FA and Unsaponifiable Composition of Five Amazonian Palm Kernel Oils

Didier Bereau[a], Bouchra Benjelloun-Mlayah[a,]*, Joseph Banoub[b], and René Bravo[c]

[a]Institut National Polytechnique de Toulouse, Ecole Nationale Supérieure des Arts Chimiques et Technologiques, Laboratoire de Catalyse, Chimie Fine et Polymères, 31077 Toulouse Cedex 4, France, [b]Science, Oceans and Environment, St. John's, Newfoundland, A1C5X1, Canada, and [c]Université des Antilles et de la Guyane, Département Scientifique interfacultaire, Laboratoire COVACHIM (Connaissances et Valorisations des Ressources Végétales), Campus de Schoelcher, 97233 Martinique

ABSTRACT: The kernel oils of five different palm species native to the Amazon basin and French Guyana were studied. Those studied were *Acrocomia lasiospatha* Wall., *Astrocaryum vulgare* C. Mart., *Bactris gasipaes* H.B.K., *Elaeis oleifera* (Kunth) Cortés, and *Maximiliana maripa* Drude. Lauric and myristic acids were found in all of the oils. Analysis of the unsaponifiable contents, especially the sterol and triterpene alcohol determinations, revealed the preponderance of sitosterol and the presence of two triterpene alcohols (cycloartenol and 24-methylenecycloartanol). Antioxidant (vitamin E) levels were present in small amounts, with the levels being more similar to olive than to palm oil.

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KEY WORDS: *Acrocomia lasiospatha,* Amazonian palms, *Astrocaryum vulgare, Bactris gasipaes, Elaeis oleifera,* fatty acid, *Maximiliana maripa,* palm oil, unsaponifiable.

More than 40% of oils consumed worldwide today are palm (lauric) oils. The two primary palm species exploited are the *Cocos nucifera* L. and the *Elaeis guineensis* N.J. Jacq. Both the mesocarp and kernels provide edible oils as well as oils that have industrial applications (e.g., soapmaking, lubricants, cosmetic creams, and surfactants, among others) (1). In this study, we have paid particular attention to five palm species occurring in French Guyana and the Amazonian basin: *Acrocomia lasiospatha* Wall, *Astrocaryum vulgare* C. Mart., *Bactris gasipaes* Kunth, *Elaeis oleifera* (Kunth) Cortés, and *Maximiliana maripa* Drude (2,3).

Acrocomia lasiospatha is a 10–15 m high, 30–35 cm diameter, spiny-stemmed palm occurring in the savannah. The fruits are brownish-yellow and the mesocarp is very mucilaginous. The palm develops fruit between July and December. *Astrocaryum vulgare* is a 10–15 m high palm with hard spines that is very common in open areas, such as fields and pastures and as secondary vegetation. The fruits are yellow-orange and drupaceous, and occur between January and August. *Bactris gasipaes* is a 10–20 m high, spiny-stemmed palm with green-yellow or yellow-orange fruits that occur between November and July. *Elaeis oleifera* palm is a (prostrate) trunk palm occurring not far from flooded areas. The red-orange drupaceous fruits occur between July and December. The *M. maripa* palm, also called *inaja* in Brazil, grows on dry, sandy sites and is generally found in open areas and secondary forest. It is a large-stemmed palm about 18 m high and 20 cm in diameter with long leaves and ovoid drupaceous fruits that are composed of a fibrous outer shell and a viscous mesocarp pulp. The fruits usually appear between January and June and may occasionally appear from October to December also. The FA composition and the unsaponifiable content of these kernel oils have been reported in several works (4–7). In the present study, these earlier results were confirmed and completed with determinations of the sterol, triterpene alcohol (TA), and total tocopherol values.

*To whom correspondence should be addressed.
E-mail: Bbenjelloun@ensct.fr

EXPERIMENTAL PROCEDURES

Materials. Fruits were harvested during the ripening season in French Guyana. They were stored at low temperature (–18°C) to avoid the enzymatic degradation of TG.

Kernel oil extraction. After shelling the pulp, the seed was ground in a mixer. A 40-g quantity of each sample was placed in an extraction thimble. The oil extraction was performed in a Soxhlet apparatus for 2 h using 200 mL hexane as solvent. The fat content was recovered by evaporation of the solvent in a rotary evaporator under low pressure. The crude oil obtained was dried and weighed.

