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Chemical, Nutritional and Toxicological Studies of Rice Bran Oil

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ABSTRACT

Rice bran oil (RBO) is an unconventional oil of very high potential availability in India. Edible grade RBO obtained by improved technology was systematically evaluated for its chemical, nutritional and toxicological properties using the protocol developed by the author.

RBO is similar to groundnut oil (GNO), in fatty acid composition, having 36% linoleic acid. The unsaponifiable matter of RBO (4·1%) is higher than GNO. Nutritional evaluation was carried out in weanling albino rats of the Wistar strain by feeding a diet containing 20% protein and 10% RBO, and adequate in other nutrients, for 3 months. A similar group with 10% GNO served as a control group. Growth, feed efficiency, mineral balance, fat absorption and serum haematology were comparable in both RBO- and GNO-fed animals. Serum and liver lipids showed a marked lowering effect in rats fed RBO, indicating its hypocholesterolemic effect. The hypocholesterolemic effects of RBO and its unsaponifiable matter were evaluated in a separate experiment in hypercholesterolemic diet-fed rats as well as in humans and were reported earlier.

Toxicological studies of RBO by the food safety evaluation protocol of WHO/FDA/DGHS were carried out in rats for three generations. The reproductive performance was comparable to GNO-fed animals. The results indicated that there were no abnormalities in any of the parameters studied, indicating the safety of RBO for human consumption.

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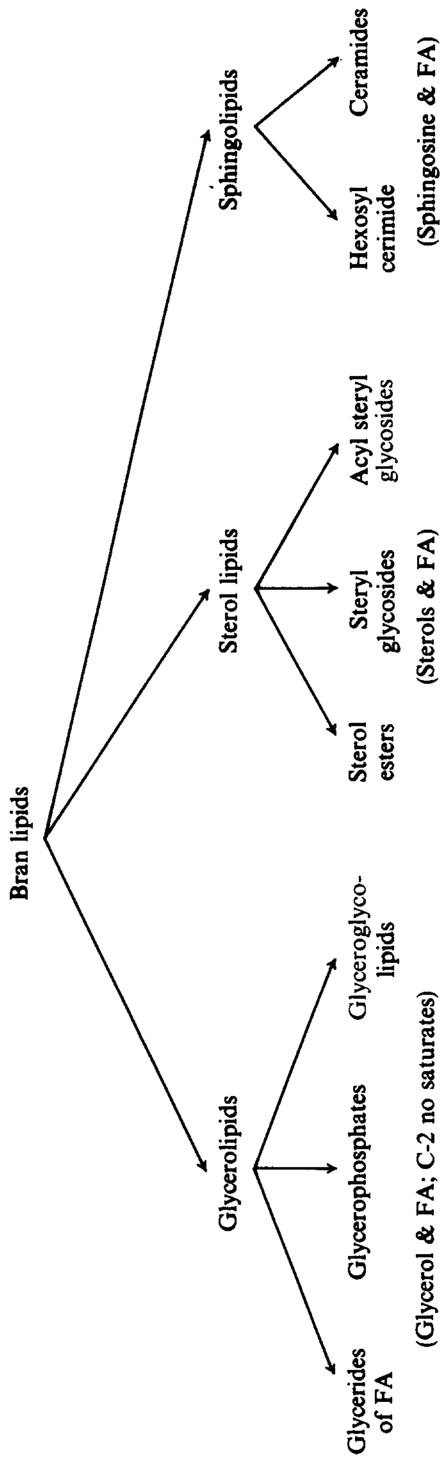

Fig. 1. Structural and metabolic relationships of bran lipids (after Fujino, 1978).

