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ORIGINAL PAPER

Characterization of *Pentadesma butyracea sabine* Butters of Different Production Regions in Benin

Fidèle P. Tchobo · Armand K. Natta · Bruno Barea · Nathalie Barouh · Georges Piombo · Michel Pina · Pierre Villeneuve · Mohamed M. Soumanou · Dominique C. K. Sohounhloue

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Abstract *Pentadesma* butter (*Pentadesma butyracea*, *sabine*, *clusiaceae*) is an extract of the kernels of tree fruits in West Africa and similar to shea butter. The study of the fatty acid composition, triacylglycerols, sterols and tocopherols of *Pentadesma* butter was carried out on seeds collected in ten production areas in Benin. The results obtained show that the composition in fatty acids is characterized by the presence of stearic acid and oleic acid, which represent nearly 96% of the total fatty acids. The triacylglycerols profile of the different butters is marked by the overwhelming presence of the triacylglycerols SOS and SOO. The unsaponifiable fraction shows, for the sterolic composition, a predominance of stigmasterol (nearly 68% of the total sterols) whilst the β-tocopherol is the main tocopherol.

F. P. Tchobo · M. M. Soumanou · D. C. K. Sohounhloue
Ecole polytechnique d'Abomey-Calavi,
Laboratoire d'Etude et de Recherche en Chimie Appliquées (LERCA) Unité de recherche en génie enzymatique et alimentaire, Université d'Abomey-Calavi,
01 BP 2009 Cotonou, Benin
e-mail: msoumanoufr@yahoo.fr

A. K. Natta
Faculté d'Agronomie,
Laboratoire d'Etudes et de Recherches Forestières (LERF),
Université de Parakou, BP 123 Parakou, Benin

F. P. Tchobo · B. Barea · N. Barouh · G. Piombo · M. Pina · P. Villeneuve (✉)
Lipotechny Laboratory,
UMR IATE, CIRAD, TA 40/16 73,
rue J. F. Breton, 34398 Montpellier Cedex 5, France
e-mail: villeneuve@cirad.fr

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Introduction

Rich and diversified tropical flora provides a multitude of forest products (fruits, seeds, leaves, etc.), which contribute to the nutritional and economic balance of rural populations [1, 2]. In West Africa, about 350 species of wild edible fruits have been recorded and described [3, 4]. The seeds and the fruits found are generally rich in lipids and provide the main part of the fatty acid needs of the populations, especially the farming population. Vegetable oils are generally provided by palm fruits, cotton, soy, peanut, palm kernel and cocoa seeds. In some regions, other plants are sources of fats and are of economic interest for the population, for example, *Vitellaria paradoxa* (shea) and *Pentadesma butyracea*.

Pentadesma butyracea Sabine is a tree from the *clusiaceae* family, with a height of about 20 m and with a distribution area reaching from Sierra Leone to the Congo [5–7]. This is a dense forest species that is found in the center and north of Benin in forest galleries and along water ways [8]. The fresh kernels consumed like kola [1] are rich in edible butter similar to shea butter. *Pentadesma* butter is used in traditional medicine as massage oil, in skin and hair care and in the manufacture of soap for its softening, lubricating and healing qualities [9].

Previous studies on the fatty acid composition of *Pentadesma* butter from the Korhogo region of the Ivory Coast and in Ghana have been characterized by the presence of over 95% of stearic and oleic acids whereas the sterolic fraction showed a predominance of Δ-5 sterols, with stigmasterol being the main sterol [9, 10]. In this context, the

studies carried out in Benin on *Pentadesma* concentrated, on the one hand, on the socio-economic appreciation and the sustainability of the *Pentadesma butyracea Sabine* in forest galleries [1] and, on the other hand, on the distribution area and the ecological factors determining its presence [8]. The composition of *Pentadesma* butter does vary depending on the distribution area and ecological factors [8]. The object of the present was to determine the influence of the geographic origin on the fatty acids, triacylglycerols, sterols and tocopherols compositions of *Pentadesma* butter from Benin from seeds collected in ten regions where in Benin.

