) and palm oil after further chemical refining (chemically-refined palm oil, CRPO) were included in the diet of rats and the effects on plasma and liver lipids were studied and compared. No differences in body weight gain or organ weights were observed as between the rats fed the PRPO and the CRPO diets for two months. Differences in plasma total cholesterol, high-density lipoprotein cholesterol and phospholipid content as between the rats fed the PRPO diet and those fed the CRPO diet were not evident. Liver total cholesterol, phospholipid, phosphatidylcholine hydroperoxide, phosphatidylethanolamine hydroperoxide and α-tocopherol contents were also not significantly different as between the rats on the two diets. Liver and plasma triglyceride levels did differ between rats on the two palm oil diets, however. The results show that differences between the dietary effects of PRPO and CRPO on the tissue lipid profiles in the rats were relatively small.

INTRODUCTION

At present, 90% of all palm oil produced is used as edible oil (American Oil Chemists' Society, 1993). Both physically-refined and chemically-refined palm oil are commonly available (Cottrell, 1991). In Malaysia and Indonesia, food grade physically-refined palm oil is commercially available, whereas in Japan, food grade physically-refined palm oil, which is imported, is further refined with phosphoric acid treatment, deacidification with alkali, bleaching and deodorization before being made commercially available. The reasons for this situation are merely commercial.

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Chemical refining reduces free fatty acids to lower levels than does physical refining (Young *et al.*, 1986; Duff, 1991). The quality of palm oil refined by the two methods is commonly judged from physical and chemical parameters (Swoboda, 1985; Stage, 1985; Willems and Padley, 1985). However it is far from clear whether physically-refined or chemical-refined palm oil is nutritionally better.

The effects of diets containing palm oil have been studied very intensively, both in humans (Marzuki *et al.*, 1991; Ng *et al.*, 1991; Wood, 1992; Sundram *et al.*, 1992) and in animals (Imaizumi *et al.*, 1990 and 1991; Choi *et al.*, 1993). However, there is no evidence on the comparative nutritional effects between physically-refined palm oil (PRPO) and palm oil after further chemical refining (CRPO). This paper compares the effects of dietary PRPO and CRPO on plasma and liver lipids in rats after a two-month feeding period.

MATERIAL AND METHODS

Care of animals

Male Sprague-Dawley rats (4-week old, 70–80 g) were obtained from Funabashi Farm Co. (Chiba, Japan). The animals were given free access to a commercial diet (Funabashi Farm F-2 pellet) for four days and were randomly divided into four dietary groups, respectively given physically-refined palm oil (PRPO), palm oil after further chemical refining (CRPO), soya bean oil (SO) and canola oil (CO). The experimental diets contained (g/kg diet): casein, 200; DL-methionine, 3; corn starch, 150; sucrose, 452; cellulose, 50; oils, 100; vitamin mixture (AIN-76A; Oriental Yeast Co., Tokyo, Japan), 10; mineral mixture (AIN-76A), 35. The diets were prepared freshly every five days and kept at –25°C to prevent oxidation. The fatty acid compositions and physico-chemical properties of the oils are shown in *Table 1*. The components of the diets were purchased from Oriental Yeast Co. Ltd except the oils, which were purchased from Nippon Oils & Fats Co. Ltd (Tokyo, Japan). The oils were kept at –25°C under nitrogen until used.

The rats were housed individually in hanging stainless steel cages at a temperature of 22°C–24°C, in a well-ventilated and light-controlled room (12-h light-dark cycle). Every day each rat received 18 g–21 g of diet, on average 20 grams. The crumbled food was placed in a plastic container. By this means no food was observed falling into the batch of droppings, and food remaining in the container amounted to only about 1 g–1.5 g when 21 g food was provided. The amount of food provided on a given day was made equal to the maximum intake of the rats fed physically refined palm oil during the previous day. Rats were given free access to water and weighed every other day.

After two months the rats were fasted 24 hours and then killed according to the *Guide for the Care and Use of Laboratory Animals* of the National Institute of Health, USA. Blood was collected through the abdominal aorta with a heparinized syringe and was centrifuged at 3000 rpm to obtain plasma. The livers were perfused *in situ* with ice-cold 0.15 M saline, isolated, washed in cold 0.15 M saline and kept at –80°C until used.

Biochemical assays

Liver total lipid was extracted with a mixture of chloroform and methanol (2:1, v/v) (Miyazawa *et al.*, 1992). Plasma total cholesterol, high density lipoprotein cholesterol, phospholipids and triglycerides were measured enzymatically using commercially available kits (total cholesterol E-test, HDL-cholesterol E-test, phospholipids test, and triglyceride E-test; Wako Pure Chem. Ind. Ltd, Osaka, Japan). Liver total cholesterol and triglyceride contents were measured enzymatically as in the case of plasma, except that the liver total lipid was dissolved in dioxane/isopropanol (50/50, v/v) (Cho, 1983). Protein was determined by the modified Lowry method of Hartree (1972). Liver α-tocopherol was measured by normal phase HPLC, using the method of Abe *et al.* (1976). Liver thiobarbituric acid-reactive substance (TBARS) were measured by the method of Ohkawa *et al.* (1979). Liver phosphatidylcholine hydroperoxide (PCOOH) and phosphatidylethanolamine hydroperoxide (PEOOH) were measured by a chemiluminescence detection-HPLC method (Miyazawa *et al.*, 1992).

