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Original Study of the Biochemical and Oil Composition of the Cambodia Nut *Irvingia malayana*

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Analysis of the biochemical composition of *Irvingia malayana* was carried out. This Cambodian nut contains 7.5% water and 70% oil. Most of the fatty acids are saturated and include 42% C12:0 and 41.8% C14:0; the sterol composition is similar to that of other vegetable oils. This oil is less rich in α-tocopherol than in γ-tocopherol. Analysis of the solid content of the oil with respect to the temperature by NMR shows a fast fall of solid content around its fusion range at 38−39 °C. The main differences in the properties of the indigenous Cambodia nut from other known oleaginous seeds are in its selenium content, fatty acid composition, fusion temperature profile, and content of antioxidants. These important characteristics can soon make possible its application in pharmacology, cosmetics, the margarine industry, etc.

KEYWORDS: ***Irvingia malayana*; Cambodia nut; oil; sterol; tocopherol; fatty acid; mineral**

INTRODUCTION

The aim of this study is to find a specific and valuable character of the Cambodian nut *Irvingia malayana* that would enable it to find a place on the international market. If it is possible to launch this on a large scale, it will contribute to the economic development of Cambodia, which is a principal producer of this nut.

I. malayana is produced not only in Cambodia but also in Malaysia, Laos, Thailand, and Indonesia. This tree is known as the "pauh kijang", which means "mango of the stags" (*1*, *2*). It produces a popular and inexpensive wood in Southeast Asia (*3*). This wood is rather fragile and light. Mitsunaga et al. (*4*) found an interesting phenol composition. The nutritional value of *I. malayana* was evaluated by Laohawinit (*2*). The most interesting part of the nut seems to be its oil, which is used for food applications (*5*, *6*). Its antimalaria effects have also been reported by Ayudhaya et al. (*7*).

No information was found concerning the analysis of sterols and tocopherols of the Cambodian nut. We studied these interesting molecules in the oil. Phytosterols interfere with the absorption of cholesterol and prevent the increase in serum cholesterol (*31*−*36*).

Vitamin E is the name given to a group of eight molecules called tocopherols. It functions as a powerful antioxidant, which protects human cells and fatty molecules against free radicals, which could damage cellular membranes, skin, and cellular DNA, causing various cancers and degenerative diseases.

In combination with other antioxidants, it inhibits free radicals and prevents oxidation of polyunsaturated fatty acids (*45*). By neutralizing free radicals and by stabilizing cell membranes, vitamin E plays a role against cancer (*46*−*50*), immunizing disorders, and premature aging.

The family of *Irvingia* is very widespread in Africa, where the majority of the studies were carried out on the species *Irvingia gabonensis* (*8*, *9*). Laboratoires Sérobiologiques recently deposited a patent on the use of *I. gabonensis* in cosmetics (*10*).

The Cambodian name of this nut is "chambak" or "krabok". Jacquat (*11*) also gives for *Irvingia malayana Oliv.* ex A. Benn. the names of "ka bok", "cha bok", "tra bok", "cham-noh", "saang", "bok", "maak bok", "ma muen", "muen", "ma luen", and "lak-kaai". It is botanically classified in the family of Irvingiaceae with the Latin name *Irvingia malayana* (*12*). Soepadmo and Wong (*13*) gives a synonym, *Irvingia harmandiana* (van Tiegh.) Pierre ex Lecomte.

This nut is collected in great quantities in the majority of the forest areas of Cambodia. In this work the biochemical composition of this nut was analyzed with the aim of better utilization of this valuable raw material.

MATERIALS AND METHODS

The samples of the Cambodian nut used in this work were gathered in February 2000 from the province of Siem Reap, an area of Cambodia made famous by the temple of Angkor Wat. Since the beginning of the analyses, in April 2000, the nut samples were stored in a cabinet at ambient temperature with an average temperature of 25 °C. All of the analyses were done in 2 months.

Mineral Composition. Total ash was obtained by calcination of 1 g of sample at 525 °C for 90 min. Mineral elements were dosed by plasma emission spectroscopy from Varian Vista ICP (*14*, *15*). Analysis of total nitrogen was carried out by carbonization according to the method of Dumas using an Elementary analyzer Leco FP 528 (*16*).

Moisture Content. The moisture content of *I. malayana* nut determination was carried out in triplicate by drying at 105 ± 2 °C during 24 h.