Experimental Procedures

Materials

Pentadesma butyacea kernels were collected from ten locations in Benin (Table 1). *Pentadesma* fruits (2 kg, around 50 fruits) were collected on the ground in the various geographical chosen fields at the same fruit maturity. The kernels (150 units, around 1.3 kg) were then extracted from the fruits and sun-dried (moisture content <10%). Before analysis, kernels were oven dried at 80 °C for 24 h and ground with a mechanical millstone.

Oil Extraction

Samples of ground kernels (500 g) were weighed and extracted four times with hexane (7 h) using a continuous Soxhlet extractor. The fat content was gravimetrically measured after removal of the solvent by rotary evaporation under vacuum. Extraction was run in triplicate (three times on 500 g samples each) on the original iso kernels.

Table 1 Different sample numbers and corresponding collection areas

Sample no.	Collection sites Villages	Regions
BP1	Agbassa	Tchaourou
BP2	Prekete	Bassila
BP3	Penelan	Bassila
BP4	Penessoulou	Bassila
BP5	Wannou	Bassila
BP6	Bassila 1	Bassila
BP7	Manigri	Bassila
BP8	Bassila 2	Bassila
BP9	Peperkou	Toucountouna
BP10	Natitingou	Natitingou

Determination of FFA Content

The NF T 60-204 Method was used for the determination of FFA content of all oils [11]. Oil samples (2 g ± 0.1 g) were dissolved in a mixture ether/ethanol (50:50 v/v) and titrated with alcoholic potassium hydroxide (0.1 N).Titration were completed in triplicate (three times on each of the three extracted oils).

Fatty Acid Analysis

In a 25-ml round bottom flask, oil samples (10 mg) were added to sodium methylate solution (3 mL) containing phenolphthalein. The mixture was refluxed for 10 min and 3 mL methanolic HCl were added until phenolphthalein discoloration occurred. The mixture was refluxed again for 10 min and cooled to room temperature. Hexane (8 mL) and water (10 mL) were added and the organic phase recovered, dried over anhydrous sodium sulfate and filtered for subsequent GC analysis An Agilent 6890 series GC apparatus provided with a Supelcowax 10 capillary column (SGE, Courtaboeuf, France) with the following characteristics: length, 30 m; internal diameter, 0.32 mm, film thickness 0.25 μm, was used. Fatty acid methyl esters were directly injected into the GC. The carrier gas was helium with a flow rate of 1 mL/min, and a splitting ratio of 1/80. The injector temp was 250 °C and that of the FID was 270 °C. The temperature settings were as follows: 150–225 °C at 5 °C/min, and then held at 225 °C for 2 min. Fatty acids were identified by comparison with commercially available fatty acids standards.

HPLC Triacylglycerol (TAG) Profiles

The HPLC pump (P1000XR), auto sampler (AS1000), and an evaporative light scattering detector (ALLTECH 500 ELSD) are Thermo-Finnigan products (Courtabœuf, France). The separations were performed on two INTERCHIM C18 columns (5 μm; 4.6 × 250 mm, Modulo-cart QS LICHROSPHER 5 ODS 2) in series. The eluent used was a gradient of mixture acetone/acetonitrile (50:50 v/v (A)) and chloroform (B). Elution was carried out at a solvent flow rate of 1 mL/min with a linear gradient as follows: 0 min 100% A, 60 min 80% A, 80 min 80% A, returning to the initial conditions within 15 min and holding at these conditions for 15 min. The effluent was monitored with an ELSD detector, with the following settings: evaporator temperature 30 °C, air pressure 44.2 psi. The triacylglycerols were identified by comparing retention times to pure standards purchased from Sigma-Aldrich (Paris, France).

Table 2 Oils and unsaponifiables and free fatty acid content of the different *Pentadesma* samples

Samples	Oil content (%)	Unsaponifiable content (%)	FFA (% oleic acid)
BP 1	45.7 ± 0.6	1.0 ± 0.2	1.11 ± 0.1
BP 2	41.2 ± 0.9	0.8 ± 0.1	0.62 ± 0.1
BP 3	43.0 ± 0.7	1.2 ± 0.1	0.66 ± 0.1
BP 4	41.9 ± 0.7	1.3 ± 0.2	0.50 ± 0.1
BP 5	41.7 ± 0.3	1.8 ± 0.1	0.95 ± 0.1
BP 6	46.3 ± 0.6	0.8 ± 0.1	0.55 ± 0.1
BP 7	47.3 ± 0.8	1.1 ± 0.1	2.19 ± 0.1
BP 8	39.1 ± 0.9	1.0 ± 0.1	1.29 ± 0.2
BP 9	46.1 ± 0.8	1.6 ± 0.1	0.65 ± 0.1
BP10	41.1 ± 0.8	0.9 ± 0.1	2.36 ± 0.1