INTRODUCTION

Rice is the major cereal consumed in India. Rice bran is a by-product of the rice milling industry. Bran is the part between paddy husk and endosperm representing 10% of the rough rice kernel, and is obtained during polishing of rice. Bran consists of 15–20% oil. Rice bran oil (RBO) is an unconventional oil of high potential availability in India. Ninety million tonnes (MT) of rough rice are produced annually in India. Rice bran oil produced annually is 150 000 MT, out of which only 15 000 MT are of edible grade and the rest of the oil is diverted for industrial purposes. This is because of the high lipase activity of the bran causing deterioration of the lipids. High FFA and high unsaponifiables render the oil non-edible. The unsaponifiable fraction also contains appreciable amounts of waxes. As India spends a heavy foreign exchange budget on importing vegetable oils for edible purposes, unconventional oils of high potential are being evaluated (Rukmini, 1987). Rice bran oil is extracted for edible purposes in countries such as Japan, China, Korea, Pakistan, Taiwan and Thailand. Stabilization of rice bran has also been developed recently in India with the collaboration of the Brady Company, USA. Improved Japanese technology by a miscella refining process has been taken up by the Indian Government and as a result we are now able to produce edible grade rice bran oil in appreciable amounts. The edible grade rice bran oil thus obtained conforms to the Govt of India Central Committee of Food Standards (CCFS) regulation of FFA content, but the unsaponifiable matter is still high (4–6%). CCFS permits an unsaponifiable matter level of 3% or below for safe edibility.

Thus, a study has been undertaken to evaluate the safety of RBO with unsaponifiable matter around 4% following a comprehensive and systematic protocol which has been developed to evaluate safe edibility (Rukmini, 1986). Several unconventional oils were evaluated by this protocol and reported earlier (Rukmini *et al.*, 1982; Rukmini & Vijayaraghavan, 1984; Rukmini & Udayasekhara Rao, 1986; Rukmini, 1987). Bran lipids were classified into groups of glycerolipids, sphingolipids and sterol lipids by Fujino (1978). Their structural and metabolic relationships are as indicated in Fig. 1. The glycerolipids were analysed and their nutritional and toxicological implications are evaluated and reported now.

MATERIALS AND METHODS

Edible grade rice bran oil was obtained from a local factory for our entire experiment. The technology adopted for producing edible grade oil is as follows: (1) solvent extraction of fresh rice bran, (2) dewaxing the crude oil by miscella phase; (3) double solvent miscella refining using hexane and isopropanol; (4) distillation and recovery of dewaxed neutral RBO; (5) bleaching under vacuum; (6) deodorization at 200°C and absolute pressure 10–12 mm Hg.

Physico-chemical parameters of RBO were determined by AOAC methods (AOAC, 1973). Fatty acid composition was determined by GLC (Varian 3700) of the methyl esters on a DEGS column on Chromsorb W (45–60 mesh) and FID, isothermally at 200°C. Peak areas were calculated by triangulation. Phytosterols, γ-oryzanol, tocopherols and squalene were analyzed by GLC (Varian 3700) using an OV-17 column. Sigma Standards of the above were used for comparison.

Nutritional evaluation

Weanling albino rats of the Wistar strain were divided into two groups of 30 animals each (15♀ + 15♂) and fed a diet containing 20% protein from casein and 10% RBO or groundnut oil (GNO), and which was adequate in all nutrients, for 15 weeks. Animals were caged individually and food and water supplied *ad lib.* Weekly food intake and body weights of individual animals were recorded. Towards the end of 15 weeks animals were transferred to metabolic cages for three days and faeces were collected for three days. Diet and faeces were analyzed for nitrogen, calcium and phosphorus (AOAC, 1984). Apparent retention of these nutrients was calculated from the diet intake and faecal excretion. Cholesterol (Abel & Brodie, 1952) and triglycerides (Bergmeyer, 1974) were estimated in the liver and serum of all animals at the end of the study.

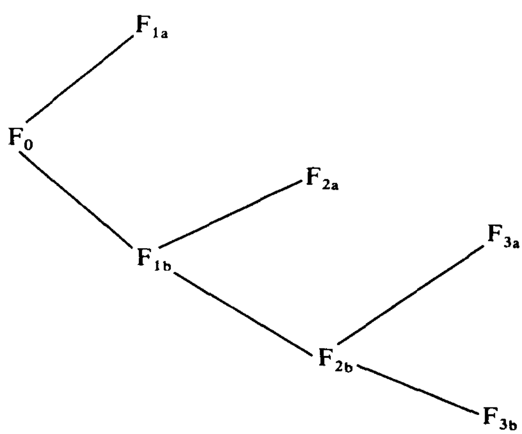

Fig. 2. Propagation of generations.

