

The initial peroxide content of crude avocado and olive oil was found to be 5.85 and 5.10, respectively. From the results obtained for storage "on the shelf" exposed to daylight and those exposed to fluorescent light, it seems that crude avocado oil is more sensitive to photo-oxidation than crude olive oil. This can be explained by the high chlorophyll concentration in crude avocado oil and by the fact that crude olive oil contains higher amounts of polyphenols, serving as antioxidants, than crude avocado oil (16). On the other hand, no effects have been apparent in the samples oxidized in the dark, even though the natural antioxidant polyphenols exist in greater concentration in the crude olive oil than in the crude avocado oil. This may be due to chlorophyll acting as an antioxidant in the dark (18). The rate of peroxide formation in the dark was much lower than that obtained with crude oils that underwent oxidation at the two additional conditions tested. The gradual decrease of chlorophyll content in oils stored in the dark can be attributed to the presence of hydroperoxides, formed as a result of an earlier oxidation, reacting with chlorophyll.

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## ✿ Chemical and Nutritional Studies on *Terminalia bellirica* Roxb. Kernel and Its Oil

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*Terminalia bellirica* Roxb. is a valuable tree of Indian forests. The seeds are valued medicinally and also industrially, for tanning purposes. The kernels, which are not currently used for edible purposes, have 40% oil and 35% protein. The oil extracted from the kernels is sweet-smelling and has palmitic (35%), oleic (24%) and linoleic (31%) acids as major fatty acids. The proximate principles, antinutritional factors and amino acid composition of the protein of the kernel are analyzed. Short term feeding of the oil at 10% level in a 10% casein protein diet to rats for 4 weeks resulted in growth comparable to that observed with animals fed a similar diet containing 10% groundnut oil. The protein utilization of casein used in the diet, as judged by the protein efficiency ratio (PER) and net protein utilization (NPU), was not adversely affected by the *T. bellirica* oil in the diet. The liver and heart lipid profiles of both the groups as reflected by the parameters, total lipids, total cholesterol and triglycerides content were comparable except for the heart triglycerides of the TBO-fed group, which were elevated. The absorption of nutrients like calcium, phosphorus and nitrogen was not adversely affected by the intake of *T. bellirica* oil. *T. bellirica* oil is absorbed to the same extent as groundnut oil. The results of this preliminary

study indicate that *T. bellirica* kernel oil may be used for edible purposes because it is a good source of linoleic acid. However, long term toxicological studies are necessary to establish its safety before it can be recommended as an edible oil for human consumption.

Feeding a diet containing 10% *T. bellirica* kernel protein as a raw diet as well as a cooked diet to rats, mice and chicks resulted in low food intake and death in all three species, probably due to heat stable antinutritional factors in the kernel.

An acute shortage of traditional edible oils in India has created considerable interest in developing new sources of oils and fats and in evaluating their nutritional and toxicological properties to establish their suitability for edible purposes. Studies on *Hibiscus sabdariffa* oil, *Cleome viscosa* oil and mango kernel oil have been reported (1,2) from these laboratories.

*Terminalia bellirica* Roxb. (*combretaceae*) is a valuable tree of Indian forests (3). The fruit is known as myrobalan and is one of the constituents of 'triphalan,' an indigenous system of medicine (4), which is considered to cure a variety of disorders. The fruit provides a valuable export

market for the tanning and dyeing industry. Row and coworkers (5) investigated the fruit from which they isolated ellagic acid and gallic acid. The kernel is a by-product containing 40% oil and 35% protein. The oil is used in soap making and, by poorer classes, as a substitute for ghee (3).

## EXPERIMENTAL PROCEDURE

The mature fruits were purchased from the local market. The kernels were manually removed from the fruits. The kernels are deep yellow, their size and shape resemble the chick pea (Kabuli chenna) and they are soft and taste like groundnut. The oil was extracted with n-hexane in a Soxhlet extractor continuously for 36 hr and the solvent removed completely under vacuum. A pale yellow, sweet smelling oil was obtained.

**Chemical analysis of kernels.** Defatted kernels of *Terminalia bellirica* were ground to pass through 60 mesh. Protein content of the resulting meal ( $N \times 6.25$ ) was estimated by the Kjeldahl method. Fat, ash and minerals were determined according to AOAC procedures (7). Trace elements were determined in an atomic absorption spectrophotometer (Varian Techtron model AAS 1000) (8). Trypsin inhibitor activity was determined by the method of Kakade (9) and hemagglutinin activity by the method of Osawa and Matsumoto (10). For amino acid analysis ground defatted kernel sample was hydrolyzed with 6N constant boiling hydrochloric acid at 110 C for 24 hr in an evacuated sealed ampule. After hydrolysis, excess acid was removed by repeated flash evaporation under reduced pressure. The analysis was carried out by ion exchange chromatography in an automatic amino acid analyzer (11).