Unsaponifiable Content

The unsaponifiable content was determined in accordance with the corresponding AFNOR method NF T 60.205-2 [12]. Samples (5 g) were saponified with 50 mL alcoholic potassium hydroxide (1N) under reflux for 1 h. The unsaponifiable components were separated from the soap in a separator funnel with hexane and washed with ethanol (10%). The solvent was evaporated off and residue was dried in an oven at 103 ± 2 °C for 15 min and weighed.

GC Sterols Analysis

The sterols' composition was determined in accordance with the corresponding AFNOR method NF ISO 6799 [13]. Sterols were extracted from 0.5 g of oil. The sample was saponified using 5 mL alcoholic potassium hydroxide (0.5N), under reflux for 15 min with cholesterol as internal standard. The unsaponifiable components were separated from the soaps on an alumina column (10 g) and washed with diethyl ether (35 mL). The solvent was evaporated and the residue was dissolved in chloroform (1 mL). The separation of sterols from other unsaponifiable components was carried out by thin-layer chromatography. The plates were developed with a mixture of chloroform/diethyl ether (90:10 v/v). The sterols bands were extracted with chloroform and analysed by GC.

The sterol solution was injected into a GC 8000 series (FISONS Instruments) equipped with FID detector and

Table 3 Composition (%) in fatty acids of the different *Pentadesma* butters

Fatty acids	Samples									
	BP 1	BP 2	BP 3	BP 4	BP 5	BP 6	BP 7	BP 8	BP 9	BP10
Palmitic acid	3.6	3.2	3.3	2.9	3.9	2.7	3.4	2.9	2.6	3.9
Palmitoleic acid	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1
Stearic acid	43.2	47.1	47.0	38.4	43.2	45.5	46.3	42.8	45.7	42.4
Oleic acid	52.0	48.7	48.7	57.6	51.6	50.8	49.3	53.2	50.6	52.5
Linoleic acid	0.7	0.6	0.5	0.6	0.7	0.5	0.6	0.7	0.6	0.7
Linolenic acid	0.2	0.2	0.1	0.2	0.2	0.2	0.1	0.2	0.2	0.2
Arachidic acid	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2

Table 4 Composition (%) in triacylglycerols of the different *Pentadesma* butters

Samples	Triacylglycerol				
	POO	POS	OOO	SOO	SOS
BP 1	0.3 ± 0.1	1.6 ± 0.1	2.8 ± 0.1	45.2 ± 0.9	50.1 ± 0.8
BP 2	0.2 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	38.3 ± 1.0	58.8 ± 1.4
BP 3	0.2 ± 0.1	1.9 ± 0.1	1.4 ± 0.1	35.1 ± 1.5	61.4 ± 1.5
BP 4	0.3 ± 0.1	0.9 ± 0.1	6.5 ± 0.1	55.2 ± 0.2	37.1 ± 0.3
BP 5	0.3 ± 0.1	1.4 ± 0.1	2.8 ± 0.1	42.3 ± 0.4	53.2 ± 0.5
BP 6	0.2 ± 0.1	1.1 ± 0.1	2.0 ± 0.1	43.4 ± 1.3	53.3 ± 1.3
BP 7	0.3 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	39.2 ± 1.0	56.7 ± 1.1
BP 8	0.2 ± 0.1	1.0 ± 0.1	3.0 ± 0.1	48.8 ± 0.6	47.0 ± 0.6
BP 9	0.2 ± 0.1	1.0 ± 0.3	2.1 ± 0.1	43.4 ± 1.5	53.3 ± 1.7
BP10	0.5 ± 0.1	2.0 ± 0.3	3.1 ± 0.1	44.5 ± 0.5	49.9 ± 0.4

SAC5 Supelcowax capillary column (SGE, Courtaboeuf, France) (length, 30 m; internal diameter, 0.25 mm, film thickness: 0.25 μm). Helium with a flow rate of 1.5 mL/min and splitting ratio 1/100 was used as the carrier gas. Injector and FID temperatures were set at 300 °C. The oven temperature was 285 °C for the 30 min run. Identification of sterols was done by the use of standards purchased from Sigma-Aldrich.

