Review Article

Red palm oil in experimental atherosclerosis

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Oil obtained initially in harvesting the fruit of the oil palm is red due to its content of carotenes, tocopherols and tocotrienols. Table 1 presents the average fatty acid, carotene and tocotrienol composition of red palm oil (RPO). In the past, the compounds imparting the red colour to the oil have been removed and sold separately under the name of palmvitee. Palmvitee has been shown to lower cholesterol levels in human subjects[1,2] (Table 2). Tocotrienols have also been shown to lower the cholesterol levels of chickens,[3] rats[4] and pigs.[5]

Tocopherols have been shown to inhibit atherogenesis in rabbits[6,7] and monkeys.[8] Antioxidants, in general, are considered to have anti-atherosclerotic properties. Gey *et al.* examined data from 16 populations in the WHO/MONICA Core study and found no significant correlation between mortality from ischemic heart disease (IHD) and levels of plasma cholesterol or blood pressure.[9] However, in 12 of the populations, the levels of vitamin E exhibited a strong inverse correlation with IHD. When all 16 populations were examined there was a modest association between plasma cholesterol levels and IHD mortality, but the inverse correlation between IHD mortality and plasma vitamin E levels was much stronger.[9]

In epidemiological studies of dietary antioxidant intake and the risk of coronary disease Stampfer *et al.*[10] observed a significant trend towards risk reduction with increasing vitamin E intake in a group of 87 245 nurses, and a similar observation was made in 39 910 male health professionals.[11] Carotenoid intake appears to reduce carotid artery wall thickness and plaque accretion.[12]

The following findings provided a rationale for examining the effects of RPO in experimental atherosclerosis. Palm oil (PO) has been stigmatised as a hypercholesterolaemic fat because of its palmitic acid (16:0) content, despite human studies that show it does not raise serum cholesterol levels.[13,14] We have shown that the presence of palmitic acid at the SN2 position of a triglyceride renders that triglyceride more atherogenic.[15–17] Cottonseed oil contains 24% palmitic acid but only 2% is at the SN2 position. Randomisation of cottonseed oil increases the amount of 16:0 at the SN2 position to 8.3% and trebles the severity of atherosclerosis.[15] Lard and tallow both contain 20–24% palmitic acid. In lard, virtually all of the 16:0 is at the SN2 position and it is 113% more atherogenic than tallow. Randomisation of lard reduces the amount of 16:0 at the SN2 position by about 67% and reduces atherogenicity by 51%. Randomization of tallow increases the amount of 16:0 at the SN2 position by 124% and increases atherogenicity by 10%[16] (Table 3). A study of synthetic triglycerides[17] showed that 1,3-palmitoyl-2-oleoylglycerol was 53% less atherogenic than 1,2-palmitoyl-3-oleoylglycerol.[17]

We have carried out a study in which the atherogenic properties of RPO were compared with those of refined, bleached, deodorized PO (RBD-PO) and randomised PO.[18] The fatty acid compositions of the oils are shown in Table 4 and the carotenoid and vitamin E levels of RPO are given in Table 5. The fats were incorporated into semipurified diets containing 0.1% cholesterol (Table 6) and fed to rabbits (10/group) for 90 days. The results are given in Table 7. It is evident that RBD-PO is 21% less atherogenic than randomised palm oil and 15% more atherogenic than RPO. The findings, while indicative, were not striking. The study was repeated using 0.2% cholesterol and feeding was maintained for 65 days. As Table 8 shows, RBD-PO was 25% less atherogenic than randomised PO and 47% more atherogenic than RPO. RPO is rich in both carotenoids and vitamin E and this study did not indicate if one or both of the components of RPO were responsible for our findings.