Physicochemical properties. Several physicochemical indices of the extracted oils were determined. The following were evaluated according to the methods listed in the Association Française de Normalisation (AFNOR) patents (8): (i) density (AFNOR NF T 60-214); (ii) refractive index (AFNOR NF T 60-212); (iii) acid value (AFNOR NF T 60-204); (iv) PV (AFNOR NF T 60-220); and (v) unsaponifiable value (AFNOR NF T 60-205).

Determination of FA composition. The FA composition was determined by analysis of their methyl esters. The methyl esters of the FA were prepared from the oils by esterification with methanol in the presence of potash and boron trifluoride (BF_3) according to AFNOR Method NF T 60-233 (8). The methyl esters were analyzed by gas chromatography using a Carlo Erba GC 6000 (Vega Series) gas chromatograph fitted with an FID and a 30 m × 0.32 mm capillary column (DB-5-MS; J&W Scientific, Folsom, CA), 0.25 µm film thickness. Helium was used as the carrier gas at a flow rate of 40 kPa/min. The analysis was performed under the following conditions: oven temperature 80°C for 2 min, with a rise of 15°C/min to 160°C and a rise of 5°C/min to 200°C. The tem-

perature was maintained at 200°C for 20 min. The detector temperature was fixed at 220°C, and the injector was maintained at room temperature. Quantification was performed using the internal standard method.

Sterol and TA analysis. Sterols and TA had to be derivatized into silylethers prior to chromatographic analysis

(i) Sterols. The unsaponifiable content, obtained using AFNOR Method NF T 60-205 (8), was derivatized with the addition of pyridine (0.5 mL), hexamethyldisilosane (100 μL), and trimethylchlorosilane (40 μL). The sterols were then centrifuged, and the upper layer was analyzed under the following conditions: The gas chromatograph (Varian CP-3800) was fitted with a 30 m × 0.25 mm capillary column (ZEBRON ZB-5; Phenomenex, Torrance, CA), 0.25 μm film thickness, and was coupled to a mass spectrometer (Saturn 2000 GC MS/MS). The oven temperature was fixed at 150°C for 5 min, then raised to 250°C at 15°C/min and held for 20 min. The EI–MS scans were 2–45 min long with a sweep of 100–600 amu (atomic mass units) (with Electronic Impact Auto). The carrier gas (He) flow rate was 1 mL/min. The National Institute of Standards and Technology (NIST) library was used to analyze the mass spectra and to confirm the identification of compounds. Quantification of the compounds was carried out using the internal standard method.

(ii) TA. The same protocol was used for the analysis of TA.

Tocopherol and tocotrienol analysis. The unsaponifiable matter was recovered from 5 g of kernel oil, according to the above procedure, and was then diluted with 2 mL of hexane and injected onto a Spherisorb® 80 Å column (25 cm, 5 μm thickness; Waters, Milford, MA). The sample was analyzed using a Waters 486 Tunable Absorbance detector (an excitation wavelength of 292 nm). The pump and autosampler were a PerkinElmer isocratic LC Pump 250, and a rate of 1 mL/min was used. Injection was performed using a Rheodyne 7725i with a 20-μL loop. The mobile phase was hexane/2-propanol (99:1, vol/vol).

RESULTS AND DISCUSSION

Mesocarp and kernel oil extraction. The results are reported in Table 1. Oil yields from the *M. maripa* species were the highest (31%); the yields from the other oils ranged from 10 to 17%. All of the yields from these oils were rather low in comparison with yields from other palm oils (palm kernel, 48–52%; copra, 68%).

Physicochemical properties. The results presented in Table 2 show that density and refractive indices were similar for all the species tested. They were comparable to indices for palm kernel oils. The stability of the extracted oils is conveyed by the low acid values and PV, with the exception of the *B. gasipaes* species, which had higher values. The unsaponifiable matter, generally 1 to 3%, was comparable to values reported for palm kernel oils.

FA composition. As observed in Table 3, the most prevalent component of the oils was saturated FA (73–87%), with lauric (35–60%) and myristic (11–12%) acids making up the largest proportion of the saturated FA. Hence, copra and palm kernel oils have been referred to as "lauric oils."