TABLE 1. PHYSICO-CHEMICAL PROPERTIES AND FATTY ACID COMPOSITIONS OF THE OILS IN THIS STUDY

	PRPO[a]	CRPO	SO	CO
Physico-chemical				
Iodine value (meq/kg)	50.4	50.2	109.5	111.9
Acid value (meq/kg)	0.7	0.1	0.1	0.1
Peroxide value (meq/kg)	4.9	0.9	0.9	0.8
Carbonyl value (meq/kg)	34.5	25.7	26.5	26.8
Fatty acid composition[b]		g/100 g		
14:0	0.9	0.9	0.1	trace
16:0	40.0	44.9	12.9	4.8
16:1(*n*-7)	trace	trace	trace	trace
18:0	5.0	5.3	3.7	1.8
18:1(*n*-9)	44.9	40.9	24.7	53.7
18:2(*n*-6)	8.9	7.8	50.8	26.0
18:3(*n*-3)	0.3	0.2	7.8	13.7
Total	100.0	100.0	100.0	100.0
P/S ratio	0.2	0.15	3.49	6.02
(*n*-3)/(*n*-6) ratio	0.03	0.02	0.14	0.51

[a]PRPO = physically-refined palm oil;
CRPO = palm oil after further chemical refining;
SO = soya bean oil;
CO = canola oil
[b]Expressed as g/100 g fatty acids.

Statistics

The data are expressed as means and standard deviation (SD). The data were subjected to ANOVA, and statistical comparisons between treatment means were made using least significant difference (LSD) (Steel and Torrie, 1980). Statistical significance was taken as $p < 0.01$.

RESULTS AND DISCUSSION

Palm oil

Two kinds of palm oil were used in this study. One was physically-refined palm oil (PRPO) imported from Malaysia. The other was physically-refined palm oil which had undergone further chemical

TABLE 2. BODY WEIGHT GAIN AND ORGAN WEIGHTS OF RATS FED DIETS CONTAINING 10% (w/w) OILS FOR TWO MONTHS

Body and organs	Dietary group			
	PRPO	CRPO	SO	CO
Body weights				
Initial	113.3 ± 3.2	112.8 ± 3.7	112.9 ± 2.7	112.4 ± 2.8
Gain[a]	287.4 ± 12.8[b]	287.0 ± 14.7[b]	324.8 ± 16.7[c]	312.0 ± 18.2[c]
Liver	11.8 ± 1.8	12.7 ± 1.9	12.6 ± 1.5	12.3 ± 1.4
Brain	1.92 ± 0.3	1.95 ± 0.1	2.00 ± 0.1	2.04 ± 0.1
Heart	1.13 ± 0.1	1.26 ± 0.1	1.24 ± 0.2	1.32 ± 0.1
Kidney	2.46 ± 0.3	2.57 ± 0.2	2.81 ± 0.3	2.72 ± 0.2

[a]Gain over 8 weeks.
Values are means (n = 8) ± SD
[b,c] Values in a row with different superscripts are significantly different at $p < 0.05$

refining (chemically-refined palm oil, CRPO). The chemical refining of PRPO is generally practised in Japan after it is imported from Malaysia. The PRPO we used had slightly higher acid, peroxide and carbonyl values than CRPO (*Table 1*). The higher peroxide value and free fatty acid content of PRPO might relate to care and handling during the voyage (Willems and Padley, 1985). Chemical refining of PRPO seems meaningful to improve quality and stabilize the imported palm oils.

Body weight gain and organ weight

Body weight gains showed no difference between PRPO- and CRPO-fed rats. The body weight gains of the rats fed on palm oils were lower than those of the rats fed on soya bean or canola oil. Organ weights however showed no difference among the four dietary groups (*Table 2*).

Plasma and liver lipids

Slight differences were observed between the plasma total cholesterol, high density lipoprotein cholesterol and phospholipid contents as between the rats fed PRPO and those fed CRPO (*Table 3*). Although measured in the fasting state, the plasma lipid contents of the rats fed on palm oils was higher than that of the rats fed on soya bean or canola oils (*Table 3*). Differences in liver total cholesterol and phospholipid contents, as between the rats fed PRPO and CRPO, were not apparent. However, triglyceride levels were lower in plasma and higher in livers in PRPO-fed rats as compared with CRPO-fed rats.

