

Phenolic Constituents of Shea (*Vitellaria paradoxa*) KernelsSTEVEN MARANZ,[†] ZEEV WIESMAN,^{*,†} AND NISSIM GARTI[‡]

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Analysis of the phenolic constituents of shea (*Vitellaria paradoxa*) kernels by LC-MS revealed eight catechin compounds—gallic acid, catechin, epicatechin, epicatechin gallate, gallo catechin, epigallo catechin, gallo catechin gallate, and epigallo catechin gallate—as well as quercetin and *trans*-cinnamic acid. The mean kernel content of the eight catechin compounds was 4000 ppm (0.4% of kernel dry weight), with a 2100–9500 ppm range. Comparison of the profiles of the six major catechins from 40 *Vitellaria* provenances from 10 African countries showed that the relative proportions of these compounds varied from region to region. Gallic acid was the major phenolic compound, comprising an average of 27% of the measured total phenols and exceeding 70% in some populations. Colorimetric analysis (101 samples) of total polyphenols extracted from shea butter into hexane gave an average of 97 ppm, with the values for different provenances varying between 62 and 135 ppm of total polyphenols.

KEYWORDS: Shea butter; green tea catechins; gallic acid; LC-MS; antioxidants; Africa

INTRODUCTION

Shea butter, which is a fat extracted from the kernels of *Vitellaria paradoxa* Gaertner (Sapotaceae) [also known as *Butyrospermum parkii*], is becoming increasingly popular as a component of cosmetic formulations, in addition to its long-standing use as a cocoa butter substitute in the chocolate industry (1, 2). Although shea nuts are a major commodity, there are no commercial plantations of shea trees (1). Virtually all of the nuts on the international market are harvested from tree populations in the villages of seven West African countries (3), although the species is to be found in 19 countries across the vast African savanna zone extending from Senegal to Ethiopia (4).

The awareness of industry and consumers alike of the antioxidant activity of phenolic compounds (5–10) has led to an increased demand for cosmetic products with high natural antioxidant contents (11–13). A commercial promotion of shea butter stresses the high levels of UV-B-absorbing triterpene esters of cinnamic acid, tocopherols, and phytosterols (14). Analytical studies report that shea butter contains 5–15% unsaponifiables, including phytosterols (campesterol, stigmaterol, β -sitosterol, and α -spinosterol) and triterpenes (cinnamic acid esters, α - and β -amyrin, parkeol, butyrospermol, and lupeol), and hydrocarbons such as karitene (1, 15–17). However, little or no information is available on the phenolic compounds in shea kernels. Our objective was thus to characterize and quantify the most important phenolic compounds in shea

kernels and to identify the best source populations in Africa for shea butter with high concentrations of phenolic compounds.

MATERIALS AND METHODS

Chemicals and Reagents. Standard phenolic compounds were purchased from Sigma Chemical Co. (St. Louis, MO); these included gallic acid, catechin, epicatechin, epicatechin gallate, gallo catechin, epigallo catechin, gallo catechin gallate, epigallo catechin gallate, and quercetin. HPLC grade methanol and analytical grade hexane, ethyl acetate, and acetic acid were purchased from J. T. Baker (Phillipsburg, NJ).

Shea Nuts and Kernels. Shea trees bear plum-sized fruits consisting of an outer skin enclosing a soft pulp surrounding a (usually single) large seed. The seed (or shea nut) consists of a thin, brittle shell enclosing a hard, dense kernel with a high fat content. The extracted fat is known as shea butter. In our study, 40 accessions of shea nuts from 10 African countries were analyzed. Nuts were collected in the field, depulped if necessary, sun-dried, and then shipped to Israel for analysis. Upon arrival, the nuts were oven-dried at 65 °C for 48 h. Prior to extraction, the nuts were decorticated, and the kernels were ground in a Moulinex 505 coffee mill (Alençon, France).

Preparation of Extracts. For HPLC Peak Separation. The best peak separations were obtained by extracting ground kernels in methanol and evaporating the extract to dryness in a round-bottom flask with a rotary evaporator. Ethyl acetate was then added to the residue, and the contents of the flask were swirled. The ethyl acetate-soluble portion of the extract was decanted into a second round-bottom flask and evaporated to dryness. The residue was resuspended in pure methanol and syringe filtered through a 0.45- μ m Millipore disk (Teknokarma, Spain) before injection into the HPLC. Clean chromatograms were also obtained by extracting ground kernels in hexane, followed by extraction of the defatted solid kernel material with methanol.