Sterol composition. Five different sterols were observed in the oils: St_1 (*m/z* 458; $C_{30}H_{54}OSi$), St_2 (*m/z* 472; $C_{31}H_{56}OSi$), St_3 (*m/z* 484; $C_{32}H_{56}OSi$), St_4 (*m/z* 486; $C_{32}H_{58}OSi$), and St_5 (*m/z* 484; $C_{32}H_{56}OSi$). All of the sterols showed characteristic fragmentation patterns, with the fragment ions at *m/z* 73 or $[M - 73]^+$ corresponding to the loss of the radical group Me_3Si^+. The fragment ion at *m/z* 90 was due to the loss of the TMSOH group, whereas the presence of the fragment ions at *m/z* 129 or $[M - 129]^+$ originated from the cleavage of the A cycle, and *m/z* 255 was typical of a Δ5 double-bond sterol. Besides the fragmentations mentioned, St_1 showed a peak at *m/z* 345 $[M - 113]^+$ corresponding to the loss of the side chain. This latter formula was C_8H_{17}, and St_1 was characterized as cholesterol. The formula of the St_2 side chain was determined

TABLE 1
Fruit Characteristics and Kernel Oil Extraction Results

	Acrocomia lasiospatha	*Astrocaryum vulgare*	*Bactris gasipaes*	*Elaeis oleifera*	*Maximilana maripa*
Kernel/dry fruit (w/w) (%)	8.4 ± 0.6	20.1 ± 3.7	8.9 ± 0.9	19.2 ± 0.5	8 ± 1.6
Oil/kernel (w/w) (%)	17.0 ± 0.7	9.6 ± 1.2	16.4 ±1.4	15.8 ± 0.5	31.3 ± 0.4
Oil/dry fruit (w/w) (%)	1.4 ± 0.1	1.9 ± 0.2	1.3 ± 0.1	3.0 ± 0.1	2.5 ± 0.1

TABLE 2
Physicochemical Characteristics of the Extracted Kernel Oils[a]

	Acrocomia lasiospatha	*Astrocaryum vulgare*	*Bactris gasipaes*	*Elaeis oleifera*	*Maximilana maripa*
Density	0.94	0.86	0.90	0.96	0.93
Refractive index	1.451	1.435	1.451	1.453	1.449
Acid value (mg KOH)	2.2	5.1	12.2	3.4	2.6
PV (meq O_2/kg oil)	8.2	9.0	68.6	6.6	4.0
Unsaponifiable value (%)	0.7	0.7	0.8	0.5	0.6

[a]Results are mean values of three determinations ± 5%.

TABLE 3
FA Composition of the Kernel Oils[a]

	Acrocomia lasiospatha	*Astrocaryum vulgare*	*Bactris gasipaes*	*Elaeis oleifera*	*Maximilana maripa*
Caproic	0.9	Trace	Trace	Trace	Trace
Caprylic	6.4	Trace	Trace	Trace	3.8
Capric	5.9	Trace	Trace	Trace	4
Lauric	38.5	43.5	60.6	35.6	40.5
Myristic	10.7	28.6	18.9	25.6	25.5
Palmitic	7.4	7.5	6	9.7	9
Palmitoleic	Trace	—	—	—	Trace
Stearic	4.1	—	Trace	1.6	2.4
Oleic	21.3	13.6	12.9	21.1	10.8
Linoleic	2.9	3.3	Trace	5.4	2.4
Linolenic	1.9	—	—	—	—

[a]Results are mean values of three determinations ± 1%.

to be C_9H_{19}, corresponding to the mass of 127 [M – 255 – TMSOH]$^+$; St_2 was therefore determined to be campesterol. In St_3, the side chain was also calculated as having a mass of 139 and a formula of $C_{10}H_{19}$ as well as a double bond in the side chain occurring in C22, C24, or C25. Fragment ions were not observed at *m/z* 401 [M – C_6H_{11}]$^+$ or at *m/z* 386 [M – C_7H_{14}]$^+$, corresponding to C23–C24 or C22–C23 cleavages and MacLafferty rearrangements, and the double bond localization was tentatively assigned to C25 or C24(28). Therefore, the double bond was located at C22 and the identity of St_3 was assigned as stigmasterol. St_4 was a sterol with a side chain mass equal to *m/z* 141; it was assigned [M – 255 –TMSOH]$^+$ with the formula $C_{10}H_{21}$. This was tentatively assigned to the structure sitosterol. In the mass spectrum of St_5, we noticed the presence at of an ion at *m/z* 345 assigned as [M – 139]$^+$, corresponding to the side chain loss, and the formula $C_{10}H_{19}$ was assigned. At this stage the determination of the presence of the double bond could not be predicted. This could be situated on several positions, mainly Δ22, Δ24 or Δ25. However, the presence of the fragment ion at *m/z* 386, corresponding to the loss of a C_7H_{14} radical group, enabled us to conclude that it was a Δ24(28) double bond. Therefore, two structures were in agreement for St_5, according to the double bond configurations, either a *cis*-configuration leading to avenasterol or a *trans*-configuration leading to fucosterol. Unfortunately, this is not elucidated and will be determined by ^{1}H NMR analysis.

