Lipid Classes, Fatty Acids, and Tocopherols of Leaves of Six Edible Plant Species†

Rudravarapu Sridhar and Gollamudi Lakshminarayana*

Indian Institute of Chemical Technology, Hyderabad 500007, India

Young leaves of six plants, namely *Tamarindus indica* (tamarind), *Oxalis corniculata* (Indian sorrel), *Rumex vesicarius* (bladder dock), *Alternanthera sessilis*, *Trigonella foenumgraecum* (fenugreek), and *Talinum triangulare* (Ceylon spinach), yielded, on extraction with chloroform–methanol 1.16, 1.47, 0.47, 0.54, 1.50, and 2.42% lipids (dry weight), respectively. Silica gel column chromatography yielded 37.0–80.5% neutral lipids (NL), 21.7–44.7% glycolipids (GL), and 1.2–26.5% phospholipids (PL). Capillary gas–liquid chromatography (GC) showed the major fatty acids to be 18:2 and 18:3 in NL, 18:3 in GL, and 18:3 followed by 18:2 and 16:0 in PL. *T. triangulare* leaf lipids contained medium-chain saturated fatty acids (C_{10}–C_{14}) in an appreciable amount (36.7%). GC–mass spectrometric and ultraviolet and infrared spectrophotometric analyses did not reveal the presence of γ-linolenic (GLA), trans, conjugated, or other unusual fatty acids. High-performance liquid chromatography showed the presence of α- and β-tocopherols but not other tocopherols and tocotrienols.

Leaves used in culinary preparations are rich sources of essential fatty acids, namely linoleic (18:2*n*–6) and linolenic (18:3*n*–3) acids (Lakshminarayana et al., 1984; Rao and Lakshminarayana, 1988). The present paper reports the contents of major lipid classes, their constituent fatty acids, and tocopherols in composite samples of young edible leaves of six plant species at a stage normally available to the consumer for culinary purposes. These are *Tamarindus indica* (L.) of Caesalpinaceae (tamarind), *Oxalis corniculata* (L.) of Oxalidaceae (Indian sorrel), *Rumex vesicarius* (L.) of Polygonaceae (bladder dock), *Alternanthera sessilis* (DC) of Amaranthaceae (syn. *A. triandra*, *A. denticulata*, *A. repens*), *Trigonella foenumgraecum* (L.) of Caesalpinaceae (fenugreek), and *Talinum triangulare* (Willd) of Portulacaceae (Ceylon spinach).

EXPERIMENTAL PROCEDURES

Materials. Tender leaves of plants grown in agricultural plots, which were harvested early in the morning, wrapped in wet jute bags, and transported within 4 h to the market, were purchased. *T. indica* and *O. corniculata* leaves were 10–15 days old, and the others were approximately 35–45 days old. Standard methyl esters of fatty acids were purchased from Nuchek Prep. Inc., Elysian, MN. Methyl ester of γ-linolenic acid (GLA) (18:3*n*–6) was purchased from Sigma Chemical Co., St. Louis, MO. Silica gel G (60–120 mesh) for column chromatography was purchased from Acme Synthetic Chemicals Ltd., Bombay, India. Column packing for GC was obtained from Supelco Inc., Bellefonte, PA. Palmvitae, a concentrate of tocopherols and tocotrienols, was donated by Palm Oil Research Institute, Kuala Lumpur, Malaysia. Standard tocopherols were purchased from Sigma.

Extraction of Total Lipids. The leaves were cleaned in running water and dipped in hot water at 95 °C for 2–3 min to inactivate the enzymes (Haverkate and Van Deenen, 1965). Total lipids were extracted according to the method of Kates (1986) using chloroform–methanol (1:2 v/v). Chlorophyll and other pigments were removed from the chloroform solution by adsorption on a short column (1.2-cm diameter) of a mixture (3–5 g) of activated charcoal and Celite 545 (2:1 w/w) (Khor, 1979).

Separation of Lipid Classes. Total lipids were fractionated into neutral lipids (NL), glycolipids (GL), and phospholipids (PL) by silica gel column chromatography (Carroll, 1976) using chloroform, acetone, and methanol, respectively, and estimated gravimetrically.

Table I. Total Lipid and Lipid Class Contents[a] of Edible Leaves

plant species	total lipids	NL	GL	PL
T. indica	1.16	0.93	0.07	0.16
O. corniculata	1.47	0.60	0.74	0.13
R. vesicarius	0.47	0.17	0.17	0.13
A. sessilis	0.54	0.30	0.15	0.09
T. foenumgraecum	1.50	0.98	0.38	0.14
T. triangulare	2.42	1.78	0.61	0.03

[a] Grams per 100 g of dry weight.