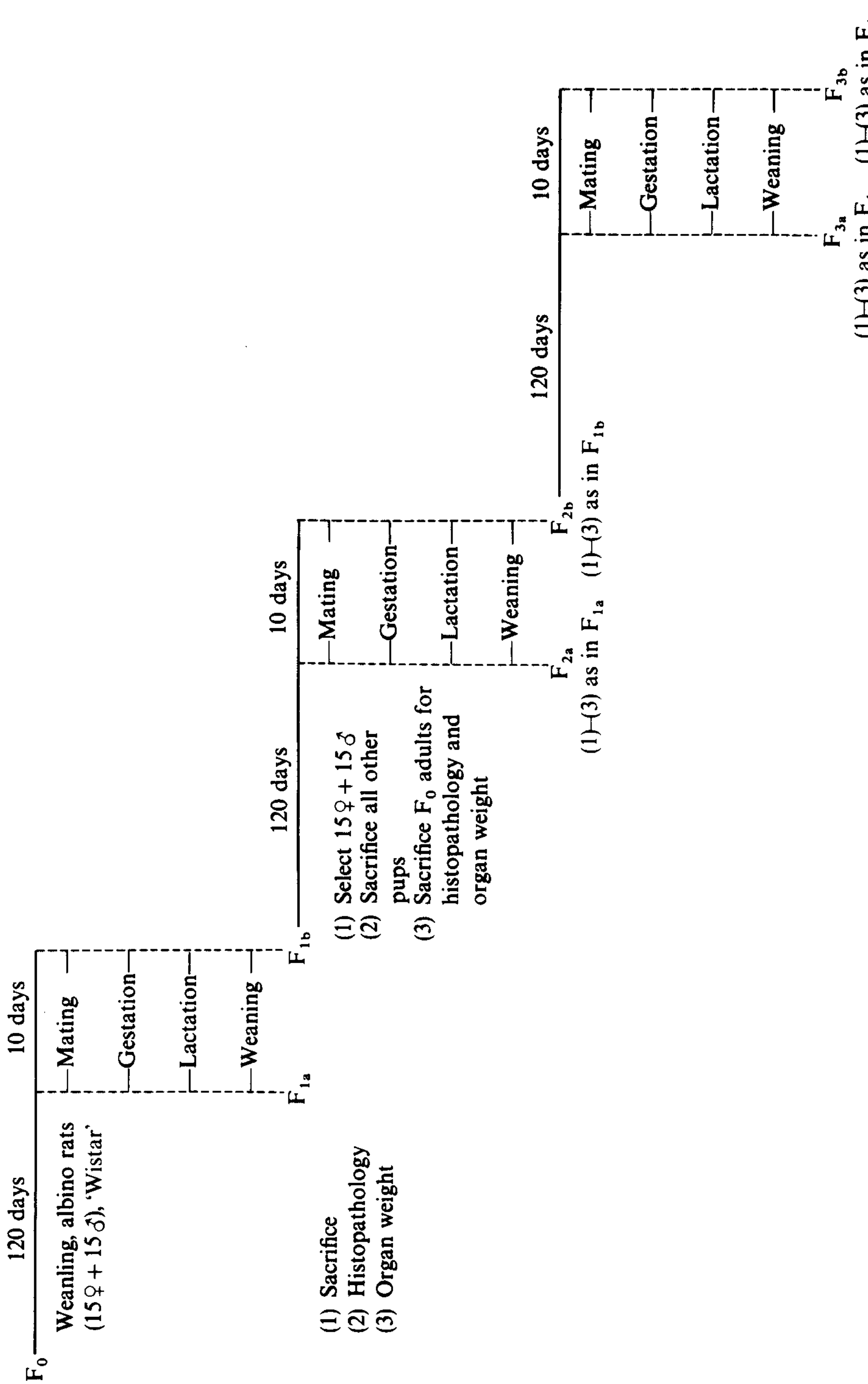

Fig. 3. Multigeneration breeding studies.

Toxicological studies

Three generation studies in rats were carried out as described in Figs 2 and 3 (Rukmini *et al.*, 1982).