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Biochemical Analysis. Amino acids were determined by RP-HPLC of derived amino acid residues using a Pico-Tag C18 column (3.93 mm i.d. × 15 cm, Waters). Samples (50 mg) were hydrolyzed under vacuum in 6 N HCl for 24 h at 110 °C in sealed tubes. Amino acids were then reacted with phenylisothiocyanate (PITC) with 20 mL of an ethanol/triethanolamine/PITC mixture (ratio 7:1:1:1) to form phenylthiocarbamyl amino acids. Elution was performed with a gradient of acetonitrile in 0.14 M sodium acetate buffer (eluant A, 100% buffer; eluant B, acetonitrile 60% in buffer v/v).

The factor relating the quantity of proteins to the quantity of nitrogen was calculated by accounting for the percentage of amino acids (*17*).

Oil was extracted from 20 g of Cambodian nut for 6 h by Soxhlet with hexane. The solvent was removed by evaporating and then flushing with nitrogen gas.

Fatty acid composition was performed by GLC (Ceinstruments, model GC 8000 Top) after methylation according to the formerly named standard NF T60-233, now NF IN ISO 5509 (*18*). Column Omegawax 250 is a grafted phase PEG of 30 m and diameter of 0.25 mm. The stationary phase has a thickness of 0.25 μm. The carrier gas was helium. Split injector temperature was 250 °C. Flame ionization detector temperature was 260 °C. The column was heated to 100 °C for 5 min and then a temperature program was applied at 10 °C/min to 230 °C.

Sterol analysis was performed on the unsaponified portion of the oil according to the standard NF ISO 6799 (*19*). The unsaponifiable portion is extracted from oil according to ISO standard 3596-1 (*20*). Silyl sterols are analyzed by GLC on a 30 m capillary column DP1701 (methylpolysiloxane, Chrompack) with a diameter of 0.32 mm heated at 245 °C. The stationary phase has a thickness of 0.15 μm. Carrier gas was hydrogen. Injector and flame ionization detector temperatures were 285 °C.

Tocopherol analysis was done according to standard ISO/FDIS9936 (*22*) on a liquid chromatograph (Spectra System P1000 XR) equipped with a Spectra System FL 3000 fluorescence detector used with a wavelength of excitation of 290 nm and emission of 330 nm. The HPLC column is a Hypersil of 250 mm and 4.6 mm diameter. Particles had an average diameter of 5 μm. The mobile phase is a mixture of 99% hexane and 1% 2-propanol. Its flow was fixed at 1 mL/min.

The solid content of the oil according to the temperature was determined by NMR on a Brucker Minispec PC120 spectrometer. Oil was melted at 80 °C and then homogenized and introduced into a 10 mm diameter and 177 mm long tube. The tubes were then put in an oven at 60 °C for 1 h and then placed in an ice bath at 0 °C for 60 ± 2 min. Then the tubes were dried and placed in a controlled bath at the lowest measurement temperature. After 30 min, the sample was transferred quickly to the NMR apparatus. The samples were then placed in another bath, controlled at the second temperature of measurement higher than the first one. After 30 min, the NMR spectra was measured. An identical procedure was used for all other temperatures.

RESULTS AND DISCUSSION

Mineral Composition. The moisture content of *I. malayana* nut was found to be 7.5 ± 0.2%. This content appears to be normal for a nut and varies with the environmental conditions.

Ash content obtained by calcination in triplicate was found to be 5.0 ± 0.1%.

Mineral composition does not show any particularly high content of any of the common minerals and is very close to the mineral composition of Grenoble walnut (**Table 1**).

I. malayana shows only half the phosphorus, double the calcium, magnesium, and iron, and 5 times more sodium than the Grenoble walnut. However, the selenium content is interesting (1.9 μg/g) because this mineral element has a role in the prevention of oxidative attacks and thus in the prevention of cancer (*22*). This content is higher than those observed in cereal grains (0.11 μg/g), green vegetables (0.01 μg/g), roots (0.005 μg/g), or fruits (0.005 μg/g) (*23*, *24*) and close to that observed in soybean (0.8−1.3 μg/g) in the United States (*25*).

Table 1. Mineral Composition of *I. malayana* (on Dry Matter)

mineral	Grenoble walnut (μg/g)	*I. malayana* (μg/g)
nitrogen	27200	23700
phosphorus	5000	2700
potassium	5000	5600
calcium	900	1700
magnesium	1280	2200
sodium	25	123
iron	45	108
copper	nd[a]	19
manganese	nd	300
zinc	nd	35
aluminum	nd	29
selenium	nd	1.9

[a] nd, not detectable.