Fatty Acid Composition by Capillary GC. Fatty acid methyl esters (FAME) of the lipid classes were prepared using 14% (w/v) boron trifluoride in methanol (Morrison and Smith, 1964) and analyzed by a gas chromatograph (Tracor 540 GC, Tracor Instruments Inc., Austin, TX) fitted with a flame ionization detector and a Nelson PC integrator (Nelson Analytical, Inc., Cupertino, CA). A flexible, fused silica capillary column (0.24 mm × 30 m) coated with SP 2330 (film thickness 0.2 μm) (Supelco) was used. The injector and detector temperatures were maintained at 225 and 250 °C, respectively. The column temperature was held at 130 °C for 5 min, increased to 170 °C (8 °C/min), held at 170 °C for 8 min, again increased to 220 °C (5 °C/min), and maintained for 10 min. Nitrogen at 15 psig (ca. 1 mL/min) was used as the carrier gas. Peaks were identified by using standard FAME and quantitated by using methyl heptadecanoate (17:0) as internal standard.

Analysis of Fatty Acids by GC–MS. Pyrrolidine derivatives of FAME were prepared according to the procedure of Vetter et al. (1971). GC–MS was carried out using a Finnigan MAT 1020 B (San Jose, CA) automated instrument. The capillary column was the same as mentioned earlier, and the column temperature was held initially at 130 °C for 5 min and then raised to 220 °C (6 °C/min). Mass spectra of the FAME and their pyrrolidine derivatives were recorded under electron impact conditions.

Spectral Methods. IR spectra of 2–6% solutions of the lipids in carbon tetrachloride were taken using sodium chloride cells of 0.1-mm path length on a Perkin-Elmer 221 spectrophotometer (Perkin-Elmer Corp., Norwalk, CT) in the 200–4000-cm^{-1} range. UV spectra of 0.1% solutions of lipids in cyclohexane were obtained on a Beckman 26 UV-visible spectrophotometer (Beckman Instruments, Palo Alto, CA).

Analysis of Tocopherols and Tocotrienols. Individual tocopherols and tocotrienols were estimated by HPLC according to the method of Gapor et al. (1986) using a Shimadzu LC-6A instrument (Shimadzu Corp., Tokyo, Japan) with a fluorescence

* Author to whom correspondence should be addressed.

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Table II. Fatty Acid Composition[a] of Neutral Lipids of Edible Leaves

plant species	10:0	12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3	20:1	20:4	22:0	24:0
T. indica		1.1	2.2		21.7		3.0	9.1	29.8	24.0	2.0	1.0	2.3	3.8
O. corniculata		1.4	1.8		19.7		0.6	13.5	24.6	34.9	0.5	2.7		0.3
R. vesicarius		2.2	2.9	0.2	17.2	0.6	3.5	4.5	19.8	48.8		0.2	0.1	
A. sessilis		2.9	8.6		26.5		3.1	3.9	22.5	31.7		0.8		
T. foenumgraecum		1.3	2.2		7.6		4.3	7.1	44.6	32.9				
T. triangulare	1.0	20.7	15.0		24.1		2.1	5.0	9.3	22.8				

[a] The fatty acid content is expressed as weight percentage of total FAME.

Table III. Fatty Acid Composition[a] of Glycolipids of Edible Leaves

plant species	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3
T. indica	0.6	0.7	7.3		1.6	0.3	9.1	80.4
O. corniculata	0.2	0.4	2.9			0.3	2.6	93.6
R. vesicarius	6.9	5.7	38.2		1.7	1.0	6.3	40.2
A. sessilis	2.0	2.5	20.7		2.0	1.9	2.9	68.0
T. foenumgraecum	0.2	0.3	5.0	0.4		0.4	0.3	93.4
T. triangulare			14.7		4.1	0.3	3.0	77.9

[a] The fatty acid content is expressed as weight percentage of total FAME.

detector. A Zorbax Sil column (4.6 × 25 cm) was used. A mixture of hexane, tetrahydrofuran, and methanol (97.35:2.5:0.15 v/v/v) was used as eluant at the rate of 0.9 mL/min. The excitation and emission wavelengths were 298 and 325 nm, respectively.

RESULTS AND DISCUSSION

Total Lipid Content and Major Lipid Classes. *T. triangulare* leaves contained the maximum amount of total lipids and *R. vesicarius* leaves the least (Table I). The amounts of total lipids were lower (0.47–2.42%) than those reported (2.7–14.9%) for other edible plant species (Ifon and Bassir, 1980; Lakshminarayana et al., 1984, 1987; Rao and Lakshminarayana, 1988). Among the total lipids of the leaves, significant amounts of NL were present ranging from 37.0% in *R. vesicarius* to 80.5% in *T. indica*. The GL content varied from 5.8% in *T. indica* to 50.3% in *O. corniculata* and the PL content from 1.2% in *T. triangulare* to 26.5% in *R. vesicarius*.