Weanling albino rats of the Wistar strain, consisting of 15 males and 15 females in each group, were fed a diet containing 20% protein, 10% RBO/GNO and adequate in all other nutrients. Animals were individually caged. Food and water were supplied *ad lib.* Weekly body weight and food intake were recorded. At the end of 100–120 days of feeding the animals were mated as in Fig. 2. Mating, gestation, lactation and weaning were followed to obtain F_{1a} pups. A week after weaning, F_0 parents were mated again to obtain mating pups F_{1b} which were propagated for further generation. The procedure was continued until F_{3b} pups were obtained (Fig. 3). Reproductive performance according to Fig. 4 was assessed in all the matings up to the third generation. At the end of the third generation the animals were sacrificed as in the protocol in Fig. 3. Before sacrificing, blood was drawn from each animal and cholesterol and triglycerides were estimated; on sacrifice, the liver was also analyzed for total lipids, cholesterol and triglycerides. Tissues such as liver, heart, lungs, kidney, ovary/testes, pancreas and thymus were weighed and fixed in formalin and subjected to histopathological studies. Statistical analysis of all the data was done by Student's '*t*' test.

1. Percentage of conception
2. Mean litter size
3. Mean birth weight of pups
4. Mean weanling weight of pups
5. Preweanling mortality
6. Number of days taken to deliver from the date of introduction for mating (IFM)

Fig. 4. Reproductive toxicology.

RESULTS AND DISCUSSION

Physico-chemical parameters of RBO and GNO are presented in Table 1. The fatty acid composition of RBO is very close to GNO especially in 18:1 and 18:2 fatty acid contents. The unsaponifiable fraction contains appreciable amounts of sterols, especially β-sitosterol, γ-sitosterol, campesterol, tocopherols and squalene. The other major portion of the unsaponifiable fraction is γ-oryzanol which consists of ferulic acid esters of triterpene alcohols cycloartenol and 2,4-methylene cycloartanol. These compounds were already reported by Tanaka *et al.* (1971).

TABLE 1
Chemical Composition of Rice Bran Oil

Fatty acid composition[a]		*Unsaponifiable matter composition (4·1%)*
Rice bran oil (%)	*Groundnut oil (%)*	
$C_{16:0}$ 22·20	14·35	γ-oryzanol, cycloartenol, 2,4-methylene cycloartanol
$C_{18:0}$ 0·84	3·10	
$C_{18:1}$ 42·04	42·63	Sterols
$C_{18:2}$ 34·20	35·93	β-Sitosterol
$C_{18:3}$ —	1·25	γ-Sitosterol
$C_{20:0}$ —	2·71	Campasterol
		Tocopherol
		Squalene

[a] Measured by GLC on 15% DEGS on Chromosorb W and TID.

Table 2 represents the growth performance of rats fed 10% GNO/RBO over three generations. The gain in body weight over 24 weeks and the feed efficiency ratio of GNO/RBO-fed animals did not show a significant difference between the groups in all the three generations.

Table 3 shows the fat absorption, and apparent retention of nitrogen, phosphorus and calcium. RBO oil in the diet did not affect the retention of these nutrients adversely and the results are comparable to those of groundnut oil-fed animals. Table 4 shows the reproductive performance of animals fed RBO/GNO for two matings in each of three generations. The percentages of conception, birth weight, litter size, weaning weight and preweaning mortality were all comparable with the animals fed GNO in both the matings in all three generations. Table 5 shows cholesterol and

TABLE 2
Growth Performance of Rats Fed 10% GNO/RBO Over Three Generations

Generations	*Gain in body weight over 22 weeks (±SEM)*		*Feed efficiency ratio (±SEM)*	
	Groundnut oil	*Rice bran oil*	*Groundnut oil*	*Rice bran oil*
I (15♀ ± 15♂)	169·3 ± 9·85	168·3 ± 8·98	20·9 ± 3·3	21·6 ± 3·34
II (15♀ ± 15♂)	166·8 ± 13·25	157·4 ± 12·74	18·5 ± 1·74	17·8 ± 3·1
III (15♀ ± 15♂)	172·0 ± 31·38	162·6 ± 27·89	25·2 ± 3·29	18·4 ± 2·69

Values are mean ± SEM.
Numbers in parentheses are number of animals.

TABLE 3
Fat Absorption and Apparent Retention of Nitrogen, Calcium and Phosphorus in Rats Fed GNO/RBO at the End of the Third Generation (6♀ + 6♂ in each group)

Fat in the diet	*Fat absorption* (%)	*Nitrogen retention* (%)	*Calcium retention* (%)	*Phosphorus retention* (%)
Groundnut oil	98·56	56·55	69·4	82·6
Rice bran oil	98·72	60·20	72·8	74·6

triglycerides of serum, liver and heart of rats fed RBO/GNO for three generations. As can be seen from the table, RBO-fed animals exhibited a low lipid profile in serum, liver and heart, indicating the hypocholesterolemic nature of RBO. Table 6 shows the organ weights expressed as percentages of body weight of animals sacrificed at the end of study. The organ weights were comparable with GNO-fed animals.

