

Regional Variation in Shea Butter Lipid and Triterpene Composition in Four African Countries[†]

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The triacylglycerol, fatty acid, and polycyclic triterpene compositions of shea butter were determined for 150 samples from the sub-Saharan countries of Mali, Burkina Faso, Nigeria, and Uganda. The compositional profiles showed high variability in all three classes of compounds. Shea butter is made up mainly of four triglycerides (TAG) differing in carbon number (CN) by two, starting from CN 50 to CN 56. The greatest source of variation was in the CN 54 TAG. Shea butter is characterized by 16 saturated and unsaturated fatty acids in greatly varying proportion, the major ones being the even homologues in the range of C₁₆–C₂₀. Oleic acid is dominant in Ugandan provenances, whereas stearic acid is dominant in West African shea butter. Acetyl and cinnamyl polycyclic triterpene means for countries ranged from 3.69 to 12.57%, with the highest values found in Nigerian provenances. Statistical comparisons of fat composition show that the geographic distance between shea populations is reflected in the degree of separation of their chemical profiles.

KEYWORDS: *Vitellaria paradoxa*; fat composition; triglycerides; acetyl and cinnamyl polycyclic triterpenes; chemometrics

INTRODUCTION

The shea tree [family Sapotaceae, *Vitellaria paradoxa* C.F. Gaertner, synonym *Butyrospermum parkii* (G. Don) Hepper] is indigenous to the savanna belt extending across sub-Saharan Africa north of the equator (1, 2). The most valued product is shea butter (Francophone usage: beurre de karité), the fat extracted from the kernels. Shea nuts have a long history as a commodity of trade (3). Processed shea butter is used primarily as a cocoa butter additive in chocolate manufacture, although it is increasingly popular in cosmetic product formulations. Within Africa, the nutritious shea fruit pulp is of dietary importance (4), whereas the bark and latex play a role in ethnomedicine (5).

Shea kernels and shea butter have been found to have high levels of phenolic compounds and tocopherols, with significant regional variation in the content of these antioxidants (6, 7). We wished to explore and determine whether significant differences in seed fat qualitative and quantitative composition exist among widely dispersed *V. paradoxa* populations. We were particularly interested in whether natural variants synthesizing

triglycerides characterized by a higher melting point or those with a higher polycyclic triterpene content could be found among the different *V. paradoxa* ecotypes or provenances. These traits are sought after by the chocolate and cosmetic industries (8–11).

The two most popular analytical techniques for the analysis of triglycerides consist of reverse phase high-pressure liquid chromatography (RP-HPLC) (12) and high-resolution gas chromatography (HRGC) (11, 13). We applied the latter technique that improved the resolution of individual triglyceride species by carbon number and allowed also the detection, on the same chromatogram, of polycyclic triterpenes (sterols, 4-methylsterols, triterpene alcohols) esterified either with acetic or with cinnamic acid (12, 13). Aiming to develop strategies to guide in the identification of shea nut populations with appropriate triterpenes and triacylglycerol composition for various food and nonfood industries, we carried out a qualitative and quantitative composition and statistical study based on over 150 samples of nuts of *V. paradoxa* provenances from the sub-Saharan countries of Mali, Nigeria, Burkina Faso, and Uganda.

EXPERIMENTAL PROCEDURES

Materials. Shea nuts were analyzed from nine locations in both Mali and Burkina Faso, two locations in Nigeria, and an aggregate regional collection from northern Uganda (see Table 1). Shea ecotypes in Uganda are considered to be *V. paradoxa* subspecies *nilotica*, whereas the rest of the samples from Mali, Burkina Faso, and Nigeria are from populations of *V. paradoxa* subspecies *paradoxa*.

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Table 1. Mean Fat Percentages of Shea Kernels from Mali, Burkina Faso, Nigeria, and Uganda