Fatty Acid Composition of NL. Table II gives the fatty acid composition of NL. The saturated fatty acid content amounted to 62.9% of total fatty acids of NL in *T. triangulare* and varied from 15.4 to 41.1% in the others. The fatty acid composition of *T. triangulare* NL was significantly different from that of other leaf NL in that it contained high amounts of 12:0 and 14:0. The compositions of leaf and seed lipids are generally quite unrelated. With a few exceptions, the presence of unusual fatty acids is confined to seed tissue (Shenstone and Vickery, 1961; Kaimal and Lakshminarayana, 1970). Young leaves of *T. indica* contained 22:0 and 24:0, together to the extent of 6.1%, as does tamarind seed kernel oil, which was reported to contain high amounts of 20:0, 22:0, and 24:0 ranging from 14.3 to 20.0% of total fatty acids (Reddy et al., 1979; Adriamantena et al., 1983). The NL of *T. foenumgraecum* contained maximum amounts of unsaturated fatty acids followed by *O. corniculata* and *R. vesicarius*. The NL of *R. vesicarius* contained 18:3 and that of *T. foenumgraecum* 18:2 in maximum amounts compared to the other NL. The NL of *O. corniculata* contained a significant amount of 20:4, which is rarely found in higher plants but reported to occur in major amounts in mosses (Gellerman et al., 1975) and ferns (Lytle et al., 1976).

Fatty Acid Composition of GL. The predominant fatty acid was 18:3 in the leaf GL, except in *R. vesicarius* which contained 52.5% saturated fatty acids (Table III).

Table IV. Fatty Acid Composition[a] of Phospholipids of Edible Leaves

plant species	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:1
T. indica			28.9			3.1	36.4	25.8	5.8
O. corniculata			10.5		0.6		4.0	84.9	
R. vesicarius	1.2	1.4	34.3	3.4		3.6	16.7	39.4	
A. sessilis			22.2			3.2	23.7	50.9	
T. foenum-graecum		0.6	11.1	1.6	2.8	0.8	20.2	62.9	
T. triangulare	0.4	0.5	21.4	0.9	7.0	4.0	0.8	65.0	

[a] The fatty acid content is expressed as weight percentage of total FAME.

Table V. Tocopherol Contents[a] of Edible Leaves

plant species	α-tocopherol	β-tocopherol
T. indica	0.28	5.24
O. corniculata	1.58	6.18
R. vesicarius	6.19	3.37
A. sessilis	22.0	6.22
T. foenumgraecum	0.87	0.37
T. triangulare	0.42	

[a] Milligrams per gram of dry weight.

Fatty Acid Composition of PL. The major saturated fatty acid was 16:0 in the PL of all the leaves studied. The PL of *R. vesicarius* contained the highest amount of saturated fatty acids, followed by *T. indica* and *T. triangulare*. Present in a significant amount in *T. triangulare* PL was 18:0, while it was absent in most other PL samples. The major unsaturated fatty acid of leaf PL was 18:3, except in *T. indica* which contained higher amounts of 18:2. The PL of *R. vesicarius* contained small amounts of cis-16:1 followed by *T. foenumgraecum* and *T. triangulare* (Table IV).

A comparison of fatty acid compositions of NL, GL, and PL fractions of leaf lipids shows that NL contain high amounts of 18:2 and 18:3, GL contain high amounts of 18:3, and PL contain high amounts of 18:3 followed by 18:2 and 16:0. The leaf lipids of some species belonging to the Boraginaceae family were reported to contain considerable amounts of stearidonic acid (18:4*n*–3) and GLA (Jamieson and Reid, 1969). GC–MS of the pyrrolidine derivatives of FAME of leaf lipid fractions has confirmed the absence of GLA or 18:4. Eicosapentaenoic (20:5*n*–3) and docosahexaenoic (22:6*n*–3) acids, reported in purslane plant lipids (Omar-Alwala et al., 1991) but negated by Lercker (1992), were also not found. Analysis of leaf lipids by UV and IR spectrophotometry has also not revealed any trans, conjugated, or any other unusual fatty acid.

Tocopherol Content. Table V gives the tocopherol contents. *A. sessilis* leaves contained good amounts of α- and β-tocopherols. *O. corniculata* and *T. indica* leaves are good sources of β-tocopherols. Other tocopherols or tocotrienols were not found in these leaves. Good amounts of tocopherols ranging from 1 to 15 mg of α-tocopherols/100 g of tissue were reported in the green leafy tops of leeks and in the leaves of mustard, nettle, parsley, spinach, turnip, and yarrow (Dicks, 1965).

The results of the present investigation indicate that the leaf lipids of plant species, namely *T. indica*, *O. corniculata*, *R. vesicarius*, *A. sessilis*, *T. foenumgraecum*, and *T. triangulare*, are rich sources of essential fatty acids (18:2*n*–6 and 18:3*n*–3) and also α- and β-tocopherols.

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