Liver PCOOH/PEOOH, TBARS and α-tocopherol

Although the acid and peroxide values of PRPO were slightly higher than those of CRPO (*Table 1*), no significant differences between liver phosphatidylcholine hydroperoxide (PCOOH) and phosphatidylethanolamine hydroperoxide (PEOOH) contents were apparent as between the rats fed PRPO and CRPO (*Table 4*). No difference was observed in liver TBARS and α-tocopherol contents either. TBARS and especially PCOOH/PEOOH values have been used to estimate the degree of lipid peroxidation in human blood plasma and erythorcytes and in the liver and brain of rodents (Miyazawa, 1993).

The present study showed that the differences between PRPO and CRPO in respect of nutritional

TABLE 3. PLASMA AND LIVER LIPID PROFILES OF RATS FED DIETS CONTAINING 10% (w/w) OIL FOR TWO MONTHS

Dietary group	Plasma				Liver		
	Triglycerides	Phospholipids	Total cholesterol	HDL cholesterol	Triglycerides	Phospholipids	Total cholesterol
		mg/dl				mg/g	
PRPO	102.7 ± 17.3[b]	148.6 ± 18.5[c]	120.6 ± 14.5[c]	35.4 ± 5.8[b]	72.3 ± 12.4[c]	26.6 ± 6.2[b]	17.4 ± 4.1[b]
CRPO	149.1 ± 20.6[c]	168.7 ± 20.2[c]	125.6 ± 20.2[c]	38.1 ± 9.7[c]	54.3 ± 10.5[b]	25.4 ± 2.3[a]	16.9 ± 2.5[b]
SO	60.7 ± 14.6[a]	104.7 ± 12.7[a]	84.4 ± 17.6[a]	27.8 ± 11.1[a]	33.4 ± 4.3[a]	31.9 ± 3.3[c]	14.1 ± 5.3[a]
CO	69.3 ± 12.9[a]	122.7 ± 10.1[b]	99.6 ± 11.3[b]	36.4 ± 4.5[b]	39.7 ± 13.2[a]	31.0 ± 3.5[c]	15.2 ± 6.1[a]

Values are means (n = 8) ± SD. Values in a column with unlike superscripts are different at $p < 0.01$

TABLE 4. LIVER PCOOH, PEOOH, TBARS AND α- TOCOPHEROL CONTENTS IN RATS FED DIETS CONTAINING 10% (w/w) OIL FOR TWO MONTHS

Dietary group	PCOOH pmol/100 mg protein	PEOOH pmol/100 mg protein	TBARS nmol MDA/100 mg protein	α-Tocopherol µg/100 mg protein
PRPO	303.0 ± 67.6	125.8 ± 61.5	438.5 ± 56.4	14.7 ± 3.6[b]
CRPO	227.9 ± 96.8	104.5 ± 44.4	489.9 ± 67.3	14.4 ± 3.1[b]
SO	214.9 ± 79.2	102.1 ± 29.8	545.4 ± 80.5	8.2 ± 2.4[a]
CO	258.5 ± 53.9	145.3 ± 60.5	415.1 ± 40.8	13.8 ± 2.3[b]

Values are means (n = 8) ± SD. Values in a column with different superscripts are significantly different at $p < 0.01$.
PCOOH = Phosphatidylcholine hydroperoxide
PEOOH = Phosphatidylethanolamine hydroperoxide
TBARS = Thiobarbituric acid-reactive substances
MDA = Malonaldehyde

effects on plasma and liver lipids in the rats were relatively small. This would be mainly due to the similarities in the fatty acid compositions of PRPO and CRPO. Accordingly, the differences observed in plasma and liver lipids between the rats fed soya bean oil or canola oil and those fed palm oil should correlate with the differences in fatty acid composition of the oils.

A high acid value has been identified as the main factor associated with an increase of the peroxide value of palm oil during handling and sea transport (Willems and Padley, 1985). Berger (1985) has shown a positive correlation between the peroxide values of palm oil imported from Malaysia and the length of time of voyage. The increase of peroxide value of palm oil during the voyage may be unavoidable at present. In this study we examined two palm oils: one was PRPO transported from Malaysia, and the other was palm oil obtained after chemical refining of imported PRPO.

The high liver and low plasma triglyceride levels induced by the PRPO as compared with the CRPO diet were probably due to the differences in the acid value and peroxide value between the two palm oils (*Table 1*) and should not be attributed only to the saturation of the palm oils. Choi *et al.* (1993) have reported that dietary soya bean oil, which is more unsaturated than palm oil, produced higher liver triglyceride levels in rats than did palm oil. A higher plasma triglyceride level with a soya bean oil diet than with a palm oil diet has also been reported in humans by Marzuki *et al.* (1991). In the present study, the liver and plasma triglyceride levels of the rats fed soya bean oil or canola oil diets were lower than in the rats fed palm oil diets.

Overall the present data suggest that physically-refined palm oil in the diet should be nutritionally equivalent to the palm oil obtained after further chemical refining of physically-refined palm oil.

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