Rice bran oil (RBO) contains about as much vitamin E as PO and RPO but its carotenoid level is very low. We then compared the atherogenic effects of PO, RPO and RBO. We included a fourth group; PO plus the carotenoid and vitamin E contents of RPO. The fatty acid composition of the fats is

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Table 1. General chemical characteristics of red palm oil

Fatty acid	%
Myristic (14:0)	0.8
Palmitic (16:0)	42.0
Stearic (18:0)	5.1
Oleic (18:1)	42.0
Linoleic (18:2)	10.0
Total carotenes (μg/g)	550
Beta-carotene	(68.2%)
Tocopherols and tocotrienols (ng/L)	468

Table 4. Fatty acid composition of palm oils[18]

Fatty acid	%	% at SN2	
		Native	Randomised
14:0	1.36	0.08	1.32
16:0	41.16	2.58	13.55
16:1 (n7)	0.23	0.02	0.01
18:0	3.96	1.08	1.00
18:1 (n9)	40.95	29.94	15.58
18:2 (n6)	11.14	8.66	4.22
18:3 (n3)	0.28	0.10	0.17
20:0	0.28	0.00	0.06
20:1 (n9)	0.15	0.00	0.05

Table 2. Serum cholesterol levels in 15 human subjects fed corn oil or palmvitee for 28 days[1]

Supplement	Total cholesterol (mg/dL)		LDL/HDL cholesterol
	Baseline	28 days	Day 28
Corn oil	290 ± 31	296 ± 31	+2%
Palmvitee	294 ± 34	249 ± 27	–15%

HDL, high-density lipoprotein; LDL, low-denstiy lipoprotein.

Table 3. Fat randomisation and experimental atherosclerosis: influence of percentage 16:0 at the SN2 position

Fat	Palmitic acid (16:0) (%)		Atherosclerosis†		Reference
	Total	SN2	Aortic arch	Thoracic aorta	number
Lard (N)	21.4	21.3	2.69	1.75	16
Lard (R)	21.4	7.6	1.50	0.69	
Tallow (N)	24.8	3.8	1.29	0.79	16
Tallow (R)	24.8	8.5	1.50	0.79	
Cottonseed oil (N)	24.0	2.0	0.58	0.13	15
Cottonseed (R)	23.8	8.3	1.40	0.71	

†Graded visually on a scale of 0–4. N, native; R, randomised.

Table 5. Carotenoids and vitamin E of red palm oil[18]

Carotenoids	512 p.p.m. (mg/kg)		Vitamin E	730 p.p.m. (mg/kg)	
Isomer	p.p.m.	%	Isomer	p.p.m.	%
β-Carotene	247	48.2	α-Tocopherol	241	33.0
α-Carotene	199	38.9	α-Tocotrienol	119	16.3
Cis α-carotene	13	2.5	β-Tocotrienol	17	2.3
Phytoene	7	1.3	γ-Tocotrienol	235	32.2
Lycopene	6	1.3	δ-Tocotrienol	116	15.9
Other	40	7.8			

shown in Table 9 and the carotenoids, vitamin E and non-saponifiables are detailed in Table 10. Rabbits (10/group) were fed the semipurified diets (Table 6) for 90 days.

Another compositional aspect in which rice bran oil differs from other fats is its relatively high content of non-saponifiables, mainly phytosterols. These sterols were esterified to commonly found fatty acids as well as more rare ones such as ferulic acid. The sterol content of the fats is presented in Table 11. RBO lowers cholesterol levels in hamsters,[19] cynomolgus monkeys[20] and humans.[21,22] Table 12 summarises the findings of the study in which the atherogenic effects of PO, RPO, RBO and PO to which carotenoids, tocotrienols and tocopherols had been added (reconstituted RPO) were analysed. Serum cholesterol levels were similar in all groups and triglycerides were highest in the rabbits fed palm oil plus carotenoids and vitamin E. PO was 138% and 150% more atherogenic than RPO and RBO, respectively. As all of the test groups contained similar

Table 6. Semipurified atherogenic diet

Ingredient	g/100 g	Calories (%)
Casein	25.00	25.6
D-L-Methionine	0.30	–
Sucrose	39.85	40.9
Palm oil	14.00	32.3
Corn oil	0.50	1.2
Cellulose	15.00	–
Mineral mix	4.00	–
Vitamin mix	1.00	–
Choline bitartrate	0.25	–
Cholesterol	0.10	–

levels of vitamin E (751 ± 67 p.p.m), the difference in atherogenicity cannot be attributed to vitamin E content. The carotenoid level of RPO is very high compared to the level in PO, a difference that might explain the PO–RPO difference. RBO also contains very low levels of carotenoids but its effects can be explained on the basis of its phytosterol content. RBO is also considerably more unsaturated. The anomalous group is the one to which carotenoids and vitamin E had been added. Assuming carotenoid levels are the basis for our findings, that group should have been no more atherogenic than RPO, whereas it was 108% more atherogenic than RPO and only 13% less atherogenic than

