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# Fatty Acid and Sterol Compositions of Malagasy Tamarind Kernel Oils

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## ABSTRACT

The oil contents of six samples of Malagasy tamarind (*Tamarindus indica* L.) kernel were determined by hexane extraction (6.0–6.4%) and chloroform/methanol extraction (7.4–9.0%). The protein contents were very low (trace–0.1%). Investigation by gas liquid chromatography revealed 15 fatty acids, mainly palmitic (14–20%), stearic (6–7%), oleic (15–27%), linoleic (36–49%), arachidic (2–4%), behenic (3–5%) and lignoceric (3–8%) acids. Testing for the sterol fraction enabled seven sterols to be separated and quantitatively analyzed by gas liquid chromatography. The main sterols were β-sitosterol (66–72%), campesterol (16–19%) and stigmasterol (11–14%).

## INTRODUCTION

*Tamarindus indica* L. (Caesalpiniaceae) is cultivated in many parts of the world: India, Florida, Egypt, Sudan, Formosa, Southeast Asian countries (1) and Madagascar. This tree is probably indigenous to this last country (2) and possibly to some parts of South India (3). The fruit is a flat pod which on ripening gives an edible pulp. Brown and flat seeds are decorticated and powdered kernel, commercially known as tamarind kernel powder (TKP), is largely used in the textile industry (1) but also in food industries because of the occurrence of a characteristic tamarind seed polysaccharide which is able to form jellies with sugar concentrates over a wide pH range (1). The TKP contains 6–8% oil, which has been investigated for its fatty acid composition by several authors (4–10). Large differences in results have been observed in the fatty acid composition of the oil and in the protein content of the TKP between the Indian (4, 5, 8–10) and the Egyptian (7) products.

In Madagascar, tamarind trees grow almost all along the western part of the island. Fruit pulp is consumed by the population but the seeds are lost. Since this unused TKP may represent a potential agroeconomical interest for Madagascar, we have investigated the chemical characteristics of six seed samples of tamarind trees growing in various parts of the island. The fatty acid and sterol compositions were also analyzed using gas liquid chromatography (GLC) and combined GLC-mass spectrometry (GLC-MS).

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## EXPERIMENTAL PROCEDURES

### Materials

Tamarind fruits were collected during 1981 in six different areas of Madagascar island (Ambanja, Ambato-Boeni, Ambila, Majunga, Miandrivazo and Tulear). The tamarind kernel powders were prepared in the following manner: the seeds were washed with water in order to free them from attached pulp. The seeds were treated with concentrated sulfuric acid (36 N) and warmed at 80–100 C, for 30 min. The testae were dissolved slowly and the remaining white kernels were ground into powder.

### Physicochemical Methods

Moisture, total proteins, ash and unsaponifiable matter contents were determined according to NFT 60-201; NFT 18-100; NFT 60-209 and NFT 60-205 Norms (11), respectively. Oils were extracted either with hexane, or with chloroform/methanol (2:1, v/v) in a Soxhlet apparatus for 10 hr by a modified Folch method (12). The solvent was removed using a rotary vacuum evaporator.

### Analysis of Fatty Acid Composition by GLC

Fatty acid methyl esters were prepared by saponification of oils and acid-catalyzed methylation using $BF_3$-$CH_3OH$ (10%, Fluka, Switzerland) according to NFT 60-233 Norm (11). Purification of the methyl esters was done as previously described (13). Commercial saturated even-numbered methyl esters (Fluka), unsaturated and polyunsaturated (Sigma, St. Louis, MO) were used as standards for the identification of methyl esters together with vegetable oils (e.g., peanut, olive, sunflower).

A Girdel 30 gas chromatograph (Girdel, France) equipped with a flame ionization detector was used for the

analysis. The column employed was a 40 m long, 0.30 mm id, 0.15 $\mu$m $d_f$, glass capillary column coated with Carbowax 20M. Temperatures used were 180 C for the column and 255 C for inlet and detector ovens. Inlet pressure of hydrogen used as carrier gas was 0.8 bar (split 40 mL/min). Peak areas were integrated by a LTT ICAP 5 electronic integrator (Girdel, France).