	Mali	Burkina Faso		Nigeria		Uganda	
shea	fat content (%)	shea	fat content (%)	shea	fat content (%)	shea	fat content (%)
Sirakorola	mean	41.17	Souly	mean	46.90	Kontagora	mean
	SD (n = 7)	0.09		SD (n = 8)	0.08		SD (n = 8)
Sebekoro	mean	42.80	Touroum	mean	47.58	Mokwa	mean
	SD (n = 7)	0.04		SD (n = 4)	0.17		SD (n = 9)
Gouani	mean	48.59	Yasso	mean	51.32		
	SD (n = 7)	0.07		SD (n = 6)	0.05		
Massala	mean	44.80	Sapone	mean	47.64	SD of mean	0.01
	SD (n = 7)	0.06		SD (n = 8)	0.04	SD of all samples (n = 17)	0.04
Niamana	mean	45.10	Peni	mean	55.18	mean of all samples (n = 17)	47.76
	SD (n = 7)	0.06		SD (n = 8)	0.03		
Koumantou	mean	51.33	Lan	mean	48.08		
	SD (n = 7)	0.06		SD (n = 5)	0.05		
M'Peresso	mean	41.90	Tambissini	mean	46.22		
	SD (n = 6)	0.08		SD (n = 9)	0.06		
Badougou	mean	43.99	Siniena	mean	49.19		
	SD (n = 7)	0.13		SD (n = 7)	0.06		
Fourou	mean	48.10		SD of mean	0.03		
	SD (n = 9)	0.04		SD of all samples (n = 55)	0.07		
SD of mean		0.03	mean of all samples (n = 55)				
SD of all samples (n = 64)		0.07					
mean of all samples (n = 64)		45.41					

Fat Content. Nuts were oven-dried and decorticated, and the kernels were crushed in a mortar and ground in a coffee mill. Samples thus prepared were weighed and extracted with hexane at room temperature for 3 h, and the fat content was determined gravimetrically after removal of the solvent by rotary evaporation (14).

Fat Class Detection and Separation. A variety of analytical techniques were used to explore the general chemical composition of shea butter, including thin-layer chromatography (TLC) done with silica gel G 60 (Merck). Spots were visualized by UV-visible spectrophotometry (254 nm), elution of triterpene fractions via silica gel G 60 column, followed by GC (Carlo Erba 5160 Mega), GC-MS (HP5988A), and NMR (Bruker model AC 300 spectrometer, Bruker Instruments, Inc., Karlsruhe, Germany) for individual component identification. The polycyclic triterpene components were identified by comparison of GC retention times, MS fragmentation patterns, and ¹H NMR using CDCl₃ as solvent data reported in the literature (discussed elsewhere). This paper focuses on composition comparisons of regional provenances using HRGC.

Triacylglycerol (TAG) and Polycyclic Triterpene GC Profiles. TAGs and polycyclic triterpene derivatives of the oils (butter) were analyzed using HRGC (12, 13, 15, 16). Samples (20 mg) were dissolved in 2 mL of isoctane, and 2 μ L of this solution was subjected to HRGC. A Carlo Erba 5160 Mega series gas chromatograph was equipped with a fused-silica capillary column, Quadrex 007-65 HT-15-0.1F (15 m \times 0.25 mm i.d., 0.1 μ m), FID, and a split/splitless injector. The column oven temperature was programmed from 270 to 290 $^{\circ}$ C at the rate of 2 $^{\circ}$ C/min and from 290 to 345 $^{\circ}$ C at 8 $^{\circ}$ C/min, was held for 18 min at 345 $^{\circ}$ C, was raised from 345 to 350 $^{\circ}$ C at 2.5 $^{\circ}$ C/min, and finally was held at 350 $^{\circ}$ C for 5 min when the last peak came out. Detector and injection ports were maintained at 360 and 320 $^{\circ}$ C, respectively. The inlet pressure of hydrogen as carrier gas was 50 kPa. Peaks were identified by comparison with standards and retention time.

Fatty Acid Analysis. Fatty acid methyl esters were prepared by using KOH methylation (14, 17). A sample (0.25 g) of oil was dissolved in hexane (2.5 mL) and the flask shaken for 30 s to complete solution. Methanolic KOH (0.3 mL, 2 N) was added, and the flask, covered with a glass cork, was shaken vigorously for 60 s. The organic layer containing the fatty acid methyl esters was separated from the methanolic phase after centrifugation for 5 min at 3500 rpm.

The organic layer containing the fatty acid methyl esters was removed and used for GC analysis. A Carlo Erba HRGC 8560 Mega 2 series gas chromatograph was equipped with a Supelco-SP-2380 capillary column (60 m \times 0.32 mm i.d., 0.20 μ m film thickness) and with a FID (*t* = 250 $^{\circ}$ C). The conditions were as follows: oven temperature program, 1 min at 70 $^{\circ}$ C, raised from 70 to 165 $^{\circ}$ C at 22 $^{\circ}$ C/min, held for 10 min at 165 $^{\circ}$ C, raised from 165 to 200 $^{\circ}$ C at 5 $^{\circ}$ C/min, held for 10 min at 200 $^{\circ}$ C, raised from 200 to 245 $^{\circ}$ C at 8 $^{\circ}$ C/min, and finally held at 245 $^{\circ}$ C for 15 min; the inlet pressure of the hydrogen as carrier gas was 50 kPa; injection by “on column”.