Table 7. Atherogenic effects of RBD palm oil, randomized palm oil or red palm oil†[18]

	Palm oil group		
	RBD	Randomised	Red
Serum lipids (mg/dL)			
Total cholesterol	213 ± 34	250 ± 53	317 ± 42
% HDL-C	14.6 ± 2.22x	9.3 ± 1.58	8.4 ± 1.16*
Triglycerides	60 ± 11	76 ± 20	57 ± 6
Atherosclerosis‡			
Aortic arch	1.00 ± 0.29	1.20 ± 0.27	0.85 ± 0.23
Thoracic aorta	0.90 ± 0.28	1.20 ± 0.27	0.80 ± 0.25

Data ± SEM. †Rabbits (10/group) were fed 0.1% cholesterol for 90 days. ‡Graded visually on a scale of 0–4. *$P < 0.05$. HDL-C, high-density lipoprotein cholesterol; RBD, refined, bleached, deoderised.

Table 8. Atherogenic effects of RBD palm oil, randomized palm oil or red palm oil†[18]

	Palm oil group		
	RBD	Randomised	Red
Serum lipids (mg/dL)			
Total cholesterol	620 ± 36[a,b]	779 ± 29[a]	769 ± 31[b]
% HDL-C	6.8 ± 0.42	6.4 ± 0.88	5.5 ± 0.58
Triglycerides	70 ± 52	122 ± 13[c]	87 ± 12
Atherosclerosis‡			
Aortic arch	1.63 ± 0.23[d]	2.13 ± 0.18[e]	1.00 ± 0.16[d,e]
Thoracic aorta	1.31 ± 0.28	1.81 ± 0.30	1.00 ± 0.31

Data ± SEM. †Rabbits (8/group) were fed 0.2% cholesterol for 65 days. Diet was as shown in Table 6, with 0.1% sucrose replaced by cholesterol. ‡Graded visually on a scale of 0–4. [a–e]Values within a horizontal row and bearing the same letter are significantly different at $P < 0.05$. HDL-C, high-density lipoprotein cholesterol; RBD, refined, bleached, deoderised.

Table 9. Fatty acid composition of test oils

Fatty acid	Palm oil (%)	Red palm oil (%)	Reconstituted palm oil (%)	Rice bran oil (%)
12:0	0.29	0.27	0.29	–
14:0	0.95	0.96	0.96	0.31
16:0	39.12	33.68	39.15	14.50
16:1 (n9)	0.16	0.20	0.16	0.16
18:0	3.85	3.30	3.91	1.66
18:1 (n9)	43.62	47.05	44.33	43.59
18:2 (n6)	11.32	13.55	10.86	36.59
18:3	–	0.56	–	1.76
20:0	0.33	0.31	0.33	0.68
22:4	–	0.13	–	0.13
Unidentified	–	0.58	0.01	0.12
Iodine value (calculated)	61.00	69.00	60.00	111.00

Table 10. Carotenoid and tocopherol content of test oils

	Palm oil	Red palm oil	Reconstituted palm oil	Rice bran oil
Carotenoids (p.p.m.)	1.3	706.1	781.3	11.5
Vitamin E (p.p.m.)				
α-Tocopherol	158.3	161.9	266.3	136.3
α-Tocotrienol	155.1	197.9	267.6	112.2
β-Tocopherol	–	–	–	8.8
γ-Tocotrienol	223.5	284.5	337.3	340.1
δ-Tocotrienol	49.8	69.4	79.5	27.3
Total (p.p.m.)	620.0	731.5	972.4	681.4
Unsaponifiables (%)	0.47	0.57	0.53	4.39