### Hydrogenation of Fatty Acid Methyl Esters

Platinum oxide deposited on charcoal (25 mg) was added to a mixture of fatty acid methyl esters (500 mg) in hexane (6 mL). Hydrogenation was achieved by stirring the mixture for 16 hr at ambient temperature under a slight positive pressure of hydrogen. Ten saturated fatty acid methyl esters were identified by GLC. The composition for the Tulear oil sample was: 14:0, tr; 15:0, tr; 16:0, 12.1%; 17:0, 0.2%; 18:0, 77.4%; 20:0, 2.8%; 21:0, tr; 22:0, 3.0%; 23:0, tr and 24:0, 4.4%.

### Analysis of Sterol Composition by GLC

Sterols were separated from other unsaponifiable matter by thin layer chromatography (TLC) (14). Unsaponifiable matter in isopropyl ether was washed with water to neutrality. The organic phase was dried and evaporated under a stream of nitrogen with a rotary vacuum evaporator. The residue was dissolved in carbon tetrachloride to give a 5% solution. Testing was done by depositing 150 $\mu$L of the solution on a 0.25 mm thick, 60 F 254 silica gel plate (Merck, Darmstadt, W. Germany) and developing using an ethyl ether/chloroform (10:90, v/v) solvent system. Cholesterol used as standard was spotted for the identification of sterols. The developed plate was sprayed with Rhodamine-B and bands were examined under 366 nm ultraviolet (UV) light. The sterol band was traced and sterols were scraped off and extracted with dichloromethane. Trimethylsilyl ether (TMS) derivatives of components (50 $\mu$L of a 0.5 mL pyridine, 0.45 mL of hexamethyldisilazane (HMDS) and 0.3 mL of trimethylchlorosilane (TMCS)) were then injected at 265 C into a 40 m long, 0.29 mm id, 0.15 $\mu$m $d_f$, glass capillary column coated with OV17. Temperatures used were 280 C for inlet and 270 C for detector ovens. Relative retention times (RRT) were expressed against cholesterol-TMS.

### Analysis of Sterols by Combined GLC-Mass Spectrometry

Pure 24-methylene cholesterol and fucosterol were extracted from *Laminaria digitata* and *Fucus serratus*, respectively (15), and authentic commercial specimens of cholesterol, $\beta$-sitosterol, campesterol and stigmasterol were used for the identification of sterols. Analyses were performed on a Girdel-Ribermag R10-10B gas liquid chromatograph-mass spectrometer (GLC-MS) (Ribermag, France). The Girdel 30 chromatograph was fitted with a 25 m silica capillary column (0.33 mm id) coated with OV1701 (0.1 $\mu$m $d_f$). Operating conditions were: column, 250 C; inlet, 270 C; ion source, 250 C; helium as carrier gas, 0.5 bar; ionizing voltage, 70 eV. A Sidar system was used for the data computation.

## RESULTS AND DISCUSSION

The white kernel (54–60%) of the tamarind seed is covered by a brown testa (40–46%), as shown in Table I. Moisture, total proteins and ash content of tamarind kernel were determined. The most surprising result was the total protein content (N x 6.25) obtained (trace–0.1%) which is very low and in contradiction with some previous results (1, 9). Such low protein content was also observed by Morad et al. (7) in Egyptian tamarind cake. The six samples of Malagasy tamarind kernels when extracted with hexane gave 6.0–6.4% of oil and 7.4–9.0% with chloroform/methanol (Table I). The amount of neutral lipid is in good agreement with Rao and Srivastava (1) and Pitke et al. (5) but higher content (16.25%) was given for the Egyptian tamarind seeds (7). Upper oil content observed with chloroform/methanol extraction shows the presence of polar lipids. Indeed, Pitke et al. (8) have analyzed such polar lipids of tamarind kernel oil and isolated phosphatidylcholine and phosphatidylethanolamine. The unsaponifiable matter is quite similar in the hexane extracted oil (3.1–3.9%) and the chloroform/methanol extracted oil (3.1–3.6%), as shown in Table I.