For LC-MS Quantitative Analysis. A simpler method was used, which is the basis of all the shea kernel data presented in this paper.

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One gram of ground kernels was weighed into a 50-mL centrifuge tube, and 20 mL of pure methanol was added. The tubes were vortexed briefly and then placed horizontally on an MRC orbital shaker model TS-400FC (Holon, Israel) and shaken at 150 rpm for 24 h. The tubes were then centrifuged at 3000 rpm for 10 min. The supernatant was poured off into 20-mL glass vials, which were placed overnight in a freezer at $-15\text{ }^{\circ}\text{C}$ to precipitate the dissolved fat out of the methanol. Extracts were syringe filtered, transferred to 1.5-mL vials, and injected into an LC-MS at room temperature.

Liquid Chromatography—Mass Spectrometry. Quantitative analysis of sample extracts was performed using an Agilent 1100 series HPLC (Palo Alto, CA) with a G1314A UV detector and a 250×4 mm, $5\text{ }\mu\text{m}$, Lichrospher RP-18 column (Merck, Darmstadt, Germany). The mobile phase consisted of (A) methanol and (B) 0.1% aqueous acetic acid. The flow rate was 1 mL/min with a gradient profile consisting of B with the following proportions (v/v) of A: 0–30 min, 5–35%; 30–35 min, 35–65%; 35–50 min, 65–100%; 50–55 min, 100%.

Mass spectra were obtained with a Bruker MS Esquire 3000 Plus (Billerica, MA) with an electrospray source and ion trap detector operated in negative ion mode. The system was run on Bruker Daltonics Data Analysis 3.0 software.

Optimization Procedures. Different mobile phases and gradients were evaluated in an HPLC system comprising a Varian ProStar 240 solvent delivery module and a ProStar 330 PDA detector (Walnut Creek, CA) monitored at 280 nm. After effective peak separation had been achieved, LC-MS analysis was used. The molecular weights of the compounds giving the major HPLC peaks were determined by MS and compared with those of known phenolic compounds. Solutions prepared for each standard were injected into the LC-MS system, and MS-MS fragmentation spectra of the standards were recorded in the system library. MS analysis was optimized for each LC peak time and target compound so that the fragmentation spectra of peak compounds could be isolated and compared with library records. Comparisons were scored with the Bruker Daltonics system software on a 1000 point scale, with 1000 being a perfect match.

Standard Calibration. Stock solutions were prepared by dissolving precisely weighed standards in methanol. Dilutions were made to obtain solutions of combined standards ranging from 6 to 500 ppm. Calibration curves were generated for each standard by plotting concentrations versus peak areas using the system software. Regression equations from the calibration curves were used to quantify concentrations of each compound in the shea kernel extracts. The figures were multiplied by the dilution factor to obtain the concentration of the compounds in the kernel.

Colorimetric Analysis. The total polyphenol content of shea butter was estimated by colorimetric analysis using the Folin–Ciocalteu reagent method of Gutfinger (18). Shea butter samples from 101 trees were prepared by extraction of dry kernels with hexane, as follows. From samples of shea butter heated in vials to $40\text{ }^{\circ}\text{C}$, subsamples of 0.5 g were taken and dissolved in 5 mL of hexane. Three successive 2-mL quantities of aqueous methanol (60:40 v/v) were added, and the solution was vortexed each time for 2 min. After each addition, the methanolic phase was pipetted off. The combined methanolic solution was evaporated to dryness at $40\text{ }^{\circ}\text{C}$ in a rotary evaporator. The dry residue was resuspended in 1 mL of methanol. An aliquot of 0.05 mL was combined with 2.5 mL of distilled water and 0.125 mL of Folin–Ciocalteu reagent. After 3 min of mixing, 1 mL of saturated sodium carbonate was added. The contents were mixed and made up to volume (5 mL). After 1 h, absorbance was read at 730 nm against a blank in a Jasco V-530 UV–vis spectrophotometer (Tokyo, Japan). Caffeic acid was used to calibrate the concentration as a function of absorbance. This method is the basis of all shea butter analyses presented in this paper.

Statistical Analysis. Analysis of variance (Fisher LSD) was performed with Statistica 6.0 software (19) to determine significant differences between samples and regions.