Table 11. Sterol content of test oils

Sterol	Palm oil	Red palm oil	Reconstituted palm oil	Rice bran oil
Unsaponifiables (%) in oil	0.47	0.57	0.53	4.39
Cholesterol	3.9	1.7	0.0	–
Campesterol	17.7	20.1	20.4	12.4
Stigmasterol	13.0	13.5	13.0	9.4
β-Sitosterol	49.8	54.2	49.8	23.4
Δ^5-Avenasterol	3.3	3.0	4.3	2.1
Δ^7-Stigmasterol	3.2	2.6	3.9	15.8
Δ^7-Avenasterol	4.5	2.9	4.1	–
Ergosterol	2.5	1.9	2.6	21.4
Fructosterol	2.2	–	1.9	0.6
Unidentified	–	–	–	14.9

Table 12. Atherogenic effects of various oils†

	Palm oil	Red palm oil	Reconstituted palm oil	Rice bran oil	*P*-value‡
Weight gain (g)	84 ± 103	243 ± 117	–27 ± 146	251 ± 97	NS
Liver weight (g)	54.4 ± 1.32	52.5 ± 3.82	51.8 ± 4.14	56.6 ± 2.06	NS
Liver (% of bodyweight)	2.16 ± 0.09	1.95 ± 0.10	2.12 ± 0.12	2.12 ± 0.09	NS
Serum lipids (mg/dL)					
Cholesterol	383 ± 42	407 ± 55	424 ± 62	383 ± 70	NS
Triglycerides	72 ± 15[a]	90 ± 22	148 ± 24[a]	86 ± 20	NS
Atherosclerosis§					
Aortic arch	1.20 ± 0.19	0.67 ± 0.19	1.00 ± 0.20	0.75 ± 0.20	NS
Thoracic aorta	0.80 ± 0.15[a,b]	0.17 ± 0.12[a,c]	0.75 ± 0.11[c,d]	0.05 ± 0.05[b,d]	0.0001
% Area	10.8 ± 1.72[a,b]	4.7 ± 2.01[a]	9.2 ± 1.32[c]	3.5 ± 0.81[b,c]	0.003

Data ± SEM. †Rabbits (10/group) were fed semipurified diets containing 0.1% cholesterol and 13% test oil for 90 days. ‡ANOVA. §Graded visually on a scale of 0–4. [a–d]Values within a horizontal row and bearing the same letter are significantly different at $P = 0.05$ by *t*-test.

PO. Could it be possible that the fine structure of RPO is different from the reconstituted RPO? Analysis of the rabbit livers indicated that extraction from rabbits fed RPO was much more difficult than from those fed reconstituted RPO. One can speculate that the carotenoids and vitamin E that are presented by naturally obtained RPO are bound or absorbed differently to the same components of the reconstituted RPO. Could there be an intrinsic distribution of the carotenoids and vitamin E in RPO that cannot be duplicated by simply adding and mixing the various components? If this is the case, our findings may add a new dimension to studies of the effects of dietary antioxidants.

Although epidemiological data suggest that vitamin E and/or carotenoids may be protective against coronary heart disease and lung cancer, experimental studies show a different picture. Greenberg *et al.*[23] studied mortality associated with low plasma levels of β-carotene and the effects of oral supplementation (50 mg/day). The study involved 1188 men

and 532 women. Treatment was for a median period of 4.3 years and the median follow-up period was 8.2 years. Subjects in the upper quartile for plasma levels of β-carotene at the beginning of the study had the lowest risk of death from all causes. The dietary β-carotene supplement did not reduce all-cause or cardiovascular mortality. Two trials of the effects of vitamin A and E and β-carotene on the incidence of lung cancer in smokers have also yielded data relating to cardiovascular disease. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC)[24] found 5% fewer deaths in men given α-tocopherol supplements and 11% more deaths in those given β-carotene. The other trial found slightly higher cardiovascular mortality in subjects given supplements of vitamin A and β-carotene.[25] The carotenoid and vitamin E delivery system in the trials cited above can be compared to that in rabbits fed reconstituted RPO; the component being tested is presented in an isolated form rather than in its natural state. Perhaps we still have something to learn about nature's packaging.

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