



# Lipids from the seeds of seven Fijian plant species

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Lipids have been isolated from the seeds of seven plants indigenous to Fiji. The fatty acid essential to human growth, linoleic acid, was found to be the main fatty acid in the seeds of three, namely, *A. pavonina*, *A. moluccana*, and *S. taccada*. Seeds of *A. pavonina* contained two longer-chain fatty acids, lignoceric acid (24:0) (30.3 mol%) and cerotic acid (26:0) (7.5 mol%), which were characterised by GC-MS. The seed oil of *A. moluccana* seems unique in that it contains high levels of the fatty acids 18:2 (43.8 mol%) and 18:3 (25.8 mol%) and was the only seed oil to contain gamma-tocopherol. The seed oil of *D. vitiense* was the only oil that contained delta-tocopherol.

## INTRODUCTION

Lipids from seeds are important dietary constituents of the Fijian people. The seeds of the following plants are eaten by the indigenous Fijians, who make up about 50% of the population of Fiji: *Adenanthera pavonina*, *Barringtonia edulis*, *Dracontomelon vitiense*, *Inocarpus fagifer*, and *Terminalia catappa*. Indo-Fijians who were brought to Fiji from India by the British and who now form about 49% of the Fiji population do not eat these seeds but typically consume more saturated fats, such as ghee (clarified butter). Hospital records in Fiji for ischaemic heart disease showed that, for every one indigenous Fijian, twelve Indo-Fijians were admitted in 1970 (Jansen, 1991). A recent study undertaken between 1989 and 1991 showed that, in Fijian patients suffering from heart attacks, 81% were Indo-Fijians, whereas only 15% were indigenous Fijians. With the current alarm over the link between dietary lipids and atherosclerosis and other health problems such as ischaemic heart disease, we undertook to analyse the lipids of the seeds eaten by the indigenous Fijians, who are less prone to heart diseases than the Indo-Fijians. In the present investigation, we have also examined the lipids of the seeds of *Aleurites moluccana*, and *Scaevola taccada*. The seed oil of *A. moluccana* has been used as a fuel for lamps, and its use as a cooking oil is currently being investigated (Sotheeswaran, 1992). The

seeds of *S. taccada* are reportedly used as a contraceptive agent by Kiribati women (Taitai *et al.*, 1984). The lipid analyses were performed by using an HPLC-FID technique (Moreau *et al.*, 1990) that separates various lipid classes, and the fatty acids were also analysed by GLC and GC-MS. We also report the tocopherol composition of the seed oils. Tocopherols are known to function as antioxidants that inhibit the oxidation of metabolites such as vitamin A, unsaturated fatty acids, and phospholipids.

## MATERIALS AND METHODS

Seeds from the following plants were collected in the field (their Fijian names are given in parentheses): *Adenanthera pavonina* (Lele), *Aleurites moluccana* (Lauçi), *Barringtonia edulis* (Vutu), *Dracontomelon vitiense* (Tarawau), *Inocarpus fagifer* (Ivi), *Scaevola taccada* (Vevedu), and *Terminalia catappa* (Tavola). Seeds of *A. pavonina*, *B. edulis*, *D. vitiense*, *I. fagifer*, and *T. catappa* are commonly eaten and can also be purchased from the local market during certain seasons.

Lipids were extracted from crushed seeds (5–10 g) with hexane by using Soxhlet extractors. The hexane was removed by using a rotatory evaporator at reduced pressure. Seeds were extracted in Fiji, and the lipid extracts were packed under nitrogen and mailed to the USA for analyses. HPLC analyses of lipid classes in the oils were performed by a previously described technique (Moreau *et al.*, 1990). The HPLC system used for analysis of phytosterols and diacylglycerols

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consisted of an ISCO model 2350 HPLC pump, equipped with an ISCO Model 2360 Gradient Programmer, and a Tracor Model 945 Flame Ionization Detector. The mobile phase was a ternary gradient (Moreau *et al.*, 1990) of iso-octane–isopropanol–water, and the column (3 × 100 mm) contained 7- $\mu$ m Li-Chrosorb Si-60. For the analysis of individual tocopherols, the mobile phase was an isocratic mixture of hexane–isopropanol (99:1), and the column (3 × 100 mm) was a Chromsep Cartridge system (Chromapack Inc.) packed with 7- $\mu$ m Hypersil APS. Under these conditions, the retention times of authentic standards of alpha, beta, gamma, and delta tocopherols were 2.8, 6.2, 7.3, and 12.6 min, respectively. Phytosterols were also analyzed in this isocratic system, and they exhibited a retention time of 5.0 min. All phytosterols were eluted together. Thin-layer chromatography was performed by spotting samples on scored pre-adsorbent silica-gel G TLC plates (Analtech, 20 × 20 cm, 250  $\mu$ m). The plates were developed in hexane–diethyl ether–acetic acid (70:30:1.5 v/v/v). After development, plates were air-dried and visualized by placing them in a chamber of iodine.