The results of the above study indicated that RBO had a similar chemical composition to GNO, similar nutritional qualities to GNO and the absorption of the fat or retention of nitrogen or calcium or phosphorus were not impaired due to RBO in the diet. The reproductive performance also was found to be normal as compared with that of GNO-fed animals in both matings in all three generations (Table 4). The lipid profile of RBO-fed animals indicated hypocholesterolemic action. This was confirmed in a specially designed experiment where hypercholesterolemic rats were fed RBO at the 10% level for a period of 8 weeks. Significant lowering of total cholesterol, low density lipoproteins and very low density lipoproteins was observed both on cholesterol-containing and cholesterol-free diets. High density lipoprotein was increased but triglycerides were decreased. Liver cholesterol and triglycerides were also reduced. Faecal excretion of neutral sterols and bile acids was increased after ingestion of rice bran oil (Sarma & Rukmini, 1986). In another experiment, supplementation of the diet with the unsaponifiable fraction of rice bran oil (0·4%) in a hypercholesterolemic diet brought about a similar hypercholesterolemic effect (Sarma & Rukmini, 1987). Furthermore, these effects were confirmed in humans (Raghuram & Rukmini, 1987). Twelve patients with high cholesterol and triglyceride levels were selected from the Coronary Heart Disease ward from a local hospital. These subjects were using either palm oil or groundnut oil or combinations of these oils initially. These patients were not taking any hypolipidaemic drugs. They were advised to use rice bran oil as their normal cooking medium and the quantity was also not restricted. Edible grade rice bran oil was supplied by the Institute. Initial levels of serum cholesterol and triglycerides and the same parameters after 15 days and one month of using rice bran oil were

TABLE 4

Reproductive Performance of Rats Fed With 10% GNO and RBO in Three Generations

Details	*First generation (F_0)*				*Second generation (F_{1b} Adults)*				*Third generation (F_{2b} Adults)*			
	First mating (F_{1a})		*Second mating (F_{1b})*		*First mating (F_{2a})*		*Second mating (F_{2b})*		*First mating (F_{3a})*		*Second mating (F_{3b})*	
	GNO (12)	*RBO (12)*	*GNO (12)*	*RBO (12)*	*GNO (12)*	*RBO (12)*	*GNO (12)*	*RBO (12)*	*GNO (15)*	*RBO (15)*	*GNO (12)*	*RBO (12)*
Conception (%)	100	100	91·67	100	83·3	100	83·3	100	77·8	93·33	80·0	100
Mean litter size	7·83	8·33	9·27	10·33	9·8	9·42	8·10	7·0	6·42	6·78	—	7·13
Mean birth weight (g)	5·44 ± 0·05	5·48 ± 0·03	5·16 ± 0·04	5·06 ± 0·04	5·36 ± 0·10	4·94 ± 0·08	4·90 ± 0·08	5·43 ± 0·08	5·4 ± 0·15	5·21 ± 0·06	4·42 ± 0·15	6·9 ± 0·06
Mean weanling weight (g) (21 days)	19·1 ± 0·42	21·12 ± 0·51	18·9 ± 0·41	22·00 ± 0·52	18·3 ± 0·40	17·98 ± 0·57	26·26 ± 1·56	24·69 ± 0·46	37·4 ± 1·90	32·3 ± 1·45	36·32 ± 1·09	25·34 ± 0·66
Pre-weanling mortality (%)	8·51	—	7·84	10·48	34·67	25·66	71·60	16·88	55·56	30·53	39·19	14·58

Values of birth and weanling weights are mean ± SEM.
Numbers in parentheses are number of animals.