RESULTS

Compound Identification. From the HPLC chromatogram of the phenolic extract from shea kernels (Figure 1), eight

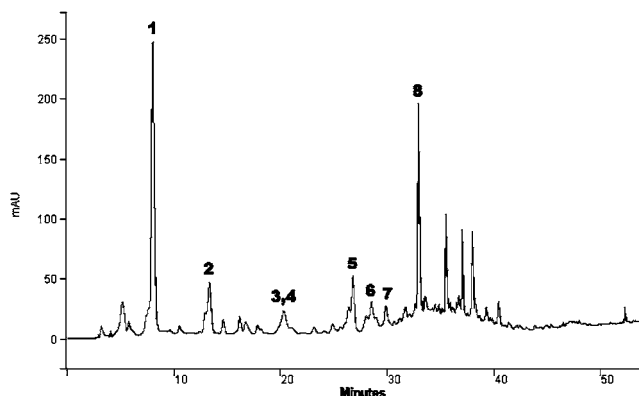


Figure 1. HPLC chromatogram (UV detector 280 nm) of the phenolic fraction extracted from shea kernels: 1, gallic acid; 2, gallo catechin; 3, epigallocatechin; 4, catechin; 5, epigallocatechin gallate; 6, epicatechin; 7, gallo catechin gallate; 8, epicatechin gallate.

Table 1. HPLC Retention Times and Molecular Weights of Phenolic Compounds Found in Shea Kernels

compound	RT (min)	MW
gallic acid	8.0	170
gallo catechin	12.9	306
catechin	20.8	290
epigallocatechin	20.8	306
epigallocatechin gallate	26.7	458
epicatechin	28.1	290
gallo catechin gallate	30.6	458
epicatechin gallate	32.4	442
quercetin	39.3	302

compounds of the catechin family (Table 1) were identified by their MS peaks. Quercetin and *trans*-cinnamic acid were also detected. The MS fragmentation patterns of 10 of the HPLC peaks scored perfect matches with the target standards in the system library. The prominent early peak visible in the HPLC chromatograms (Figure 1) of samples from all provenances was identified as gallic acid, the average concentration of which was 1135 ppm (or mg/kg) of dry shea kernels. This value comprised 27% of the mean total content of nine phenolic compounds (4071 ppm), with considerable variation between regional means. Gallo catechin was the next most prevalent compound (986 ppm or 23% of the average total), followed by epigallocatechin (16%), epigallocatechin gallate (13%), gallo catechin gallate (7%), and catechin, epicatechin, and epicatechin gallate (3% each). Quercetin was present only in trace amounts, averaging 0.1% of the total phenolic compounds.

Variation between Provenances. A comparison of six compounds of the catechin family in 40 samples of shea kernels from 23 sites in 10 African countries is given in Table 2. Samples from the greater Lake Chad basin area of Chad and northern Cameroon (N'Djamena, Kousseri, and Zidim) had very high concentrations of these compounds, with the N'Djamena samples having the highest amounts of total catechins (7274–8042 ppm). Although we do not have sufficient replications to warrant definitive conclusions, we note that the Lake Chad basin area is the hottest and driest of the sites sampled. It is possible that the shea trees in this area are also affected by residual salinity created by historical fluctuations in the surface area of Lake Chad.

Samples from the much cooler and wetter highlands of Guinea and west Cameroon also had high total contents of the six major catechin compounds (Figure 2). Provenances from lowland West Africa, with intermediate rainfall and temperature, had

Table 2. Concentrations (Parts per Million) of Gallic Acid, Gallic acid (GC), Epigallocatechin (EGC), Epigallocatechin Gallate (EGCG), Epicatechin (EC), and Epicatechin Gallate (ECG) in Dry Shea Kernels^a