According to the mass spectra analysis, five different sterols [cholesterol, campesterol, stigmasterol, sitosterol, and Δ5 avenasterol (or fucosterol)] were present (Fig. 1), with sitosterol being the major (63–81%) component (Table 4). Δ5-Avenasterol (or fucosterol) has been identified as a second major component in the *A. lasiospatha, A. vulgare,* and *B. gasipaes* species. All these kernel oils made up about 25–45% of the unsaponifiable matter and had a sterol content comparable to the sterol content of coconut and palm kernel oils (between 1000 and 3800 ppm).

TA. Two main compounds were observed: AT_1 (*m/z* 498; $C_{33}H_{58}OSi$) and AT_2 (*m/z* 512; $C_{34}H_{60}OSi$). The EI–MS of AT_1 gave a fragmentation typical of a terpenic alcohol, which indicated the loss of a methyl radical or TMSOH group or either groups simultaneously, forming the fragment ions at *m/z* 484, 408, and 393, respectively. A fragment pattern characteristic of TA was observed for AT_1: the fragment ion at *m/z* 297 [M – C_8H_{15} – TMSOH]$^+$, corresponding to the loss of the

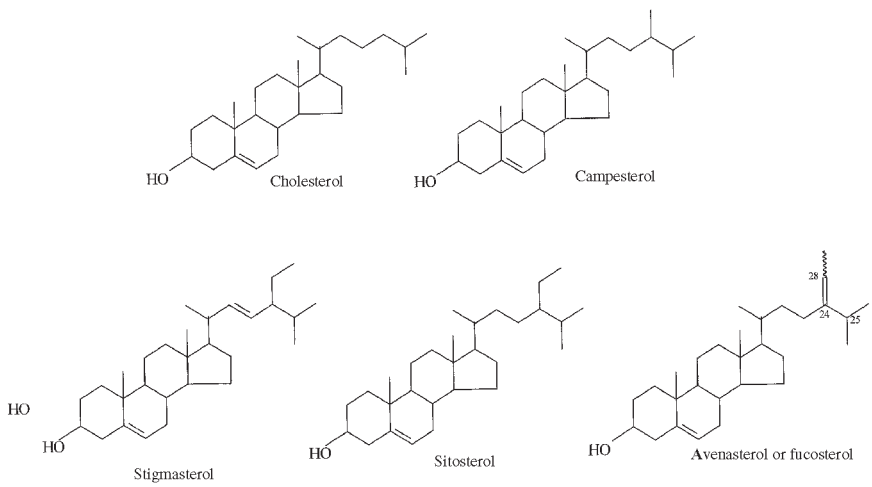

FIG. 1. Different sterols isolated in the kernel oils.

TABLE 4
Sterol Composition of the Extracted Kernel Oils[a]

Sterol (ppm)	*Acrocomia lasiospatha*	*Astrocaryum vulgare*	*Bactris gasipaes*	*Elaeis oleifera*	*Maximilana maripa*
Cholesterol	Trace	Trace	5–7	Trace	44–50
Campesterol	108–130	84–87	59–61	62–70	63–79
Stigmasterol	63–65	35–40	35–37	159–174	93–99
Sitosterol	2480–2664	1353–1363	1468–1492	1352–1512	746–958
Δ5-Avenasterol or fucosterol	732–860	638–648	434–440	95–103	142–170
Total	3383–3719	2110–2138	2001–2037	1668–1859	1088–1356

[a]Results are mean values of three determinations.