HPLC Tocopherols Analysis

Tocopherols were analysed by HPLC in accordance with the AFNOR Method ISO 9936 [14]. Typically, 20 mg of sample was dissolved in 5 mL *n*-hexane and analyzed by HPLC. The separation of the compounds was achieved with a hypersil Silica (Si 60.5 μm, 256 × 4.6 mm) column. The eluent was a mixture of hexane/dioxane (97:3 v/v) with a flow rate of 1 mL/min and the injection volume was 20 μL. The wavelength of excitation and of emission was fixed at 290 nm and 330 nm, respectively for the spectrofluorimetric detector (FL 3000). Tocopherol standards (Sigma-Aldrich) were injected to identification. Software for data processing was in accordance with the AFNOR method ISO 9936.

All analyses mentioned above were carried out in triplicate and results are given as mean values of three determinations.

Results and Discussion

The lipid and unsaponifiable content and the acid index (% in oleic acid) are shown in Table 2. This oil content varied between 40 and 50%, which is comparable to that of shea kernels [15]. The *Pentadesma* butters obtained were light yellow in colour, solid at room temperature with acidity between 0.5 and 2.4%. The sample butters had a very low unsaponifiable level (<2%), which concurred with the literature [9, 10].

Pentadesma butter was characterized by seven saturated or unsaturated fatty acids (Table 3), palmitic (P), palmitoleic (Po), stearic (S), oleic (O), linoleic (L), linolenic (Ln) and arachidic acid (A), in different proportions. The fatty acid composition of the sample butters was mainly stearic (38–47%) and oleic (48–58%) acids and represented nearly 96% of the total fatty acids. Palmitic acid was present in an average proportion of 3% and linoleic acid was between 0.5 and 1%. The other fatty acids had percentages lower or equal to 0.2%. It should be noted that the fatty acid composition of the butters agrees with those obtained by Dencausse et al. [9] and Adamako [10]. One special feature was sample BP 4, which had an oleic acid content of 58% and stearic acid content of lower than 40%.

Table 5 Composition in sterols of the different *Pentadesma* butters

Samples	BP1		BP2		BP3		BP4		BP5		BP6		BP7		BP8		BP9		BP10	
Sterol	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g
Brassicasterol	2.7	5.3	5.0	11.0	3.3	5.0	3.1	4.8	3.8	7.3	4.0	7.6	4.4	7.1	2.7	5.6	3.3	5.8	5.3	7.1
Campesterol	18.6	35.5	25.4	56.0	16.1	24.2	19.3	29.9	21.2	40.6	20.8	39.8	20.0	32.0	25.1	51.9	28.4	49.1	22.5	30.5
Stigmasterol	69.2	132.2	59.0	130.2	68.2	102.2	68.9	106.8	68.6	131.1	68.7	131.8	67.3	107.6	65.7	135.8	59.2	102.3	65.7	89.1
β- Sistosterol	5.3	10.2	6.7	14.8	8.5	12.7	5.3	8.2	3.0	5.7	4.5	8.6	6.3	10.1	4.7	9.8	6.4	11	6.5	8.7
Δ^5-Avenasterol	–	–	1.4	3.0	0.7	1.1	0.7	1.1	1.0	1.9	0.7	1.3	0.6	0.9	0.2	0.5	–	–	–	–
Spinasterol	0.7	1.4	0.9	1.9	0.3	0.3	–	–	–	–	0.2	0.4	0.3	0.4	0.3	0.7	0.7	1.3	–	–
Δ^7-stigmasterol	2	3.9	1.1	2.5	1.8	2.6	0.6	1	–	–	0.4	0.7	0.4	0.6	0.7	1.3	1.0	1.7	–	–
Δ^7-Avenasterol	1.2	2.3	0.5	1.1	0.9	1.4	2.1	3.2	2.3	4.3	0.8	1.5	0.6	1.0	0.5	1.0	1.0	1.7	–	–
Total		190.8		220.5		149.5		155		190.9		191.7		159.7		206.6		172.9		135.4