The fatty acid composition of the six Malagasy tamarind kernel oil samples were determined as methyl esters by GLC using a glass capillary column coated with Carbowax 20M. The corresponding equivalent chain length (ECL) was calculated for each methyl ester peak. We investigated 17 fatty acids which on hydrogenation gave 10 linear saturated fatty acid methyl esters (see experimental procedures). A good agreement was observed between the fatty acid percentage before and after hydrogenation (taking into account the sum of saturated plus unsaturated fatty acids which have the same carbon number). The differences between raw and purified methyl esters were insignificant. Small differences were observed in the fatty acid composition of the hexane extracted oils and the chloroform/

**TABLE I**

**Proximate Analysis of Various Malagasy Tamarind Kernels**

| Seed origin | Ambanja | Ambato boeni | Ambila | Majunga | Miandrivazo | Tulear |
|---|---|---|---|---|---|---|
| Kernel/testa ratio | 60/40 | 54/46 | – | – | 55/45 | 55/45 |
| Moisture (%)[a] | 8.4 | 8.1 | 8.7 | 8.5 | 8.5 | 9.2 |
| Protein (%)[a,b] | – | tr. | tr. | 0.1 | – | – |
| Ash (%)[a,b] | 2.3 | 2.4 | 2.3 | 2.5 | 2.5 | 2.2 |
| Oil (%)[a,b] | | | | | | |
| Hexane extraction | 6.2 | 6.2 | 6.4 | 6.4 | 6.0 | 6.1 |
| Chloroform/methanol extraction | 9.0 | 9.0 | 8.8 | 8.7 | 7.4 | 8.6 |
| Unsaponifiable matter in oil (%)[a] | | | | | | |
| Hexane extraction | 3.6 | 3.9 | 3.1 | 3.2 | 3.5 | 3.7 |
| Chloroform/methanol extraction | 3.1 | 3.3 | 3.2 | 3.5 | 3.6 | 3.6 |

[a]Percent by weight.

[b]Dry matter basis.

methanol extracted oils. Linoleic acid was significantly higher in hexane extracts (42.8–57.6%) than in chloroform/methanol extracts (36.0–48.6%).

Complete results of the fatty acid composition of the chloroform/methanol extracted oils are given in Table II. The main fatty acids were palmitic (14.9–19.4%), oleic (15.3–26.3%) and linoleic (36.0–48.6%) acids. Stearic (6.2–7.0%), arachidic (2.3–3.3%), behenic (3.1–4.8%) and lignoceric (3.9–8.0%) acids were detected in small amounts. The presence of odd-numbered fatty acids such as pentadecanoic, heptadecanoic, heptadecenoic, heneicosanoic and tricosanoic acids was observed in minute amounts. As shown in Table II, the variation in composition for the six samples investigated is slight and our results are in agreement with those given by Badami et al. (4), Reddy et al. (9) and Pitke et al. (10) for the main fatty acids.

Seven sterols were investigated using an OV17 glass capillary column. The RRT were expressed against cholesterol-TMS and the compositions of sterol fraction of 3 samples investigated are given in Table III. Identification was made using mixtures of known sterols as standards (15) and GLC-MS of the trimethylsilyl ether derivatives. The configuration at C-24 of the sterols possessing an asymetric carbon atom at the 24 position (campesterol, stigmasterol and β-sitosterol) was not determined. The use of $^{1}H$ and $^{13}C$ NMR spectroscopy (16, 17) or gas chromatography with peculiar conditions (18) is necessary to assign a configuration at C-24. The more important were β-sitosterol (66.6–71.3%), campesterol (16.0–18.1%), stigmasterol (11.2–13.9%). Cholesterol, 24-methylene cholesterol, fucosterol and $\Delta_5$-avenasterol were detected in minute amount in all samples.

