Plant Foods for Human Nutrition 38: 145–153 (1988)

© Kluwer Academic Publishers, Dordrecht – Printed in the Netherlands

Serum and liver lipids of rats fed rubber seed oil

E. NWOKOLO,[1,3] D.D. KITTS[2] & J. KANHAI[2]

[1]Department of Animal Science, University of British Columbia, Vancouver, British Columbia, Canada V6T 2A2; [2]Department of Food Science, University of British Columbia, Vancouver, British Columbia, Canada V6T 2A2; [3]present address: Department of Animal Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5

Received 22 June 1987; accepted in revised form 27 November 1987

Key words: rubber seed oil, cholesterol, lipoproteins

Abstract. Crude rubber seed oil (RSO) was incorporated at the 5% level in diets free of cholesterol or containing 1% cholesterol, respectively. These studies were initiated for the purpose of evaluating the palatability and potential toxicity of RSO. Corn oil was used as a control. A considerable amount of unsaponifiable matter was detected in RSO. In addition, RSO was found to possess a fatty acid profile which was fairly different from that of corn oil, primarily due to a much higher content of linolenic acid and a lower content of linoleic acid in RSO. No adverse effects on food intake and average daily gain were observed in rats fed RSO in both cholesterol-free and cholesterol diets. The presence of RSO in cholesterol-free diets results in lower ($p < 0.05$) serum and liver total cholesterol levels than in control animals. A relative hypercholesterolemic effect compared to corn oil was observed however, when RSO was added to diets containing 1% cholesterol. In summary, the physicochemical properties of RSO as well as the presence of cholesterol in the diet are important factors in evaluating the cholesterolemic effect of RSO.

Introduction

Vegetable oils are of primary significance to human and animal nutrition. Tropical agricultural crops, including soybean, oil palm, peanuts, cottonseed and coconut have become universally recognized as major sources of edible oils. In addition to these already established oil seeds, there is a tremendous variety of tropical trees and shrubs whose seeds contain significant amounts of potentially edible oil. Many hitherto unknown tropical crops are increasingly becoming the focus of research attention, in an attempt to estimate their nutritional or industrial potentials.

The rubber tree (Hevea brasiliensis) is native to many tropical countries and produces seeds that contain a lipid content (43% of weight) that is equivalent to other tropical seeds such as palm kernel (44% of weight) and melon seed (49% of weight), but considerably higher than soybean (20% of

weight)[17]. Former studies on rubber seed have been primarily focused on its use in animal feeds [16, 18]. These studies have reported a high content and bioavailability of nutrients and thus a potential usefulness in animal nutrition. A similar value to human consumption has been discussed to a lesser extent by Achinewhu [1] who also demonstrated rubber seed meal to contain saponins which are similar to soybean. Since it has been estimated that in Nigeria alone the potential annual yield of rubber seed oil (RSO) is approximately 5500 t, and in Malaysia 25 times that amount, this source of oil, when refined, could contribute substantially to national requirement for vegetable oils in many tropical countries.

The present study was aimed at evaluating both the nutritional and possible toxicological effects of RSO in rats. In this context, the cholesterolemic effect of RSO was examined since the association of dietary fat intake and serum cholesterol levels have been implicated with coronary heart disease [13]. The physicochemical properties of this oil, was further studied in relation to its effects on serum and liver lipids.

Materials and methods

Rubber seed oil was obtained from the Nigerian Rubber Research Institute, Benin, Nigeria. The physicochemical properties of RSO, including its refractive index, iodine value, saponification value and unsaponifiable matter were determined using standard techniques [2]. Fatty acids were identified from their methyl esters by gas-liquid chromatography using a Varian Model 3700 GC, fitted with a SP-2330 silica capillary column (30 m × 0.25 mm ID. Supelco Inc., USA). Nitrogen was the carrier gas (40 mL/min) and the analysis was performed at 180 °C for 10 minutes at a temperature gradient that increased by 5 °C per minute to 195 °C. The proportion of principal fatty acids was calculated on the basis of the mass of their methyl esters from retention times of known standards using a Varian Model CDS 401 integrator.