sample	country	site	gallic	GC	EGC	EGCG	EC	ECG	total
194	Burkina Faso	Lan	420	512	274	456	34	62	1758
205	Burkina Faso	Lan	562	374	162	378	64	68	1608
207	Burkina Faso	Lan	520	186	68	128	22	44	968
210	Burkina Faso	Lan	368	44	16	48	8	22	506
215	Burkina Faso	Lan	478	764	360	462	42	138	2244
322	Burkina Faso	Sapone	410	408	168	34	126	62	1208
266	Burkina Faso	Siniena	380	426	408	466	46	70	1796
737	Cameroon	Bangangte	842	636	542	140	338	84	2582
730	Cameroon	Foumban	3330	884	684	608	80	82	5668
739	Cameroon	Kousseri	2620	1006	1222	390	34	54	5326
740	Cameroon	Kousseri	1230	1728	822	1558	136	268	5742
741	Cameroon	Kousseri	3656	1330	1006	500	32	64	6588
742	Cameroon	Kousseri	2012	1798	868	130	596	104	5508
743	Cameroon	Kousseri	1246	776	662	474	32	92	3282
746	Cameroon	Zidim	444	1972	1520	1592	160	482	6170
744	Chad	N'Djamena	4736	1196	1846	22	196	46	8042
745	Chad	N'Djamena	4550	680	1414	384	188	58	7274
619	Ethiopia	Gambella	202	868	440	20	170	130	1830
657	Gambia	Esaw	872	1254	440	1268	32	102	3968
627	Guinea	Fouta Djalou	460	1282	1084	1118	72	406	4422
631	Guinea	Fouta Djalou	724	1516	2286	1522	314	278	6640
634	Guinea	Fouta Djalou	440	1680	886	2	1132	46	4186
638	Guinea	Fouta Djalou	622	1838	1718	1040	70	194	5482
639	Guinea	Fouta Djalou	1706	2184	2392	2	1018	36	7338
589	Mali	Badougou	118	1312	782	1516	164	236	4128
513	Mali	Koumantou	338	464	314	754	60	102	2032
417	Mali	Sebekoro	1796	454	480	292	42	72	3136
450	Mali	Sirakorola	1112	596	644	2	136	34	2524
344	Nigeria	Kontagora	542	112	80	2	30	8	774
348	Nigeria	Kontagora	994	158	148	92	4	30	1426
350	Nigeria	Kontagora	784	90	56	14	2	6	952
353	Nigeria	Kontagora	2062	306	144	340	44	50	2946
357	Nigeria	Kontagora	2690	1554	498	278	70	24	5114
374	Nigeria	Mokwa	1810	82	80	260	26	44	2302
621	Senegal	Kedougou	386	1698	1416	1688	308	502	5998
39	Uganda	Abim	284	856	154	386	14	90	1784
12	Uganda	Kuju	146	2618	448	1554	72	198	5036
49	Uganda	Lira-Palwo	372	766	154	454	22	94	1862
35	Uganda	Unknown	132	858	176	354	16	166	1702
26	Uganda	Okwang	150	1278	434	494	18	110	2484
51	Uganda	Okwang	64	996	484	500	12	106	2162
44	Uganda	Patongo	250	2210	1010	1772	126	236	5604
	means		1116	994	685	559	145	121	3621

^a Due to similar HPLC retention times, catechin and gallic acid gallate (not shown) were quantified separately with fewer samples.

significantly lower kernel phenolic contents, with the lowest levels being those for samples from Burkina Faso.

The samples from Chad (N'Djamena), in addition to having the highest overall phenolic content, also contained very high levels of gallic acid (mean = 4643 ppm), the mean level being more than double that of any other country (**Figure 3B**). The mean gallic acid content for sites in the regional block comprising Cameroon, Chad, and Nigeria was notably higher (1480–4643 ppm of dry kernel) than that of the Mali–Burkina Faso–Guinea region (448–841 ppm) or Uganda (200 ppm). In contrast, the Ugandan samples had the highest content of epigallocatechin gallate (788 ppm), whereas the Chadian samples exhibited low concentrations of this compound (203 ppm).

Concentration of Phenolic Compounds in Kernels versus Butter. The mean concentration of catechins in dry shea kernels (LC-MS analysis) was slightly more than 4000 ppm (0.4% of the kernel weight), with a range of 2100–9500 ppm. In contrast, we found a mean of 97 ppm total polyphenols in shea butter (colorimetric analysis). The range for 101 samples was 35–915 ppm. These values indicate that 90–98% of the potential phenolic content of shea butter is lost in hexane extraction of

shea kernels. A shea butter sample from northern Uganda that had been extracted with a press followed by purification in boiling water had a comparable total polyphenol content (100 ppm), indicating a similar loss of polyphenols. These losses may be greater when one considers that our shea kernel analysis gives only the content of catechins and not that of all phenolic compounds.

We found a poor (or even negative) correlation between concentrations of catechins in shea kernels and the concentration of polyphenols in shea butter from the same source populations. The mean total polyphenol content of shea butter was highest in the samples from Burkina Faso (**Figure 3A**) and lowest in those from Chad, whereas the inverse was true for catechin content of the kernels (**Figure 2**). Ugandan shea butter also had high concentrations of total polyphenols, whereas Ugandan kernels had less than half the total catechin content of Chadian kernels. This discrepancy may be attributable to the lower solubility of gallic acid, the most hydrophilic of the 10 phenolic compounds that we identified in shea kernels, in the hexane extraction of shea butter from the kernels. Because Chadian shea kernels are very high in gallic acid, this could result in a relatively large loss of phenolic compounds during extraction.