Identification of fatty acids was done by the use of standards and was confirmed by GC-MS analysis. Percent fatty acid was obtained by dividing the peak area of the individual fatty acid by the sum of all peak areas obtained for fatty acids by using an integrator.

Statistical Analysis. Linear discriminant analysis (LDA) using SPSS software was used for direct comparisons between pairs of countries for all analyzed parameters (18). The objective was to identify the compositional factors characterizing or distinguishing each region as previously reported for Italian olive oil varieties (19, 20). ANOVA mean separations for TAG, fatty acids, and triterpene values were performed using Statistica software (21).

RESULTS AND DISCUSSION

Separation of Triglycerides and Polycyclic Triterpenes. Figure 1 shows the HRGC profile used to separate triglyceride species by fatty acid composition (described and discussed elsewhere) and fractionation of the two groups of triterpenes found in shea butter. Detection of the triterpene fractions show of one group of acetyl-esterified polycyclic triterpenes rapidly eluted from the GC column close to the origin of the chromatogram and a second group of cinnamyl-esterified polycyclic triterpenes eluted in the middle of the chromatogram just before the triglyceride fraction. In agreement with previous studies (12, 22–25), this HRGC profile reveals the presence of at least four major components in each of the triterpene fractions. Comparison of MS and ¹H NMR data of these two triterpene groups