**Chemical analysis of the oil.** The oil was analyzed for various physicochemical parameters by conventional methods (12). The fatty acid composition of the methyl esters was determined by GLC (Aerograph Model 650) using 15% DEGS column on Chromosorb-W, mesh 45-60, with a flame ionization detector. Fatty acids were separated isothermally at 200 C, and the concentrations were calculated by triangulation. The fatty acid composition was expressed as percent of the total oil.

**Short term feeding studies of the oil and meal in rats.** Weanling male Wistar rats were divided into five groups of six animals each. The diets fed to these animals were as follows: Group 1, protein-free; Group 2, 10% casein protein + 10% groundnut oil (GNO); Group 3, 10% casein protein + 10% *T. bellirica* kernel oil; Group 4, 10% *T. bellirica* kernel protein (raw) + 10% GNO; Group 5, 10% *T. bellirica* kernel protein (cooked) + 10% GNO.

All the experimental diets were complete with respect to all other nutrients. Food and water were given ad lib for four weeks. Daily food intake and weekly body weights of individual animals were monitored throughout the experimental period. Toward the end of the experiment feces were collected from individual animals for 3 days. Diet and feces were analyzed for nitrogen, fat, calcium and phosphorus (13).

Diet No. 4 was fed to two groups of weanling male mice, six in each group, raw and cooked. Twelve one-day-old male chicks were fed only the raw diet.

Rats were killed by ether anesthesia at the end of 4 weeks. After the intestinal content was removed, the whole carcass was hydrolyzed by 6N HCl at 15 lb pressure for 2 hr. Nitrogen content of the hydrolysate was deter-

mined. One gram of liver and the complete heart of each animal of groups II and III were preserved before hydrolysis of the carcass for the estimation of total lipids, cholesterol (14) and triglycerides (15). The liver and heart nitrogen contents also were estimated and added to the carcass nitrogen. Protein efficiency ratio (PER), dry matter digestibility, protein digestibility and NPU were calculated (16).

## RESULTS AND DISCUSSION

The kernels of *Terminalia bellirica* contain 40% oil and 35% protein. Table 1 presents the physicochemical contents and fatty acid composition of the oil from *T. bellirica* kernels. The linoleic acid content (30%) is similar to that in groundnut oil. There are no unusual fatty acids, as can be seen from the fatty acid composition. The fatty acid composition is comparable to that of any other edible oil. The unsaponifiable fraction, however, is high and needs further detailed study. Refining of the oil may reduce the unsaponifiable matter.

Table 2 shows the proximate principles and antinutritional factors of the kernel, and Table 3 shows the amino acid composition of the kernel protein. The protein content is higher than in many commonly consumed legumes and oilseeds. Lysine content of the protein is slightly higher than many of the cereals and lower than most of the pulses. When compared to the WHO/FAO reference pattern, the essential amino acid concentration in kernel protein appears to be lower, except that of leucine, tyrosine and phenylalanine. Arginine and glutamic acid are in unusually high levels.

**Short-term evaluation of the oil.** Table 4 represents the growth pattern of the *T. bellirica* oil/groundnut oil fed group of rats over 4 weeks. As can be seen from the table, there is no difference in the gain in body weight of animals fed groundnut oil/*T. bellirica* oil over 4 weeks. The utilization of casein protein is not affected by *T. bellirica* oil in the diet, as can be seen by PER, dry matter digestibility and protein digestibility. The NPU also is not statistically

TABLE 1

### Physicochemical Composition of *T. bellirica* Kernel Oil

Fat content of kernel	40.9%
Saponification value	195.2
Acid value	1.2
Iodine value	73.6
Unsaponifiable	8.2%

### Fatty Acid Profile of *T. bellirica* Kernel Oil (TBO)/Groundnut Oil (GNO)

Fatty acids	% Concentration	
	TBO	GNO
C <sub>16:0</sub> Palmitic	35.57	14.35
C <sub>16:1</sub> Palmitoleic	2.00	—
C <sub>18:0</sub> Stearic	8.18	3.10
C <sub>18:1</sub> Oleic	23.55	42.63
C <sub>18:2</sub> Linoleic	30.70	35.93
C <sub>18:3</sub> Linolenic	—	1.25
C <sub>20:0</sub> Arachidonic	—	2.71

different between the two groups. These results indicate that the oil compares favorably with groundnut oil in its nutritive quality. Table 5 represents liver and heart, total lipids, cholesterol and triglycerides. The liver lipid pro-