Table 2. Amino Acid Composition of *I. malayana* Protein Compared with Brazil Nut Defatted Flour and Bean Flour

amino acid	bean flour (%)	Brazil nut defatted flour (%)	*I. malayana* (%)
aspartic acid	12.8	8.3	8.5
glutamic acid	15.9	19.5	19.4
serine	6.6	4.6	6.8
glycine	3.7	4.1	8.4
histidine	2.8	2.5	1.9
arginine	6.3	14.7	6.9
threonine	5.3	2.8	5.3
alanine	4.1	2.9	6.9
proline	4.5	4.7	4.9
tyrosine	3.2	2.8	2.8
valine	6.1	5.6	5.4
methionine	0.8	6.3	1.4
cystine	0.6	2.2	1.7
isoleucine	5.0	3.7	4.4
leucine	8.8	7.4	7.2
phenylalanine	6.4	4.6	3.2
lysine	7.1	3.3	4.9

Protein Analysis. Nitrogen content obtained by total carbonization was 2.37% (**Table 1**). Protein content was determined using the factor of 6.25, commonly used to relate nitrogen content with protein content. It was found to be 14.8%. To refine this content, we calculated the factor according to the analysis of percentage of amino acids given in **Table 2**. The correct factor was found to be 7.2. On the basis of this new value the protein content was found to be 17.06%.

Each sample was analyzed in duplicate (two hydrolysates by sample, one chromatographic analysis by hydrolysate) for amino acids. Tryptophan was not analyzed. Sulfur amino acids were analyzed after performic oxidation, in the form of cysteic methionine sulfone and cysteic acid. The amino acid composition of *I. malayana* proteins (**Table 2**) resembles that of bean and Brazil nut (*26*). Lysine content is notable (4.9%), and sulfur amino acid content as methionine is a little low (1.4%), especially if it is compared with that of Brazil nut, which has an exceptionally high sulfur amino acid content (*27*).

Oil Analysis. The oil content of *I. malayana* nut is 70.3 ± 1.1% compared to 66% in Grenoble walnut (*28*) or 65−70% for Brazil nut (*29*). The rate of unsaponifiables in oil is 1.2%, which is normal for a vegetable oil.

Fatty Acid Composition. The oil from the nut was found to contain 96.2% of saturated fatty acids (**Table 3**). This oil contains primarily lauric (C12:0) and myristic (C14:0) acids. Myristic acid is seldom present in great quantity in known vegetable oils. Lauric oils, copra and palm kernel tree, traditionally used in industry, contain less myristic acid, ∼17%. *I.*

Table 3. Fatty Acid Composition of *I. malayana* Oil Determined by GC Compared with Other Oily Nuts (*29*)

fatty acid	almond (%)	walnut (%)	palm kernel oil (%)	coconut (%)	olive (%)	*I. malayana* (%)
C8:0			4	7.5		
C10:0			4	7		2.7
C12:0			50	48		42.5
C14:0			17.5	17		42.1
C16:0	10	9	8	9	13.9	7.3
C16:1			0.5	0.5	1.5	
C18:0		4	2	2	2.8	1.6
C18:1	75	20	13	7	71.8	2.3
C18:2	15	67	1	2	9.0	1.5
C18:3					1.0	
unsaturated FFA	90.0	87.0	14.5	9.5	83.3	3.8

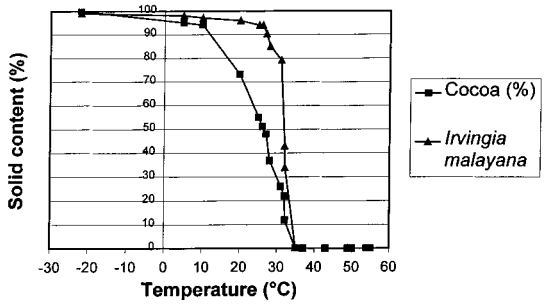

Figure 1. Solid content of *I. malayana* oil compared with cocoa butter solid content (*30*) obtained by NMR.

malayana oil is thus completely original because it contains simultaneously lauric (42.5%) and myristic (42.1%) acids. These medium-chain fatty acids have a great importance when the surfactant action is researched. This oil is thus found to be very different from other nut oils such as Grenoble walnut or almond, which are much more unsaturated.