Animal diets

Male Wistar rats (Charles River Laboratories, Quebec, Canada) weighing between 100 and 120 grams were used. Animals were individually housed in stainless steel cages in a room with controlled temperature (20–23 °C) and lighting (alternative 12 h periods of light and darkness). Animals were fed a pelleted commercial laboratory chow (Purina Lab Chow) for 4 days after arrival. Two experiments were conducted to determine the cholesterolemic

Table 1. Composition of control and experimental diets (w/w%)[a]

Ingredient	Experiment 1 (Cholesterol-free diet)		Experiment 2 (Cholesterol diet)	
	I Control	II Experimental	III Control	IV Experimental
Casein[b]	20	20	20	20
Corn starch	15	15	15	15
Sucrose[b]	49.85	49.85	48.85	48.85
Non nutritive fiber	5	5	5	5
Mineral mixture[bc]	3.5	3.5	3.5	3.5
Vitamin mixture[d]	1.0	1.0	1.0	1.0
Choline chloride	0.2	0.2	0.2	0.2
Corn oil[e]	5	–	5	–
Rubber seed oil	–	5	–	5
D-L methionine[b]	0.3	0.3	0.3	0.3
L-Lysine HCl	0.15	0.15	0.15	0.15
Cholesterol[b]	–	–	1.0	1.0

[a] There were 6 animals in each dietary group.
[b] ICN Nutritional Biochemicals Corp., Cleveland, OH.
[c] Mineral mixture. ICN 76; Nutritional Biochemicals Corp. Cleveland OH.
[d] Vitamin mixture. ICN 76; Nutritional Biochemicals Corp. Cleveland OH.
[e] Best Food brand – Div. Canada Starch Comp. Montreal, Que.

response of rats fed RSO. In experiment 1, rats were fed a cholesterol-free diet which included either corn oil (control) or RSO (experimental). In experiment 2, both the corn oil and RSO diets were supplemented with 1 percent cholesterol. The experimental diets (Table 1) were administered for 28 days. All diets were stored at 4 °C prior to daily feeding of the animals. Food intakes were recorded each day and animals were weighed once a week. At the end of each experiment, animals were lightly anesthetized with ether and blood was withdrawn from the inferior vena cava. The livers were also excised, weighed and frozen at −29 °C until latter analysis.

Analytical methods

Serum from non-fasted rats was separated by centrifugation (3000 × ***g*** for 20 minutes) and stored at −20 °C until analyzed for total serum cholesterol [22] and triglycerides [26] by enzymatic colorimetric methods (Boehringer Mannheim). Serum lipoproteins were separated in duplicate runs from pooled serum samples obtained in each group by density gradient ultracentrifugation methods [23]. Ultracentrifugation tubes were centrifuged in a SW 40 rotor for 22 h at 272 000 × ***g*** at 20 °C in a Beckman ultracentrifuge. Rat serum lipoproteins were separated at density ranges of

$1.0063 < p_{20} < 1.1019$ for Chylomicron-VLDL; $1.019 < p_{20} < 1.063$ for LDL ($LDL_1 + LDL_2$) and $1.063 < p_{20} < 1.21$ for HDL, respectively. Lipoprotein cholesterol was measured enzymatically as mentioned above. Liver lipids were extracted by the method of Folch [7] and liver cholesterol was determined by the method of Carlson and Goldfarb [4]. All statistical analysis was performed using the Student's *t*-test.

Results and discussion

The addition of RSO to both cholesterol-free and cholesterol-supplemented diets had no adverse effect on food intake (Table 3). Rats consuming the cholesterol-free diet containing RSO actually exhibited significantly ($P < 0.05$) greater average daily gains than the corn oil control animals. A similar increase in the average daily gain of rats fed RSO in cholesterol-supplemented diets was also observed; however, the differences between the two groups was not statistically significant. These results would indicate that the addition of RSO to both diets had no toxic or antipalatability effects.

Table 2. Physicochemical properties of Rubber Seed Oil

Property	Rubber Seed Oil
Refractive index (25 °C)	1.469
Iodine value (25 °C)	138.0
Saponification value	192.0
Unsaponified matter (%)	4.8
Saponified matter (%)	95.2
Fatty acid (%)	
14:0	0.08
16:0	9.57
16:1	0.14
18:0	10.60
18:1	27.31
18:2	34.90
18:3	17.30
20:0	0.57
20:1	0.18
22:0	0.15
24:0	0.12
Total saturated	20.79
Total unsaturated	79.82
Total EFA content[1]	52.20
P/S	2.25

[1] EFA = essential fatty acids.