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**TABLE II**

**Fatty Acid Composition (Area %) of Malagasy Tamarind Kernels Oils—Comparison with Data of Various Origins**

| Fatty acid | ECL[a] | This work | | | Literature | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Indian | | | | Egyptian | Unknown |
| | | Min | Mean[b] | Max | (4) | (5) | (9) | (10) | (7) | (6)[c] |
| 12:0 | | – | – | – | 0.3 | tr | tr | tr | 28.2 | – |
| 14:0 | | 0.2 | 0.2 | 0.2 | 0.2 | tr | tr | tr | 0.4 | 1.0 |
| 15:0 | | tr | tr | tr | – | – | – | – | – | – |
| 16:0 | | 14.9 | 17.4 | 19.4 | 1.1 | 14.8 | 8.7 | 9.0 | 10.2 | 25.9 |
| 16:1ω9 | 16.18 | 0.3 | 0.3 | 0.3 | – | – | – | – | – | tr |
| 17:0 | | 0.2 | 0.2 | 0.2 | – | – | – | – | – | – |
| 17:1ω9 | 17.20 | tr | tr | 0.1 | – | – | – | – | – | – |
| 18:0 | | 6.2 | 6.7 | 7.0 | 6.6 | 5.9 | 4.4 | 5.0 | tr | 9.3 |
| 18:1ω9 | 18.19 | 15.3 | 19.5 | 26.3 | 15.0 | 27.0 | 19.6 | 19.0 | 24.3 | 27.9 |
| 18:2ω6 | 18.59 | 36.0 | 42.4 | 48.6 | 55.4 | 7.5 | 50.2 | 45.0 | 34.2 | 9.1 |
| 18:3ω6 | 18.97 | tr | 0.1 | 0.2 | – | 5.6 | 2.8 | 5.6 | – | { 7.7 |
| 20:0 | | 2.3 | 2.8 | 3.3 | 3.7 | 4.5 | 9.0 | 6.2 | – | |
| 20:1ω9 | 20.16 | 0.6 | 0.9 | 1.2 | – | – | – | – | – | – |
| 21:0 | | tr | tr | tr | – | – | – | – | – | – |
| 22:0 | | 3.1 | 4.0 | 4.8 | 1.8 | 12.2 | – | – | – | 5.6 |
| 23:0 | | tr | tr | tr | – | – | – | – | – | – |
| 24:0 | | 3.9 | 5.5 | 8.0 | 5.9 | 22.3 | 4.0 | 10.8 | – | – |

[a]ECL: equivalent chain lengths of fatty acid methyl esters on Carbowax 20M glass capillary column.

[b]Mean of 6 Malagasy samples.

[c]Mean of 2 samples, unidentified substances: 13.5%.

**TABLE III**

**Sterol-TMS Analysis of Various Malagasy Tamarind Kernel Oils[a]**

| Sterol | RRT[b] | Kernel oil origin | | |
|---|---|---|---|---|
| | | Ambanja | Majunga | Miandrivazo |
| Cholesterol | 1.00 | 0.2 | 0.6 | 0.2 |
| Campesterol | 1.29 | 16.0 | 18.0 | 18.1 |
| 24-Methylene cholesterol | 1.33 | tr | tr | tr |
| Stigmasterol | 1.41 | 11.2 | 13.7 | 13.9 |
| β-Sitosterol | 1.61 | 71.3 | 66.6 | 67.1 |
| Fucosterol | 1.70 | 0.1 | 0.2 | 0.1 |
| $\Delta_5$-Avenasterol | 1.79 | 1.2 | 0.9 | 0.6 |

[a]Area % determined on an OV17 glass capillary column.

[b]RRT is expressed against cholesterol-TMS.

