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Influence of Climate on the Tocopherol Content of Shea Butter

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The shea tree, *Vitellaria paradox*a Gaertner, is the source of a commercial seed fat known as shea butter. High-performance liquid chromatography (HPLC) analysis of the tocopherol content of shea butters from different regions of Africa showed high variability between provenances and a significant effect of climate on α -tocopherol levels. The total tocopherol content (α , β , γ , and δ) in 102 shea butter samples from 11 countries ranged from 29 to 805 μ g/g of shea butter, with a mean of 220 μ g/g. α -Tocopherol, the principal form detected, averaged 64% of the total tocopherol content. Shea butters from *Vitellaria* populations situated in hot, dry climates had the highest levels of α -tocopherol (for example, a mean of 414 μ g/g in samples from N'Djamena, Chad). The lowest concentrations of α -tocopherol were found in samples from cool highland areas, especially in northern Uganda (a mean of 29 μ g/g).

KEYWORDS: Shea butter; tocopherols; temperature effects; antioxidants; Africa

INTRODUCTION

Shea butter is a common vegetable fat of the savanna zone of Africa north of the equator. The fat is extracted from the kernels of the shea tree, *Vitellaria paradoxa* C. F. Gaertner (Sapotaceae), also known as *Butyrospermum parkii* (G. Don) Kotschy. Shea nuts are exported for use primarily as a cocoa butter substitute or improver in chocolate manufacturing (1). However, with the current trend toward natural ingredients in skin products, shea butter is rapidly becoming a popular component of cosmetic formulations (1, 2).

Although shea nuts are a major commodity, they are not a plantation crop (1). The nuts on the international market are harvested from village tree populations in several West African countries (3, 4). The Vitellaria populations sourced for exports represent only a fraction of the species range, which extends across 19 countries in the vast African savanna zone extending from Senegal to Ethiopia (5). The kernel fat content, fatty acid profile, and antioxidant levels are extremely variable across this zone (6-9). This means that there is great potential to link source populations with desirable characteristics with particular market needs. Currently, the shea butter available on the international market is compositionally better suited for use in the chocolate industry than for cosmetics. This is due to the harder, more crystalline fat (higher ratio of stearic acid to oleic acid) produced by the Vitellaria populations providing the bulk of shea nut exports.

Consumer demand for natural skin products is fueled by consumer awareness of the health benefits of antioxidants coupled with a desire for natural sources instead of synthetic additives (10, 11). We reported in an earlier paper the high content of phenolic compounds in shea kernels (7). The phenolic profile of shea butter is composed of catechin family compounds similar to those found in green tea, which has gained wide attention recently as an antioxidant-rich health beverage (12-15). In this paper, we report the tocopherol content of shea butter. Tocopherols are collectively known as vitamin E and represent an important class of antioxidants (16). Our objective was to characterize shea butter tocopherol composition and to identify the best source populations in Africa for shea butter with high concentrations of antioxidants.

MATERIALS AND METHODS

Chemicals and Reagents. Standards for α -, β -, γ -, and δ -tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO). Highperformance liquid chromatography (HPLC) and analytical grade hexane and methanol 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. We analyzed shea butters extracted from 102 accessions of shea nuts from 11 African countries. Postharvest handling followed common commercial practice. Whole shea fruits were collected in the field, depulped, 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 coffee mill.

Fat Extraction. For each shea nut accession, between 3 and 10 g of ground kernels was placed in a 50 mL centrifuge tube. The tubes were filled to volume with analytical grade hexane and shaken overnight on an orbital shaker. The tubes were then centrifuged at 3000 rpm for 10 min. The supernatant was poured off into 20 mL glass vials, which were evaporated to dryness in a fume hood, leaving the partially or fully crystallized fat. The glass vials were then heated to 45 °C to drive

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 Table 1. Mean Concentration of Tocopherols in Shea Butters from