Table 3. Effect of diet on food intake, weight gain and liver weight[1,2]

	Experiment 1 (Cholesterol-free Diet)		Experiment 2 (Cholesterol Diet)	
	I Control (CO)	II Experimental (RSO)	I Control (CO)	II Experimental (RSO)
Daily food intake (gm)	20.2 ± 1.8	20.8 ± 0.7	19.3 ± 1.0	20.4 ± 1.1
Average daily gain (gm)	6.41 ± 0.02	7.30 ± 0.02*	6.73 ± 0.08	6.95 ± 0.10
Liver weight (gm)	13.8 ± 0.5	14.9 ± 0.7	13.6 ± 0.9	17.3 ± 0.3*
(% body weight)	4.77 ± 0.2	4.78 ± 0.5	4.75 ± 0.36	5.56 ± 0.13**
Condition[3]	Normal	Normal	Fatty	Fatty

[1] Means ± SEM.
[2] CO = Corn oil; RSO = Rubber seed oil.
[3] Condition was assessed by visual appearance.
* $p < 0.05$.
** $p < 0.025$.

Moreover, the observed effects of RSO on lipid metabolism could not be attributed to a severe dietary restriction due to an indigestible oil.

The physiochemical properties of RSO are presented in Table 2. RSO was found to contain a significant amount of unsaponifiable matter. This observation may only be pertinent to the lower serum cholesterol levels observed in rats fed the cholesterol-free diet (Table 4). Former studies have demonstrated plant sterols to possess an antiatherogenic property [11] by inhibiting cholesterol absorption [20]. More recently, other studies have also shown that the level of β-sitosterol in corn oil will account for the enhanced excretion of other sterols [5]. An unidentified component of the unsaponifiable matter present in rice bran oil has also been attributed to the lowering of cholesterol in rats fed both cholesterol-free and cholesterol-containing diets [21]. In the present study, both the serum and liver total cholesterol levels were significantly ($P < 0.05$) reduced and triglycerides unaffected in animals fed 5% RSO, when compared to the corn oil control animals fed the cholesterol-free diets. This particular reduction in total serum cholesterol was characteristic of a general reduction in the cholesterol composition of the various lipoprotein fractions examined, with little difference in the overall atherogenic index.

It has also been demonstrated by numerous workers that the degree of cholesterol lowering of a dietary lipid cannot be attributed simply to the amount of fat, but also the source of fat [3, 12, 19]. Both the degree of saturation and chain length of dietary triglyceride has recently been shown to be important in regulating serum and liver cholesterol levels [5]. In some cases this property has been attributed to an increased fecal sterol excretion [25]. A high percentage of polyunsaturated fatty acids (PUFA) in the diet

Table 4. Effect of diet on serum lipid, lipoprotein and liver lipids[1]

	Experiment 1 (Cholesterol-free diet)		Experiment 2 (Cholesterol diet)	
	I Control CO	II Experimental RSO	I Control CO	II Experimental RSO
Serum lipids (mg/dl)				
Total cholesterol	116.3 ± 6.55	92.5 ± 9.0*	126.4 ± 7.0	137.4 ± 8.7*
Triglycerides	127.7 ± 14.4	128.3 ± 11.2	177.3 ± 3.1	193.5 ± 8.7*
HDL cholesterol	89.6	63.0	51.4	45.3
$\frac{\text{HDL cholesterol}}{\text{Total cholesterol}}$	0.77	0.68	0.41	0.33
Chl-VLDL cholesterol[2]	16.74	14.85	24.89	58.34
LDL cholesterol[3]	20.28	16.25	40.16	31.34
$\frac{\text{HDL cholesterol} \times 100}{\text{Chl-VLDL} + \text{LDL cholesterol}}$	242	202	79	51
Liver cholesterol (mg/g)	5.06 ± 0.39	4.14 ± 0.31*	21.64 ± 1.87	23.33 ± 2.81

[1] Means ± SEM; lipoprotein cholesterol determinations represent duplicate analysis of pooled serum samples.
CO = Corn oil; RSO = Rubber seed oil.
* $p < 0.05$.
[2] Chl-VLDL cholesterol = pooled chylomicron + VLDL lipoprotein fraction $1.0063 < p < 1.1019$.
[3] LDL-cholesterol = LDL_1 + LDL_2 lipoprotein fraction ($1.019 < p < 1.063$).