Table 6 Composition in tocopherols of the different *Pentadesma* butters

Sample	Tocopherol				
	α- Tocopherol µg/g	β-Tocopherol µg/g	γ-Tocopherol µg/g	δ-Tocopherol µg/g	Total µg/g
BP 1	53.4 ± 1.1	107.2 ± 0.6	7.2 ± 0.3	26.9 ± 2.2	194.7
BP 2	29.9 ± 0.7	50.4 ± 0.5	5.0 ± 0.4	10.0 ± 0.7	95.3
BP 3	46.3 ± 0.8	40.8 ± 0.37	6.5 ± 0.8	17.0 ± 0.1	110.6
BP 4	32.8 ± 0.2	71.9 ± 1.1	3.9 ± 0.2	16.2 ± 0.5	124.8
BP 5	50.8 ± 0.4	80.4 ± 0.6	8.0 ± 0.2	20.5 ± 1.7	159.7
BP 6	34.5 ± 0.5	44.8 ± 1.6	5.3 ± 0.5	17.8 ± 1.8	102.4
BP 7	47.3 ± 0.6	93.5 ± 1.1	3.8 ± 0.7	24.9 ± 2.7	169.5
BP 8	45.1 ± 0.6	85.0 ± 2.7	7.5 ± 0.4	30.6 ± 0.5	168.2
BP 9	31.7 ± 0.3	72.6 ± 0.6	7.8 ± 0.4	23.1 ± 0.6	135.2
BP 10	96.7 ± 0.7	8.5 ± 0.2	17.8 ± 0.6	13.2 ± 1.2	136.2

The presence of five triacylglycerols (POO, POS, OOO, SOO, SOS), was observed in the samples (Table 4). SOO and SOS were the predominant triacylglycerols. The absence of tristearin (SSS) and a low content of triolein (OOO) were observed despite the high content of stearic and oleic acids. In nearly all the samples, the triacylglycerol SOS was the main triacylglycerol with a concentration of over 50% (50–62%) whereas the SOO content varied between 35 and 45%. The percentages of 57% and 49% of SOO and 37% and 47% of SOS were observed for samples BP4 and BP8, respectively. Triolein (OOO) was 3% in most samples, except in sample BP4 where it was 6%. This difference in the composition of OOO of these two samples could be explained by the high oleic acid content in BP4.

The phytosterols are important compounds in the unsaponifiable fraction of the vegetable oils and their cholesterol lowering properties are largely recognized. The average sterol content of the *Pentadesma* butter was 1,773 µg/g oil (Table 5). The lowest content was obtained in sample BP10 with 1,353 µg/g oil. The maximum content of 2,205 µg/g oil was obtained in sample BP1, which was a difference of 852 µg/g oil. Identification of the different physterols showed that it is essentially composed of Δ^5 sterols (97%). Stigmasterol (59–69%) was the main sterol, accompanied by campesterol (16–29%), β-sitosterol (3–7%) and brassicasterol (3–6%). The composition of phytosterols varied in its composition in Δ^7 sterols although they represented less than 3% of the total sterol content. This sterol composition was quite different to the one obtained by Dencausse et al. [9] for the Korhogo *Pentadesma* (Ivory Coast) which has 22% of Δ^7 sterols. This difference could be linked to the origin of the seeds and would therefore be an origin identification factor. It is worth noting that sterols (average 0.15%) and tocopherols (average 0.015%) were not the predominant compounds of the unsaponifiables (average 1.15%). According to Dencausse et al. [9], the unsaponifiables were very rich in terpenic alcohols in particular lupeol, butyrospermol and α amyrin.

Several factors linked to the environment (climate, soil, etc.) influence the tocopherol content [16]. The content of total tocopherols was between 95.3 and 194.7 µg/g (Table 6). β-tocopherol was the main tocopherol of the sample oils studied, except BP 3, of which content of α-tocopherol was slightly higher than that of β-tocopherol. Sample BP 10 had a very low β-tocopherol content.

The results obtained showed that the different components (fatty acid, triacylglycerols, sterols and tocopherols) of the *Pentadesma* seeds collected in different geographic areas of Benin were very similar to each other. However, slight differences were sometimes observed, which may allow one to search for pertinent identification criteria for the areas of origin.

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