TABLE 2

Proximate Principles and Mineral Composition of *Terminalia bellirica* Kernels

Moisture	6.4 g %
Protein content of kernels with fat	33.5%
Fat	40.9%
Ash	4.8%
Phosphorous	370 mg/100 g
Calcium	237 mg/100 g
Iron	4.3 mg/100 g
Zinc	4.0 mg/100 g
Magnesium	138 mg/100 g
Manganese	1.03 mg/100 g
Copper	1.28 mg/100 g
Chromium	0.21 mg/100 g
Trypsin inhibitory activity	620 TIU/g substance Specific Activity = 10 TIU/mg protein
Phyto hemagglutinin activity	NIL

TABLE 3

Amino Acid Composition of *T. bellirica* Kernel Protein (g/16 g N)

Lysine	3.22
Histidine	2.42
Arginine	17.50
Aspartic acid	7.06
Threonine	2.04
Serine	3.44
Glutamic acid	30.00
Proline	5.26
Glycine	6.22
Alanine	3.75
Valine	3.81
Isoleucine	3.46
Leucine	6.95
Tyrosine	2.07
Phenylalanine	3.25
Methionine <sup>a</sup>	0.72
Tryptophan <sup>a</sup>	0.86

<sup>a</sup>Methionine and tryptophan were estimated microbiologically in acid and alkaline hydrolysates, respectively.

TABLE 4

Effect of *T. bellirica* Oil on the Utilization of Casein

Group	Gain in body weight (g/4 weeks)	Protein intake (g/4 weeks)	PER	Dry matter digestibility %	Protein digestibility %	NPU
I GNO (6)	75.8 ± 4.82	21.1 ± 0.81	3.79 ± 0.10	91.0 ± 0.6	85.0 ± 1.4	72.7 ± 1.2
II TBO (6)	73.3 ± 3.69	22.5 ± 1.29	3.48 ± 0.46	91.0 ± 1.0	84.0 ± 0.84	69.8 ± 1.2

Values are mean ± SEM. The number of rats is given in parentheses.

file apparently is normal, though there is a fourfold increase in the heart triglycerides of the *T. bellirica* fed group. The significance of this needs to be established by long term studies. There is a wide variation of levels of triglycerides within the group of TBO fed animals. The GLC analysis of these heart lipids did not show an accumulation of any abnormal fatty acid in the heart. The high unsaponifiable matter may be responsible for the high triglyceride levels in the heart. This is under investigation.

Table 6 gives data on absorption of nitrogen, calcium, phosphorus and fat. There is no significant difference between the two groups, indicating *T. bellirica* oil is absorbed to the same extent as groundnut oil in these animals.

*Biological evaluation of kernel protein.* Food intake of raw as well as cooked kernel meal by experimental rats, mice and chicks was very low, around 1 g per animal per day. Rats and mice lost around 25% of their initial body weight by the end of the first week of the experiment; by the end of two weeks, all the animals receiving the raw and cooked *T. bellirica* kernel diet died. One-day-old chicks receiving the raw *T. bellirica* kernel diet died by the eighth day of the experiment. One chick which was morbid was killed; its organs, including the liver, kidney, spleen, heart and stomach, were found to be normal when examined.

These results indicate the *T. bellirica* kernel diet is not palatable even after cooking. During cooking, heat labile antinutritional factors like protease inhibitors are destroyed. Heat stable antinutritional factors may be present in kernels and may be responsible for the low food intake by the three species of animals tested here. Hence, *T. bellirica* kernels may not be useful for animal or human consumption unless they are further processed to remove the heat stable antinutritional factors present. However, the protein can be used as an adhesive in industry (3).

The results of the preliminary study on *T. bellirica* oil indicate that the kernel oil may be edible, as inferred from its chemical composition, fatty acid profile and short term biological evaluation. It appears to be a good source of linoleic acid. However, refining the oil and long term feeding studies may be necessary before recommending this oil for edible purposes.

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TABLE 5

Lipid Profile of Heart and Liver of Rats Fed 10% GNO/TBO Over 4 Weeks

Group	Organ	Total lipid g/100 g tissue	Cholesterol mg/100 g total lipid	Triglycerides mg/100 g total lipid
GNO	I Liver (6)	3.6 ± 0.54	248 ± 12.2	242.0 ± 41.5
TBO	II Liver (6)	2.4 ± 0.15	158 ± 17.3	271.1 ± 31.4
GNO	I Heart (6)	5.2 ± 0.23	212 ± 15.7	83.6 ± 24.0
TBO	II Heart (6)	4.9 ± 0.74	218 ± 7.5	330.6 ± 106.7 (range 127-400 mg)

Values are mean ± SEM. The number of rats is given in parentheses.

TABLE 6

Nitrogen, Calcium and Phosphorus Absorption in Rats

Fat in the diet	Fat absorption %	Nitrogen intake/day/rat (mg)	Absorption %	Calcium intake/day/rat (mg)	Absorption %	Phosphorus intake/day/rat (mg)	Absorption %
GNO	96.4	140	85.11	138.47	95.4	141.6	99.0
TBO	95.8	130	83.1	131.4	95.5	113.4	95.1

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