has been reported to lower plasma cholesterol [14] or affect cholesterol distribution between tissues and plasma compartments [10]. For example, lower liver cholesterol levels have been observed in animals fed diets containing safflower oil compared to more saturated oils such as coconut oil [12]. A majority of the evidence relating these effects to certain vegetable oils have been attributed to their linoleic acid (C18:2 $n = 6$) content [19]. In the present study, RSO was found to contain a considerably high proportion of α-linolenic acid (C 18:3 $n = 3$; Table 2), albeit the total essential fatty acid content of RSO (52.2%) was similar to that of corn oil (58.1%; [24]). The finding that RSO had a greater lowering effect than corn oil in a cholesterol-free diet, could suggest a possible hypolipidemic effect of linolenic acid in RSO in addition to linoleic acid. This hypothesis is supported by other studies which have shown linolenic acid to be equivalent to the unsaturated fatty acids present in fish oils in lowering cholesterol levels [6]. In addition, linolenic acid is known to suppress the metabolism of linoleic acid [8] and therefore may have indirectly influenced the cholesterol response of these animals by sparing linoleic acid.

The addition of cholesterol to both RSO and corn oil-containing diets resulted in hypercholesterolemia and lipidosis (Table 4). Other studies [9, 20] have formerly attributed hypercholesterolemia in both humans and rats fed cholesterol-containing diets to increases in cholesterol absorption, enhanced conversion of cholesterol to bile acids and excretion of neutral steroids. In contrast to the finding observed in the cholesterol-free diet, the presence of RSO in diets supplemented with cholesterol resulted in a significant ($P < 0.025$) increase in total serum cholesterol levels, when compared to control animals. This response to dietary cholesterol in rats fed RSO was characterized by a substantial increase in the serum chylomicron-VLDL cholesterol composition and a reduction in HDL cholesterol. As a result, the atherogenic index of rats fed RSO was altered considerably in the cholesterol-containing diets. Since the chylomicron-VLDL fractions represent the primary transport moieties for cholesterol from the intestine, these results imply that RSO could have facilitated the absorption of dietary cholesterol. This result would seem to contradict the apparent hypocholesterolemic affect of RSO observed in the cholesterol-free diet. On the other hand, this particular cholesterolemic response is likely specific to the exogenous supply of cholesterol in these animals and possibly reflects an alteration in the transport of cholesterol across the intestinal cell. Therefore not only the characteristic properties of RSO but also the level of dietary cholesterol are important in evaluating the cholesterolemic response of RSO. Further research is required to investigate how RSO may enhance the absorption of exogenous cholesterol.

Conclusion

In this study an attempt has been made to evaluate the nutritional and toxicological properties of an oil extracted from the rubber seed. These initial results indicate that the use of RSO as an edible oil will not be restricted by toxic or antipalatability factors. In Western countries a trend towards an increased intake of vegetable fat and particularly unsaturated fatty acids in the diet has occurred. A majority of the vegetable fats and oils consumed are derived from soybean oil which is not only an excellent source of linoleic acid but also contains a relatively high proportion of linolenic acid (7–10%, [24]). In the present study, RSO was shown to be a rich source of α-linolenic acid and to a lesser extent linoleic acid. Although its usefulness as a source of edible oil in various foods could be limited by its susceptibility to auto-oxidation and production of off-flavors, due to the high α-linolenic acid content, it is feasible that it could be utilized as a blend with other vegetable fats, such as palm kernel, which contains predominately saturated fatty acids. With the increasing evidence that now points to n-3 fatty acids as being essential nutrients, with distinctly different and important physiological functions to that of the n-6 family [15], recent recommendations have prudently encouraged an adequate amount of n-3 fatty acids in the diet, especially during pregnancy, lactation and infancy. The economic feasibility of increasing the agricultural productivity of this potential oil for its source of n-3 fatty acids remains to be determined.