TABLE 5
Cholesterol and Triglycerides of Serum, Liver and Heart of Rats Fed GNO/RBO at the End of the Three Generation Study

Sample Number	*Group*	*Serum*		*Liver*		*Heart*	
		Cholesterol (mg %)	*Triglycerides (mg %)*	*Cholesterol (mg %)*	*Triglycerides (mg %)*	*Cholesterol (mg %)*	*Triglycerides (mg %)*
1	GNO (18)	98·8 ± 2·59	142 ± 6·75	198 ± 5·92	81·2 ± 7·04	99·2 ± 15·5	70·7 ± 21·6
2	RBO (12)	86·5 ± 1·92	108 ± 4·37	182 ± 7·03	50·4 ± 10·2	76·2 ± 3·29	34·1 ± 3·87

Values are mean ± SEM.
Numbers in parentheses are number of animals.

monitored. The results of the study indicated significant decreases in serum cholesterol and serum triglycerides after the use of rice bran oil for 15 days as well as 30 days. The decrease and rate of decrease of serum cholesterol and triglycerides were also significantly high as compared to the initial levels as well as a control group. A control group consisted of normal subjects (who were using GNO/Palm oil) whose serum cholesterol and triglycerides were monitored for one month. The results are reported elsewhere (Raghuram & Rukmini, 1987*a*). The hypocholesterolemic effect of RBO in humans was indicated by Suzuki *et al.* (1984) from Japan.

Tanaka *et al.* (1971) reported γ-oryzanol present as a major fraction of unsaponifiable matter. γ-oryzanol is reported to be a mixture of cycloartenyl ferulate ($C_{40}H_{58}O_4$), 2,4-methylene cycloartanyl ferulate ($C_{41}H_{60}O_4$) and steryl ferulate ($C_{38}H_{56}O_4$). Which of the factors are responsible for the hypocholesterolemic activity is under experimentation. Toxicological

TABLE 6
Organ Weights of Rats Sacrificed at the End of Three Generations Expressed as the Percentage of Body Weight (6♀ + 6♂ in each group)

Organs	*First generation*		*Second generation*		*Third generation*	
	GNO	*RBO*	*GNO*	*RBO*	*GNO*	*RBO*
Liver	3·94 ± 0·19	3·53 ± 0·11	4·02 ± 0·21	3·93 ± 0·06	3·36 ± 0·09	3·65 ± 0·14
Heart	0·34 ± 0·01	0·33 ± 0·02	0·31 ± 0·01	0·32 ± 0·01	0·28 ± 0·01	0·29 ± 0·01
Spleen	0·19 ± 0·01	0·18 ± 0·01	0·22 ± 0·01	0·22 ± 0·02	0·20 ± 0·01	0·21 ± 0·01
Lungs	0·71 ± 0·09	0·64 ± 0·07	0·90 ± 0·12	0·84 ± 0·04	0·61 ± 0·04	0·53 ± 0·03
Kidney	0·72 ± 0·02	0·72 ± 0·03	0·76 ± 0·02	0·74 ± 0·01	0·61 ± 0·01	0·65 ± 0·02
Testes	0·98 ± 0·03	0·89 ± 0·03	0·74 ± 0·09	0·58 ± 0·08	0·92 ± 0·04	0·90 ± 0·03
Ovaries	0·24 ± 0·02	0·23 ± 0·01	0·31 ± 0·03	0·36 ± 0·02	0·35 ± 0·03	0·39 ± 0·02

Values are mean ± SEM.

studies also indicated that the oil is toxicologically safe for human consumption. The reproductive toxicology indicated no abnormalities and is comparable with groundnut oil. Mutagenicity (Polasa & Rukmini, 1987*a*) assay was negative. Snacks deep fat-fried in RBO and repeatedly heated RBO were also tested for mutagenic potentials and found to be negative (Polasa & Rukmini, 1987*b*). Acceptability, keeping quality and the oxidative stability of the oil were tested by standard protocols and found to be comparable and even better than groundnut oil.

RBO has a beneficial effect of bringing down the circulating levels of serum cholesterol and triglycerides. The higher unsaponifiable matter has no adverse effect, but a beneficial effect has been noticed with unsaponifiable matter alone (Sarma & Rukmini, 1987). It has high levels of natural antioxidants and hence has a remarkable oxidative stability and keeping quality. In addition, RBO appears to be more acceptable and earned high scores in organoleptic evaluation (Raghuram & Rukmini, 1987*b*). Thus RBO can safely be recommended as an edible oil in a situation where acute shortage of edible oil is being experienced.

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