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# Study of the Neutral Lipids of Sunflower Meal and Isolates

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## ABSTRACT

Two types of sunflower protein isolates have been obtained from prepress and solvent extracted sunflower meal. The first was obtained by precipitation (at the isoelectric point) of the alkaline extract of the meal, and washing the curd with water. In the second, the alkaline extraction was carried out in the presence of sodium sulfite, and the curd was washed with water, ethanol and acetone. Both isolates were air-dried and then dried under vacuum at 50 C. From the total lipids, obtained with 86% ethanol, the neutral lipids were separated using a column of Florisil. The lipids studied were those of the two isolates mentioned above as well as those of the original meal. The following types of compounds were separated, identified and quantified: hydrocarbons, waxes, methyl esters, triglycerides, free fatty acids, diglycerides, free sterols, and hydroxy fatty acids.

## INTRODUCTION

Due to the growth of world population, plant proteins will be more extensively used in the future. In developed countries, edible proteins are usually of animal origin, whereas in developing countries vegetable sources already account for more than 80% of protein supplies.

Traditionally, proteins from oilseeds have been byproducts of oil extraction, but now they are becoming increasingly important as foods. Oilseeds are one of the mainstays of the world food supply; sunflower being the second in importance, after soybean, as an oil source (1). Like other seeds, sunflower yields, after oil extraction, a meal which is a suitable source of proteins.

As far as we know, no previous studies have been reported on the lipid contaminants associated with sunflower proteins. Evidence for the presence of phosphatides in isolates from soybean meals was presented many years ago by Smiley and Smith (2). Eldridge et al. (3, 4) found phosphatidylcholine, phosphatidylethanolamine, saponines, sitosterol glycoside and genistein in soybean protein isolates. Neither free fatty acids nor hydroxy fatty acids were found in the lipids extracted with ethanol, which is surprising as they are rather polar compounds.

This paper describes the isolation and characterization of neutral lipids associated with the protein isolates obtained by alkaline extraction and isoelectric precipitation from sunflower meals.

## EXPERIMENTAL PROCEDURES

### Materials

Meals from prepress hexane-extracted, partially dehulled sunflower seeds were supplied by the industry. Hydrocarbons and normal alcohols were obtained from olive oil unsaponifiable (5). Pure octadecyl octadecanoate was a gift of H.K. Mangold. Pure triglycerides were obtained from olive oil and sunflower by preparative thin layer chromatography (TLC) on Silica Gel G. 1,2- and 1,3-diglycerides and cholesterol were commercial samples. A qualitative mixture of methyl ester fatty acids with different degrees of unsaturation was prepared by transesterification of an equilibrated blend of coconut oil and linseed oil. 12-Hydroxyoctadecenoic acid (ricinoleic acid) was obtained from castor oil; hydrogenation of this compound gave the corresponding saturated acid. 9- and 13-hydroxyoctadecanoic acids were obtained from linoleic acid by oxidation with soybean lipoxygenase, followed by reduction ($NaBH_4$) and hydrogenation (6).

### General Methods

Acid value and iodine value were determined with the micromethods of Gorbach (7). The colorimetric method of Vioque and Vioque (8) with N,N′-dimethyl-*p*-phenylendiamine was used for peroxide value determination. Standard methods for water, ash and fiber and the micromethod of Clark for nitrogen (9) were used. The total protein was calculated as total nitrogen × 6.25.

Free lipids refer to those extracted with hexane under continuous stirring for 6 h. Associated lipids were obtained, following the method of Nash et al. (10) by extraction with 86% ethanol at room temperature for 37 h and removing the nonlipid material according to Singh and Privett (11).

For the isoelectric point determination, 15 g of meal were extracted twice with 300 mL 0.2% NaOH solution. Forty-mL aliquots of the extract were titrated with 0.5 N HCl to various pH values ranging from 2.0 to 7.0. The curd formed was separated by centrifugation for 30 min at 4,000 rpm. The supernatant was decanted and its volume and nitrogen content were determined. The percentages of protein precipitated were plotted vs the different pH in order