References

1. Achinewhu SC (1983) The saponins content of some Nigerian oil seeds. Qual Plant Plant Foods Hum Nutr 33: 3–9
2. AOAC (1984) Official Methods of Analysis. 14th edn. Assoc. Official Analytical Chemists, Arlington, VA
3. Beveridge JMR, Connell WF, Mayer GA (1956) Dietary factors affecting the level of plasma cholesterol in humans: The role of fat. Can J Biochem Physiol 34: 441–455
4. Carlson SE, Goldfarb S (1977) A sensitive enzymatic method for the determination of free and esterified tissue cholesterol. Clinica Chimica Acta 79: 575–582
5. Clifford AJ, Smith LM, Creveling RK, Hamblin CL, Clifford CK (1986) Effects of dietary triglycerides on serum and liver lipids and sterol excrection of rats. J Nutr 116: 944–956
6. Dam H, Kristensen G, Nielsen GK, Sondergaard E (1959) Influence of dietary cholestrol, cod liver oil and linseed oil on cholesterol and polyenoic fatty acids in tissues from fasted and non-fastest chicks. Acta Physiol Scand 45: 31–42
7. Folch J, Lees M, Sloane-Stanley GHS (1957) A simple method for the isolation and purification of total lipids from animal tissue. J Biol Chem 226: 497–509
8. FAO Food and Nutrition Series (1977) Dietary fat and oils in human nutrition. No. 20. Report of an expert consultation by FAO and World Health Organization, Rome 177

9. Grundy SM, Ahrens EH (1970) Measurements of cholesterol turnover, synthesis and absorption in man, carried out by isotope kinetic and sterol balance methods. J Lipid Res 10: 91–107
10. Grundy SM, Ahrens EH (1970) The effects of unsaturated fats on absorption, excretion, synthesis and distribution in man. J Clin Invest 49: 1135–1152
11. Ikeda I, Morioka H, Sugano M (1979) The effect of dietary β-sitosterol and β-sitostanol on the metabolism of cholesterol in rats. Agric Biol Chem 43: 1927–1933
12. Kellogg TF (1974) Steroid balance and tissue cholesterol accumulation in germ-free and conventional rats fed diets containing saturated and polyunsaturated fats. J Lipid Res 15: 574–179
13. Levy RI, Rifkind B, Dennis B, Ernst N (1979) Nutrition, Lipids and Coronary Heart Disease. Raven Press, NY
14. Moore RB, Anderson JT, Taylor HL, Keep A, Frantz D (1968) Effect of dietary fat on the fecal excretion of cholesterol and its degration products in man. J Clin Invest 47: 1517–1531
15. Newringer M, Connor WE (1986) n-3 fatty acids in the brain and retina. Evidence for their essentiality. Nutr Rev 44: 285–294
16. Nwokolo E, Akpapunam M (1986) Content and availability of nutrients in rubber seed meal. Trop Sci 26: 83–88
17. Nwokolo E, Sim JS (1987) Nutritional assessment of defatted oil meals of melon (Colocynthis citrullus L) and fluted pumpkin (Telfaria occidentalis Hook) by chick assay. J Sci Food Agric 38: 237–246
18. Orok EJ, Bowland JP (1974) Nigerian pararubber seed meal as an energy and protein source for rats fed soybean meal or peanut meal supplemented diet. Can J Anim Sci 54: 239–246
19. Paul R, Ramesha CS, Gargaly J (1979) On the mechanisms of hypocholesterolemic effects of polyunsaturated lipids. Adv Lipid Res 17: 155–171
20. Raicht RF, Choen BI, Shefer S, Mosbach EH (1975) Sterol balance studies in the rat. Effects of dietary cholesterol and β-sitosterol on sterol balance and rate limiting enzymes of sterol metabolism. Biochem Biophys Acta 388: 374–384
21. Sharma RD, Rukmini C (1986) Rice bran oil and hypocholesterolemia in rats. Lipids 21: 715–717
22. Siedal J, Hagele EO, Ziegenhorn J, Wahlefeld AW (1983) Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. Clin Chem 29: 1075–1080
23. Terpstra AHM, Woodward CJH, Sanchez-Muniz FJ (1981) Improved techniques for the separation of serum lipoproteins by density gradient ultracentrifigation. Visualization by prestraining and rapid separation of serum lipoproteins from small volumes of serum. Anal Biochem 65: 42–49
24. Weihrauch JL, Brignoli CA, Keeves JB, Overson JL (1977) Fatty acid composition of margarines, processed fats and oils. Food Technol February 90–91
25. Wilson JD, Siperstein MD (1959) Effect of saturated and unsaturated fats on fecal excretion of end products of cholesterol [4-14C] metabolism in the rat. Amer J Physiol 196: 596–598
26. Ziegenhorn J (1975) Improved method for enzymatic determination of serum triglycerides. Clin Chem 21: 1627–1629