TABLE 5
Triterpene Alcohols of the Endocarp Oils[a]

Triterpene alcohol (%)	*Acrocomia lasiospatha*	*Astrocaryum vulgare*	*Bactris gasipaes*	*Elaeis oleifera*	*Maximilana maripa*
Cycloartenol	100	86	65.3	100	91.7
24-Methylenecycloartanol	—	14	34.7	—	8.3

[a]Results are mean values of three determinations ± 5%.

TABLE 6
Tocopherol and Tocotrienol Contents of the Selected Endocarp Oils[a]

Tocopherol/tocotrienol (%)	*Acrocomia lasiospatha*	*Astrocaryum vulgare*	*Bactris gasipaes*	*Elaeis oleifera*	*Maximiliana maripa*
α-T	6–7	7–16	2–3	2–3	2–3
α-T3	18–19	55–59	1–2	1–2	6–7
α-T	1–2	1–2	0–1	0–1	0–1
α-T3	0–1	14–22	0–1	0–1	1–2
α-T	4–5	4–5	—	—	1–2
α-T3	0–1	6–7	2–3	2–3	2–3
α-T	—	—	Trace	Trace	—
α-T3	—	3–4	—	—	—
Total	29–35	12–18	90–115	5–11	5–10

[a]Trace (<0.5%).

side chain and TMSOH; at *m/z* 255 [M – C_8H_{15} – C_3H_{15} – TMSOH]$^+$, the fragment originating from the cleavages of the C14–C15 and C13–C17 bonds; and the fragment ion resulting from the methyl group transfer of the latter fragment ion at *m/z* 241 [M – C_8H_{15} – TMSOH – CH_3]$^+$. Fragment ions corresponding to cleavages of the C9–C19, C9–C10, and C5–C6 bonds at *m/z* 286 were observed. They are specific ions of TA with a cyclopropane ring. The localization of the side-chain double bond in C24 was enhanced by the presence of the fragment ion at *m/z* 340, originating from the C_5H_9 loss (allylic cleavage in C24). Hence, AT_1 was classified as cycloartenol (Fig. 2).

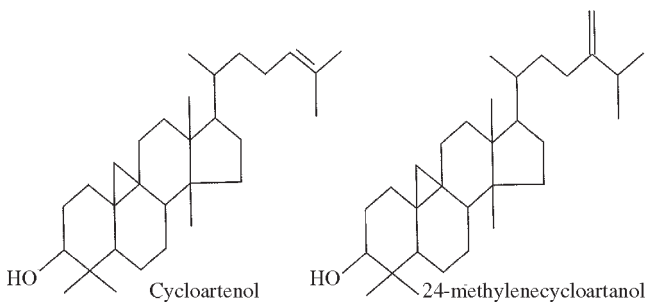

FIG. 2. Triterpene alcohols present in the endocarp oils.

Besides the usual fragment ions corresponding to methyl and TMSOH group losses, AT_2 showed the specific fragmentations of triterpene alcohols at *m/z* 297, at *m/z* 255, and at *m/z* 241. The side-chain mass was evaluated at 125 with a C_9H_{17} formula. Also, the fragment ion at *m/z* 286 was observed, and AT_2 was presumed to have a cyclopropane ring. Fragment ions were observed at *m/z* 339 [M – C_6H_{11} –TMSOH]$^+$ and at *m/z* 379 [M – C_3H_7 – TMSOH]$^+$ and were attributable to C22–C23 allylic and C24–C25 vinylic cleavages. Thus, the double bond could be considered as being in C24–C28, and AT_2 was classified as 24-methylenecycloartanol (Fig. 2).

Hence, the analysis of mass spectra revealed the presence of two tetracyclic TA in the kernel oils—cycloartenol and 24-methylenecycloartanol—although the latter was not detected for the *A. lasiospatha* and *E. oleifera* species. These alcohols are common for these types of oils.

Tocopherol and tocotrienol analysis. These results are given in Table 6. The total tocopherol content of these oils was low and ranged from 5 to 115 ppm, which is common for these types of vegetable oils. Interestingly, tocotrienols (especially α-tocotrienol) were predominant for all species (60–84%) except for the *E. oleifera* species, where α-tocopherol was a major component. Total tocopherols represented only 0.1 to 1.6% of the unsaponifiable matter.

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