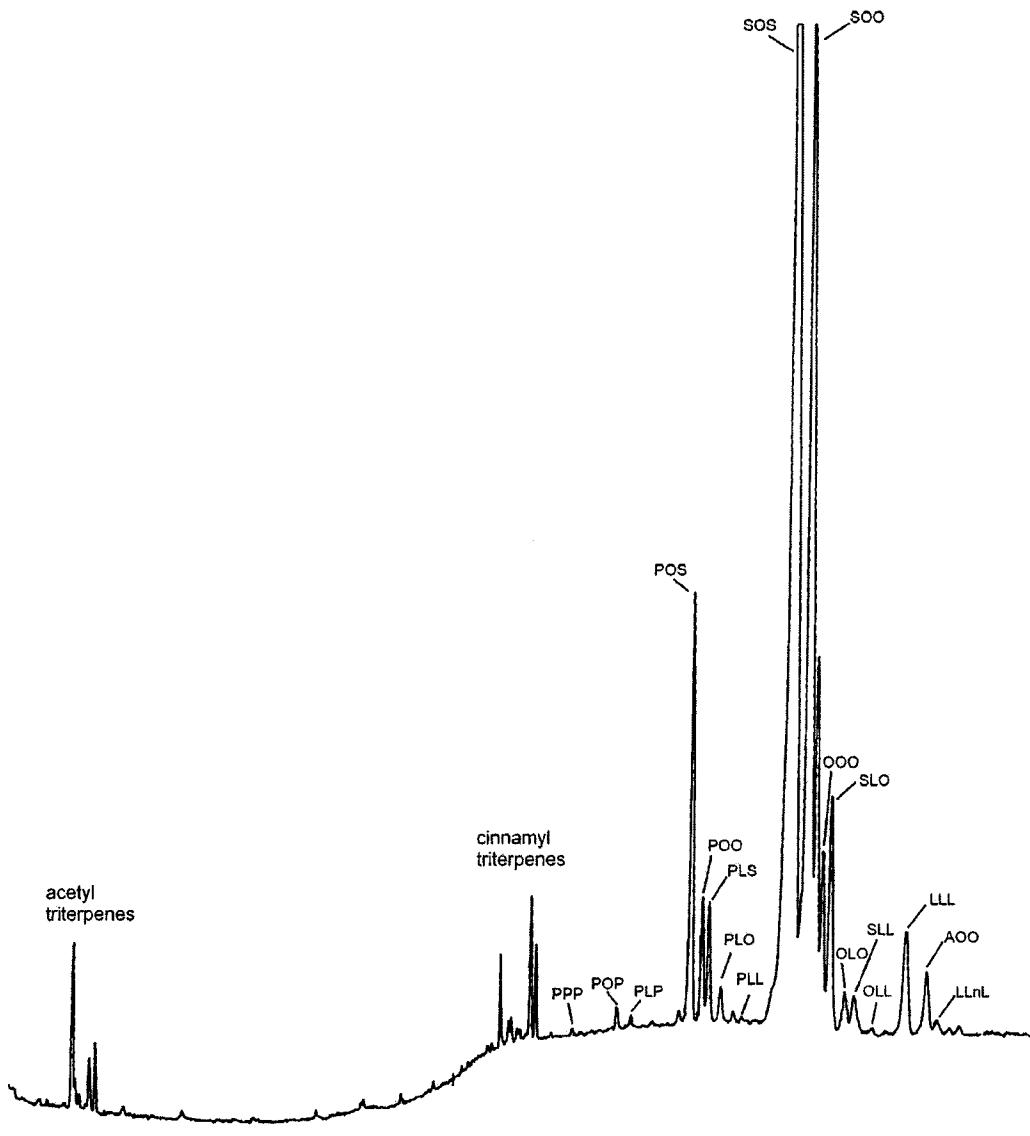


Figure 1. HRGC chromatogram showing triterpene and triglyceride peaks.

with those reported in the literature (24–28) confirmed the identification of the following trans triterpenol acetate and triterpenol cinnamate esterified polycyclic triterpene components: α -amyrin, β -amyrin, leupol, and butyrospermol, as shown in **Figure 2**. In agreement with our results, some other studies (25, 28, 29) dealing with various plant species reported on a mixture of fatty acids and cinnamic acid esters of triterpene alcohols.

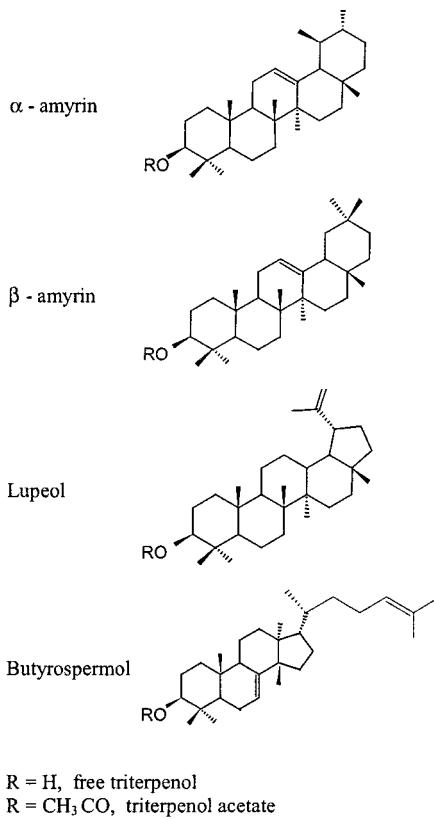
Kernel Fat Content. Data in **Table 1** show higher kernel fat contents and less variation within and between sites than reported in a previous paper (8), likely due to differences in fat determination methodology. In the present study, site means ranged from 41 to 55%, with an overall mean of 48% (158 samples analyzed). Among countries, Uganda had the highest mean kernel fat content at 53%.

Fatty Acid Composition. Shea butter fat is characterized by 16 saturated and unsaturated fatty acids (**Table 2**), although in greatly different percentages: myristic (C14:0), pentadecanoic (C15:0), palmitic (C16:0), palmitoleic (C16:1), heptadecanoic (C17:0), stearic (C18:0), elaidic (C18:1, trans), oleic (C18:1, cis), a fatty acid accompanying oleic acid (C18:1, 11 cis), linoelaidinic (C18:2, cis, trans), linoleic (C18:2, cis, cis),

arachidic (C20:0), linolenic (C18:3, cis, cis, cis), eicosenoic (C20:1n-9), behenic (C22:0), lignoceric (C24:0), and traces of caproic (C6:0), caprylic (C8:0), capric (C10:0), and cerotic (C26:0) acids. For discussion and statistical calculation, only those acids present in a sensibly consistent amount ($\geq 0.01\%$) were retained and considered.

Striking variations in fatty acid composition according to regional *Vitellaria* populations are observed only when Uganda populations are compared with those of the other countries examined. Uganda populations produce a butter in which oleic acid is the major component, amounting to an average value of 57.76%. The butter of Mali, Burkina Faso, and Nigeria has a much lower concentration of oleic acid, ranging from 42.0 to 46.2%.