For fatty-acid analyses, samples were hydrolyzed in 2.0 M methanolic KOH for 30 min at 25°C. The samples were then acidified to pH 3.0 with aqueous HCl and extracted with cyclohexane. After methylation with diazomethane, the fatty-acid methyl esters were baseline-resolved by using GLC (Hewlett–Packard 5880A; Supelco fused-silica capillary column, SP-2340: the column oven program was from 150°C to 160°C at 0.5°C/min, from 160°C to 170°C at 1.0°C/min, and from 170°C to 190°C at 1.5°C/min). A mixture of fatty-acid methyl ester standards was used to identify the common fatty-acid methyl esters. The fatty-acid methyl esters were quantified with an FID, and the concentrations were converted to mol% by correcting for the relative molecular mass. GC–MS was performed with a Hewlett–Packard model 5995B GC–MS operated at 70 Ev. For GC–MS, the column and temperature programs were identical to those described above for the GC analyses.

## RESULTS AND DISCUSSION

The levels of total extractable lipids were measured in the seeds of each of the seven species and ranged from 3 to 42% (Table 1). The seeds of all except those of *B. edulis*, *D. vitiense*, and *I. fagifer* were rich in triacylglycerols and could be considered 'oilseeds'. The seeds of *A. pavonina*, *A. moluccana*, *D. vitiense*, *I. fagifer*, and *T. catappa* contained phytosterols at sufficient levels to be detected by the Flame Ionization Detector. No steryl esters or steryl glycosides were detected. Four of the seed oils contained significant levels of diacylglycerols, and three of the seed oils contained low levels of alpha-tocopherol. *A. moluccana* was the only seed oil to contain gamma-tocopherol at detectable levels, and *D. vitiense* was the only oil to contain delta-tocopherol

**Table 1. Lipid composition of seeds from Fijian plant species**

Species	Phyto sterols* <sup>a</sup>	DAG* <sup>a,b</sup>	Tocopherols <sup>a</sup>			
			Alpha	Beta	Gamma	Delta
ppm						
<i>Adenanthera pavonina</i> (42%) <sup>c</sup> (Mimosaceae)	4.42	0	n.d.	n.d.	n.d.	n.d.
<i>Aleurites moluccana</i> (42%) (Euphorbiaceae)	0.24	0	100	n.d.	4 300	n.d.
<i>Barringtonia edulis</i> (3%) (Lecythidaceae)	0	1.77	n.d.	n.d.	n.d.	n.d.
<i>Dracontomelon vitiense</i> (5%) (Anacardiaceae)	0.29	2.65	n.d.	n.d.	n.d.	2 400
<i>Inocarpus fagifer</i> (4%) (Caesalpinaceae)	1.03	1.71	400	n.d.	n.d.	n.d.
<i>Scaevola taccada</i> (25%) (Goodeniaceae)	0	0	100	n.d.	n.d.	n.d.
<i>Terminalia catappa</i> (21%) (Combretaceae)	0.32	11.00	n.d.	n.d.	n.d.	n.d.

\* Expressed as percentage of oil (w/w).

<sup>a</sup> Remainder (not shown) consists predominantly of triacylglycerol.

<sup>b</sup> DAG = Diacylglycerol.

<sup>c</sup> Percentage composition of the seed oil with respect to the weight of the seed is given in parentheses. Data presented are the means of two separate extractions, and triplicate analysis were conducted for each extract.

n.d. = not detected (minimum limit of detection was 100 ppm).

above the detection limit of 100 ppm. Although our HPLC techniques (Moreau *et al.*, 1990) were capable of detecting polar lipids, none were detected (the minimum limits of detection were about 0.1 mg/g oil). Thin-layer chromatography confirmed the presence of triacylglycerol, diacylglycerols, phytosterols, and tocopherols in these samples. Individual diacylglycerols or phytosterols were not further characterized.

Table 2 reports the results of the total-fatty-acid analyses. As a result of the method of sample preparation, both free and esterified fatty acids are included in the analyses. The most significant observation is that the fatty acid linoleic acid (18:2 (9, 12C)) is the major component of the seeds of *A. moluccana*, *I. fagifer*, and *S. taccada*. This fatty acid is essential to human growth and development. *A. moluccana* was the only seed oil that contained high levels of linolenic acid (18:3) (25.8%), a fatty-acid that can often cause oils to be more rapidly oxidized. The seed oils of *A. moluccana*, and *S. taccada* were found to be the most polyunsaturated of the oils examined. The seed oil of *A. pavonina* was found to be the only oil that contained behenic acid (22:0) (3.2%), lignoceric acid (24:0) (30.3%), and cerotic acid (26:0) (7.5%). The fatty-acid compositions are expressed as percentage total fatty acids. The identities of the very long-chain fatty acids were confirmed by GC–MS, and the mass-fragmentation patterns for their methyl esters are reported below:

Table 2. Fatty-acid analysis of seed oils by GC

Species	Fatty acid mol % $\pm$ S.D.										
	16:0	16:1	18:0	18:1 9C	18:1 11C	18:2 9, 12C	18:3 9, 12, 15C	20:0	22:0	24:0	26:0
<i>A. pavonina</i>	9.3 $\pm$ 0.4	—	2.3 $\pm$ 0.1	14.4 $\pm$ 1.1	0.4 $\pm$ 0.1	26.0 $\pm$ 1.4	3.0 $\pm$ 0.2	1.0 $\pm$ 0.1	3.2 $\pm$ 0.3	30.3 $\pm$ 1.2	7.5 $\pm$ 0.3
<i>A. moluccana</i>	6.9 $\pm$ 0.5	—	—	20.3 $\pm$ 1.8	0.5 $\pm$ 0.1	43.8 $\pm$ 3.0	25.8 $\pm$ 1.3	—	—	—	—
<i>B. edulis</i>	43.7 $\pm$ 3.9	1.8 $\pm$ 0.2	24.8 $\pm$ 2.1	20.4 $\pm$ 0.7	0.3 $\pm$ 0.1	0.6 $\pm$ 0.1	—	3.3 $\pm$ 0.2	—	—	—
<i>D. vitiense</i>	7.1 $\pm$ 1.0	—	38.7 $\pm$ 1.7	48.1 $\pm$ 3.5	—	5.6 $\pm$ 0.2	—	0.6 $\pm$ 0.1	—	—	—
<i>I. fagifer</i>	34.6 $\pm$ 2.8	—	7.0 $\pm$ 0.2	16.4 $\pm$ 0.8	4.1 $\pm$ 0.3	34.9 $\pm$ 1.8	1.7 $\pm$ 0.2	1.6 $\pm$ 0.2	—	—	—
<i>S. taccada</i>	12.9 $\pm$ 1.4	3.9 $\pm$ 0.6	7.7 $\pm$ 0.5	22.0 $\pm$ 1.3	0.9 $\pm$ 0.2	53.2 $\pm$ 3.9	0.1	0.5 $\pm$ 0.1	—	—	—
<i>T. catappa</i>	32.4 $\pm$ 2.3	—	5.3 $\pm$ 0.4	37.3 $\pm$ 2.2	0.5 $\pm$ 0.1	24.5 $\pm$ 0.6	—	—	—	—	—

\* S.D. = standard deviation quoted for each concentration.

Data presented are the means of two separate extractions, and triplicate analyses were conducted for each extract.

Methyl ester of lignoceric (tetracosanoic) acid: *m/z* 382.80 ( $M^+$ , 6%), 143.35 (4), 129.35(2), 101.20 (3), 98.30 (3), 97.20 (6), 95.25 (3), 88.15 (4), 87.15 (6), 83.15 (10), 75.15 (34), 74.15 (100).

Methyl ester of cerotic (hexacosanoic) acid: *m/z* 410.75 ( $M^+$ , 8%), 143.35 (6%), 97.30 (6), 88.15 (4), 87.15 (67), 83.15 (12), 75.15 (44), 74.15 (100).

Analyses of two of these seed oils examined here have previously been published. The seed oil of *T. catappa* was reported to be rich in oleic, linoleic, palmitic, and stearic acids (Gupta *et al.*, 1983). Our results confirm this report and extend it by including the analyses of phytosterols, diacylglycerols, and tocopherols. A previous report also noted that the fatty acids in the seed oil of *A. pavonina* contained 25% lignoceric acid (Mubidri *et al.*, 1928). Our analyses revealed slightly higher levels of lignoceric acid (30.3%), 7.5% cerotic acid, and significant levels of phytosterols (see Table 1). No published information is available on the seed oils of the other plants reported herein. The data in this report indicate that the seed oil of *A. moluccana* may be superior to those others studied because it has high levels of the fatty acids 18:2 (43.8%) and 18:3 (25.8%). The seeds of this species also had high oil content (42%), and this oil was rich in gamma tocopherol.

The seed-lipid analyses reported in this study were obtained from seeds obtained in one growing season (1991) in one region of Fiji. Further work is required to

verify whether or not the seed-lipid analyses that were obtained in this study are representative of those that would be obtained from the same plant species grown in different regions and in other growing conditions.

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#### REFERENCES

- Gupta, R., Rauf, A., Ahmed, M. S., Jr., Ahmed, F., & Osman, S. M. (1983). Chemical screening of seed oils, *J. Oil Technol. Assoc. India*, **15**, 6-7.
- Jansen, A. A. J. (1991). Biochemical studies. In *Food and Nutrition in Fiji*, Vol. 2. An IPS Publication, University of the South Pacific, Suva Fiji, 1991, p. 299.
- Moreau, R. A., Asmann, P. T. & Norman, H. A. (1990). Analysis of major classes of plant lipids by HPLC with FID. *Phytochemistry*, **29**, 2461.
- Mubidri, S. M., Ayyar, P. R. & Watson, H. E. (1928). Seed fats, *J. Indian Inst. Sci.*, **11**, 173.
- Sotheeswaran, S. (1992). Lipids of Fiji, Unpublished work, University of the South Pacific, Suva, Fiji.
- Taitai, T., Cati, A., Tira, T. & Soetjahja, I. (1984). Traditional contraceptive study protocol prepared for the Ministry of Health & Family Planning, Kiribati, Fiji.