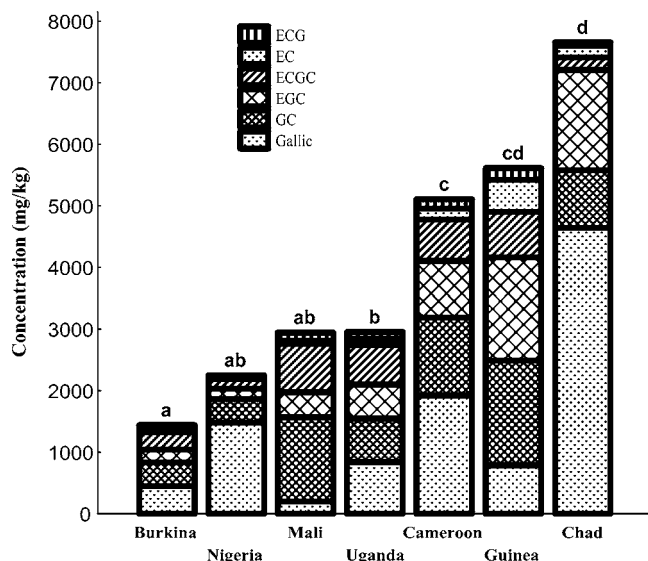


Figure 2. Distribution by country of mean concentrations of catechins in dry shea kernels. Significant differences (Fisher LSD, $\alpha = 0.05$) between countries for the total values of the six catechins are indicated by different letters. Senegal, Gambia, and Ethiopia were not included in the statistical analysis due to insufficient replications.

In contrast, the mean gallic acid contents of shea kernels from Uganda and Burkina Faso were lowest among the countries sampled, which may result in a higher relative retention of kernel polyphenols during hexane extraction. Traditional African shea butter extraction using boiling water may also result in the loss of the gallic acid fraction. Water may dissolve and remove much of the gallic acid and other hydrophilic compounds from shea butter during boiling, whereas hexane may fail to extract these compounds from the kernel along with the butter.

DISCUSSION

Shea butter has been traded for centuries in Africa, where it is used as a cooking fat and medicinal ointment by savanna peoples, who are well aware of its health benefits (20). The nuts are an export commodity and are therefore an important resource of the dry savanna belt north of the equator in Africa (21). However, until recently, very little effort has been devoted to studying this resource (22). Of the 10 phenolic compounds that we have identified in shea kernels, eight are catechins, a family of compounds that is currently receiving considerable attention in antioxidant research (23–26). The phenolic profile of shea kernels is similar to that of green tea (27). Quantitatively, the concentration of catechins in shea kernels compares favorably to the polyphenolic content of olives, which are in wide demand for their antioxidant properties (28, 29). The total content of the 10 major phenolic compounds in ripe olives, determined by an LC-MS analysis similar to ours, was reported to range from 1700 to 8100 ppm (0.17–0.81%) (30). Folin–Ciocalteu estimates of total polyphenols in extra virgin olive oil range from 150 to 800 ppm (31–33), representing a loss of 88–94% of the phenolic content of olives during oil extraction. A mean total polyphenol content of 62 ppm has been reported for refined virgin olive oil (28). This places shea butter between refined virgin and extra virgin olive oil in terms of total phenolic content, with potentially higher levels possible with an extraction method that minimizes phenolic losses. The average catechin content of shea kernels is higher than the average total polyphenol content of ripe olives. These findings should therefore be of considerable interest to the cosmetic industry.