Palmitic acid, the precursor of stearic acid in the chain elongation process from C16:0 to C18:0 (30), is present in an average concentration of 4.16% in the Ugandan shea butter, compared to an average value of $\sim 3.30\%$ for the butter samples of the other three countries. Most of the previous comments on concentrations of each single major fatty acid are confirmed by the derived percentage values of saturated fatty acid (SFA), unsaturated (USFA), monounsaturated (MUFA), and polyun-

**Figure 2.** Formulas of major polycyclic triterpenes of shea butter.

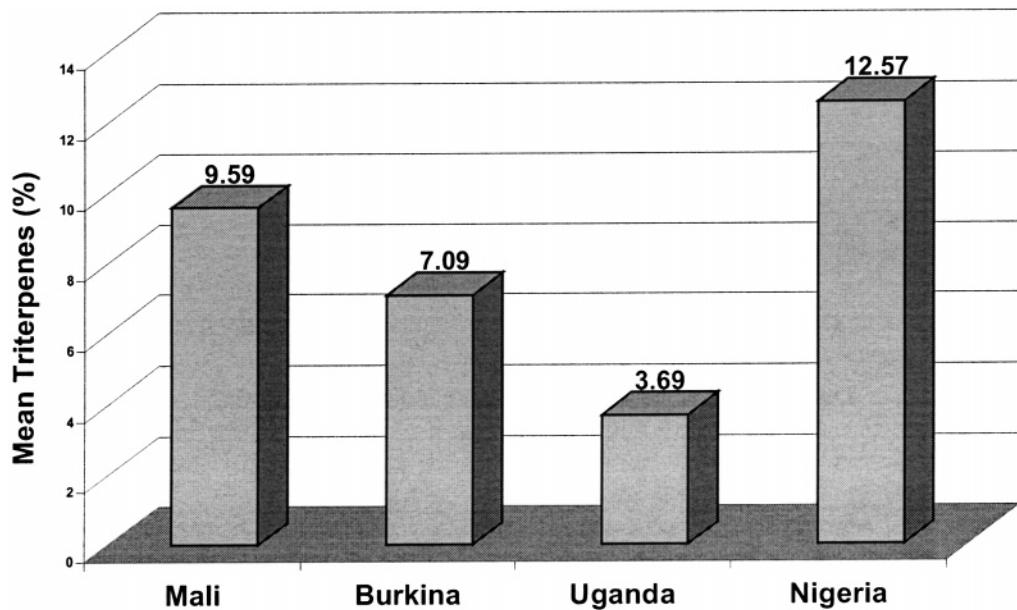
shea butter from Mali averaged 9.59%, whereas provenances from Burkina Faso averaged 7.09%. Triterpene mean separations

were statistically significant at $\alpha = 0.05$, according to Fisher's LSD test. Statistically significant differences were stronger for cinnamyl triterpenes content than for acetyl triterpenes (**Table 4**).

Integration of these data suggests that West African provenances had significantly higher levels of both acetyl and cinnamyl triterpenes than provenances of East Africa. These data should be of significant interest to the cosmetic and pharmaceutical industries (6, 7, 33).

Statistical Analysis. The chemometric statistical trends of the data reported here reflect the geographic separation among the *Vitellaria* populations examined. The LDA model recently demonstrated for accurate groups of olive oil classification (19, 20) was applied to classify samples in clusters according to the country and to identify the variables that are significant for distinguishing among the countries. Statistical comparisons of provenances by country using LDA shows that Ugandan shea butter is compositionally distinct from the West African types, with close to 100% of the samples clearly different from the others (**Table 5**). Mali and Burkina Faso provenances are closest and could be separated correctly in only 64% of the cases. Nigerian shea butter provenances could be separated from the other two West African countries with a higher level of confidence. A similar geographic pattern was reported for a study using RAPD markers to assess gene flow and regional variation across the *Vitellaria* species range (34).

The overall results of this study show that the chemical constituents of shea butter are consistent across the species range, while at the same time the relative proportions of the constituents greatly vary. Regional variants exist with high concentrations of desired classes of compounds such as triterpenes. The data complement previous findings showing high

**Figure 3.** Comparison of total triterpene fractions. Values represent the mean of all provenances analyzed for each country.**Table 5.** Comparison of Shea Butter Composition by Country Using Linear Discriminant Analysis (LDA)

paired comparisons		percentage of correct classification		selected variables
country 1	country 2	original case	cross-validation	
Mali	Burkina Faso	64.7	62.2	cinnamyl 8, linolenic acid
Mali	Nigeria	79.0	74.1	POS, PLS, linolenic acid
Nigeria	Burkina Faso	88.9	81.9	cinnamyl 8, PLS, LLL, eptadecanoic acid
Mali	Uganda	100	98.8	oleic acid, cinnamyl 10, eicosenoic and linolenic acids, PLS, SOS
Burkina Faso	Uganda	100	98.7	stearic and eicosenoic acids, cinnamyl 10, acetyl 5, PLO, SOS
Nigeria	Uganda	97.4	100	SOS, stearic acid, OOO, arachic acid, SLL

endemic levels of catechins and tocopherols in some provenances or ecotypes, together with regionally characteristic triglyceride profiles (6–8).

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