Solid Content According to the Temperature by NMR. The fusion range of this oil was found between 38.6 and 39.4 °C. It presented a fast drop in solid content around its fusion range and reached 0% of solid toward 43 °C (**Figure 1**). This followed the same pattern as that of cocoa butter but at a higher temperature (*30*). The cocoa butter has the advantage of being completely molten at 35 °C and is thus pleasant in the mouth, whereas *I. malayana* oil is not completely liquefied at 43 °C. The rapid descent of the solid content can make this oil a candidate for many industries. It would, however, be necessary to modify its range by ∼0−10 °C so that the oil becomes more malleable outside refrigerators.

Sterol Composition. I. malayana's phytosterol composition (**Table 4**) is similar to that of other oleaginous seeds (*37*, *38*). Its content in β-sitosterol is high and is near that of olive and almond oils. However, its stigmasterol content is higher than that in olive and almond oils. Sitosterol takes part in the conversion of linoleic acid into polyunsaturated fatty acids. This reaction is essential for the conversion of omega 6 fatty acids into prostaglandins and leukotrienes. Prostaglandins and leukotrienes are involved in the immune system and assist in reduction of thromboembolic problems by reducing platelet aggregation and in the reduction of the anti-inflammatory drug metabolites (*39*). β-Sitosterol also takes part in the reduction of cholesterol levels (*40*, *41*). In the human body, there is a declination of DHEA production with age. This hormone is responsible for the synthesis of estrogen, progesterone, testosterone, cortisol, and others (*42*). Recent research reports that β-sitosterol improves urinary problems (*43*). Stigmasterol is a natural steroid that takes part in the chain of progesterone and androgens synthesis (*44*).

Table 4. Composition of Sterol Fractions of *I. malayana* Oil Determined by GC Compared with Olive Oil and Almond Nut Oil Sterol Fractions (*37*, *38*)

composition	olive oil (%)	*I. malayana* (%)	almond nut oil (%)
cholesterol	0.23	0.5	–[a]
campesterol	3.2	11.8	4
stigmasterol	1.3	20.6	3
cholestenol	–	–	–
β-sitosterol	81	62.7	77
sitostanol	2.3	–	–
Δ5-avenasterol	2.7	3.9	12
Δ7-stigmastenol	0.5	0.4	2
Δ7- avenasterol	–	–	2

[a] –, not found.

Table 5. Composition of Tocopherol Fractions of *I. malayana* Oil Determined by HPLC Compared with Olive Oil and Walnut Oil Tocopherols (*29*)

tocopherol	*I. malayana* (%)	olive oil (%)	walnut oil (%)
α-tocopherol	10.5	84.2	1.7
β-tocopherol	–[a]	5.3	1.8
γ-tocopherol	89.5	10.5	88
δ-tocopherols	–	–	8.5

[a] –, not found.

Tocopherol Composition. The tocopherol composition of *I. malayana* oil indicates a smaller quantity of α-tocopherol (10.5%) than γ-tocopherols (89.5%). Curiously, β-tocopherol and δ-tocopherols were not found. *I. malayana* had a ratio of α-tocopherol (10.5%)/γ-tocopherols (89.5%) inverse to that of olive oil (84.2/10.5) and also a ratio γ-tocopherol (0%)/α-tocopherol (84.2%) opposite that of walnut oil (88/1.7) (**Table 5**).

Conclusion. The biochemical composition of *I. malayana* is rather traditional and approaches those of other common nuts. Its oil, on the other hand, is different in its high fusion range. Its fusion range around 39 °C is rare for a vegetable oil. Ninety percent of *I. malayana* fatty acids are saturated with 41.8% myristic acid and 42% lauric acid. Among oils used industrially, only oils of the palm kernel tree and copra have such a great quantity of C12:0. Few oleaginous seeds have such a great quantity of C14:0. It has also an abrupt fall of solid content similar to that of cocoa butter but at a temperature of 39 °C as compared to 33 °C for cocoa butter oil. Its composition in sterols and tocopherols is also interesting for the treatment of cholesterol, cardiovascular diseases, or cancer. These qualities make *I. malayana* oil an interesting basic product for margarine, cosmetic, and pharmacology industries. A recent European law that allows vegetable oil at 5% in chocolate could open an industrial use for this oil.

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