 Different Provenances in Africa

		μ g/g shea butter					
country	provenance	α -toc	β -toc	γ -toc	δ -toc	total toc	N
Uganda	Okwang	23	9	46	0	78	1
Uganda	Adwari	25	8	0	35	68	1
Uganda	Abim	30	9	37	21	96	2
Uganda	Kuju	35	4	0	5	44	2
Uganda	Olilim	37	0	75	0	112	1
Uganda	Patongo	38	9	84	35	164	1
Burkina Faso	Lan	136	10	16	23	200	9
Burkina Faso	Sapone	177	7	17	7	208	10
Nigeria	Kontagora	160	26	103	60	349	10
Mali	M'Peresso	111	4	23	28	167	10
Mali	Sebekoro	168	22	9	26	225	10
Ethiopia	Gambella	65	0	0	0	65	1
Senegal	Passi	65	14	0	38	117	1
Senegal	Kedougou	166	0	20	47	233	1
Guinea	Fouta	122	9	19	63	213	10
Gambia	Esaw	97	120	4	33	253	2
Ghana	Savelugu	101	23	12	49	184	10
Cameroon	Bangante	59	17	24	90	189	6
Cameroon	Foumban	61	5	29	34	129	4
Cameroon	Kousseri	260	33	51	129	495	5
Chad	N'Djamena	414	0	222	0	786	1
mean concentration		112	16	38	34	208	
mean relative percent		64	7	14	15	100	
minimum provenance mean		23	0	0	0	44	
maximum prove	nance mean	414	120	222	129	786	

off the remaining solvent and to melt the extracted fat prior to homogeneous recrystallization.

Sample Preparation. Glass vials of shea butter were reheated to 45 °C until all crystals were dissolved. Using a Pasteur pipet, a 1 g subsample from each vial was weighed out into a new 20 mL glass vial and 10 mL of HPLC grade hexane was added. Vials were vortexed until all of the fat was dissolved. A fraction of this solution was syringe-filtered through 0.45 μ m Millipore disks (Teknokarma, Spain) and transferred to 1.5 mL vials before injection into the HPLC.

HPLC Analysis. Quantitative analysis of sample extracts was performed using an Agilent 1100 series HPLC (Palo Alto, CA) with a G1314A UV detector and a 250 mm \times 4 mm, 5 μ m Spherisorb SS NH₂ column (Regis Technologies, Morton Grove, IL). The detection wavelength was 295 nm. The mobile phase consisted of an isocratic solution of 75% hexane and 25% ethyl acetate with a flow rate of 1 mL/min.

Standard Calibration. Stock solutions were prepared by dissolving α -, β -, γ -, and δ -tocopherol standards in methanol. Following IUPAC procedures (*16*), the absorbance of the standard solutions was measured at 292 (α), 296 (β), and 298 (γ , δ) nm and the concentration of tocopherols (μ g/mL solution) was calculated by dividing absorbance values by prescribed factors of 0.0076 (α), 0.0089 (β), 0.0091 (γ), and 0.0087 (δ). The concentration of tocopherols in samples was determined in relation to the peak areas of standards. Results were adjusted for the average specific gravity of shea butter (0.90) and expressed as μ g tocopherol/g shea butter.

Statistical Analysis. Analysis of variance (Fisher LSD) was performed with Statistica 6.0 software (*17*) to determine significant differences between samples and regions.

RESULTS

Total tocopherol content varied from a low of 29 to a maximum of 805 μ g/g shea butter, with a mean of 220 μ g/g across all samples (102 samples from 21 sites in 11 African countries). The means for different provenances are given in **Table 1**. The relative proportions of α -, β -, γ -, and δ -tocopherols were variable, including samples from the same source population. The principal tocopherol was the form α , with a mean of 64% of the total tocopherol content, followed by δ (15%), γ (14%), and β (7%).

Table 2. Mean Concentration of $\alpha\mbox{-}{\rm Tocopherol}$ in Shea Butters from Different Climate Zones

	mean annual	mean α -toc		
climate	temperature (°C)	μg/g	N	
cool moderate warm hot	20–22 27–28 29–31 32+	44 98 145 299	20 4 59 8	



Figure 1. Comparison of mean α -tocopherol levels in extracted shea butter with mean June temperatures during final maturation stage of shea fruits (92 samples from 22 sites). Statistical significance (Fisher LSD, α = 0.05) is noted.

Influence of Climate. Provenances with hot, dry climates have a strikingly higher tocopherol content than warm, moderate, and cool areas. This trend is seen most clearly with α -tocopherol (**Table 2**), where the mean of each climate category is almost double that of the next coolest zone. Our temperature information is drawn from NOAA (National Oceanographic and Atmospheric Administration) Africa maps and temperature tables (18). A line plot giving analysis of variance mean separations for tocopherol levels under seven different generalized June temperatures is given in **Figure 1**. Because shea fruits mature in June across most of the species distribution area (1), mean June temperatures were used to plot a rough relationship with α -tocopherol content. A more precise correlation between climate and tocopherol levels in shea butter would require detailed local temperature and soil moisture data.