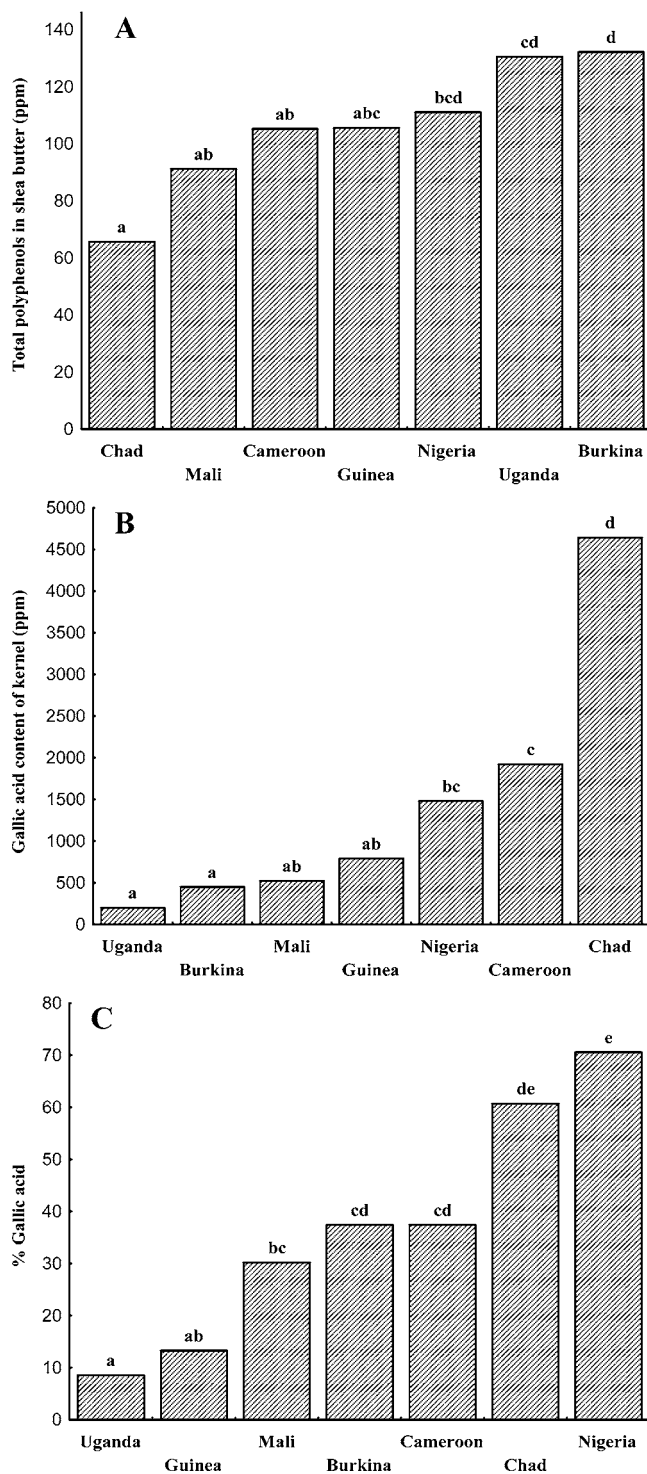


Figure 3. Comparison of country means for (A) total polyphenol content of shea butter, (B) gallic acid concentration in dry shea kernels, and (C) percentage of gallic acid out of six catechins in shea kernels. Significant differences (Fisher LSD) are indicated at $\alpha = 0.20$ (A) and $\alpha = 0.05$ (B, C). Countries for which insufficient numbers of samples were available are not included in the statistical analysis.

Our results show that the overall concentration and the relative percentages of different phenolic compounds in shea kernels vary from region to region. The overall concentration of phenolic compounds in shea kernels may be linked to the level of environmental stress in the source population, with the highest phenolic concentrations occurring in *Vitellaria* trees at the upper and lower temperature limits of the species. This phenomenon

has been widely reported in other species (34–36). It is likely that the relative proportions of the different phenolic compounds are genetically based, as suggested by the occurrence of high gallic acid levels in shea populations in a contiguous geographic area (Chad–Cameroon–Nigeria) that includes both hot and cool zones (Figure 3C). This finding is in keeping with reports of phenolic profiles characteristic of particular cultivars or regional populations in other plants (37, 38).

Our data indicate that *Vitellaria* populations in the Lake Chad basin and in the West Cameroon and Guinea highlands may be especially promising resource areas for high-phenolic shea butter, followed by some sites in the highlands of northern Uganda. At present, these areas have little or no involvement in the international shea nut and shea butter trade. In contrast, the lowland West Africa zone, where most of the shea trade is centered, has relatively low kernel phenolic levels. However, within the West African zone, we would expect to find higher phenolic concentrations in *Vitellaria* populations in the hotter, drier Sahel belt, on the basis of the trend found in the samples we analyzed. Our findings indicate that shea butter extraction and refining processes will need to be modified in order to retain higher levels of phenolic compounds in the final products.

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