We excluded 10 shea butter samples from the Fouta Djalon highlands of Guinea from the temperature analysis. We previously reported that the seed fat of this *Vitellaria* population has a much lower proportion of unsaturated fatty acids than is usually found in cool, highland areas (6, 8). On the basis of French colonial records (19), this population appears to be very recent and is most likely derived from shea trees with similar fat composition (6) growing in adjacent lowland areas (8). The mean α -tocopherol content of Fouta Djalon shea butter samples was 122 μ g/g, while the mean annual temperature was around 20 °C (1, 8).

DISCUSSION

The results show that shea butter is a potentially rich source of natural vitamin E for cosmetic formulations. About two-thirds of the vitamin E found in shea butter occurs in the form of α -tocopherol (**Table 1**), which some studies have found to have

the highest antioxidant activity among the tocopherols (20), although this is not always the case (21). The α -tocopherol content appears to be directly related to the temperature of the climatic zone from which the butter is provenanced (Figure 1). Total tocopherol content in shea butter from N'Djamena, Chad, averaged 786 μ g/g, while the median total tocopherol content of Ugandan highland shea butter was 87 μ g/g. The difference between the two locations was greater for α -tocopherol: 414 μ g/g from Chad vs a mean of 29 μ g/g from Uganda. This observation shows that shea trees in the hottest part of the Sahel zone may produce shea butter containing almost 10 times as much vitamin E and 15 times as much α -tocopherol as Vitellaria populations in cool highland areas. This indicates that the amount of α -tocopherol in shea butter increases as both total tocopherols and the percentage of α -tocopherol increase with temperature.

The effect of temperature on tocopherol content of vegetable oils is well-known. Increased tocopherol content of oils during seed maturation under high temperatures or depressed tocopherol levels under lower temperatures have been reported for canola, soybean, sunflower, oats, and flax (22-24). There are reports of a decrease in total tocopherols in response to elevated temperature in some crops (24) or increases in the percentage of one tocopherol without affecting the total tocopherol level (22). Elevated temperature and drought were found to increase α -tocopherol in olives (25). Our data show that the tocopherol content of shea butter is similarly influenced by temperature during seed maturation and perhaps also by drought. It should be noted that the hottest zones in our survey area are also the driest.

The anomalous data from the Fouta Djalon highlands of Guinea suggest that in this population there may be a genetic component overriding the influence of climate. We note that this *Vitellaria* population appears, based on historical records, to be recently derived from the adjacent lowlands (8, 19). Because both the fatty acid profile and the mean tocopherol levels resemble that of the lowland *Vitellaria* population, it is possible that the new population has not yet adapted to the cool highland environment.

Tocopherols represent an important class of antioxidants. We previously identified and quantified eight catechin family phenolic compounds in shea kernels by LC-MS fragmentation spectra and peak area measurements (7). These compounds represent another major antioxidant group. Each of these antioxidant classes shows a different relationship to climate. Phenolic compounds are typically produced in plants in response to stress, which may occur at both higher and lower temperatures (26, 27). Tocopherol content increases with temperature, while phenolic compounds in shea kernels follow a parabolic curve, with elevated levels occurring under both cool, wet and hot, dry conditions (Figure 2). The lowest phenolic levels are found under unstressed, mesic growth conditions. Shea nut yields are also reported to be highest under intermediate temperature and rainfall conditions (19). Overall, the highest antioxidant levels (both tocopherols and polyphenols) are found in shea populations in the hottest, driest areas. Areas with intermediate temperatures have intermediate levels of tocopherols in shea butter coupled with low kernel polyphenol levels. Under cool temperatures, the trend in the two antioxidant classes is markedly different (Figure 2): low levels of tocopherols occur in shea butter while moderately high polyphenol levels are found in shea kernels.

These findings should be useful for identifying potential source populations for high antioxidant shea butter. The use of



Figure 2. Effect of climate on the concentration of α -tocopherol in shea butter as compared with the phenolic content (total catechins) of shea kernels. The values represent the means of provenances sampled within each climate category.

shea butter in cosmetic formulations should increase as trade links develop with these promising areas, which currently have little or no involvement in international shea nut commerce.

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