

PHYSICO-CHEMICAL CHARACTERISTICS OF SHEA BUTTER (*Vitellaria paradoxa* C.F. Gaertn.) OIL FROM THE SHEA DISTRICTS OF UGANDA

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ABSTRACT

Shea oil is a vegetable oil obtained from the seeds of the shea tree (*Vitellaria paradoxa* C.F. Gaertn.). It constitutes an important source of fat in food and cosmetics. Although shea oil can be marketed both locally and internationally, increasing demand worldwide for exportable products calls for their certification. Characterization of shea oil is one step towards developing its certification system. In this study, the physico-chemical characteristics of shea oil in different shea zones of Uganda were assessed. Samples of shea fruits were collected between the months of June-August 2007 in the districts of Pader, Lira, Katakwi and Arua representing Acholi, Lango, Teso, and West Nile shea zones, respectively. Seed oil was extracted by Soxhlet apparatus using n-hexane solvent and analysed for colour, refractive index, viscosity, oil content, acid value, peroxide value, saponification value, iodine value, α -tocopherols and fatty acid profile. Shea oil content, colour, refractive index and viscosity ranged from 41-54%, orange to orange-yellow, 1.670-1.690 and 2.4-2.8 cP, respectively. Acid and peroxide values ranged between 2.3-12.59 mgKOH/kg and 2.10 to 2.50 meq/kg, respectively. Saponification, iodine and α -tocopherols values were between 160 mgKOH/g and 192mgKOH/g, 39.21 I₂/100 and 41.37 I₂/100g and 26.3-44.4 mg/100g, respectively. Fatty acid profile for palmitic, stearic, oleic, linoleic and arachidic fatty acids ranged between 6.52-8.12%, 28.65-30.94%, 55.54-57.63%, 6.18-7.79% and 0.65-0.90%, respectively. Although there was significant variation in the oil yield ($P \leq 0.05$), the physico-chemical characteristic and fatty acid profile showed no significant variation in the shea zones of Uganda ($P \leq 0.05$). The fact that physico-chemical characteristics of shea oil from the different shea zones of Uganda are comparable to other high value edible vegetable oils indicates its suitability as raw material for food, cosmetic and pharmaceutical products. This characterization is a bench mark for monitoring the quality of shea oil from Uganda and can be used to enhance its local and international trade.

Key words: Shea, oil, physico-chemical, Uganda, *Vitellaria paradoxa*

INTRODUCTION

Worldwide, natural vegetable oil and fats are increasingly becoming important in nutrition and commerce because they are sources of dietary energy, antioxidants, bio-fuels and raw material for the manufacture of industrial products. They are used in food, cosmetic, pharmaceutical and chemical industries. Vegetable oils account for 80% of the world's natural oils and fat supply [1]. Edible oil in Uganda is mainly imported, an indication that oil production in Uganda is lower than the demand of 45,000-50,000 MT and which is growing at 3% per annum [2].

Although the major raw materials for production of vegetable oil in Uganda are sunflower, palm, soya bean and cotton seeds, existence of indigenous plants that contain oil are well known among rural communities. With increasing awareness of the importance of vegetable oils in the food, pharmaceutical and cosmetic industries, there is need to focus on indigenous plant species to meet the increasing demand. *Vitellaria paradoxa* (the shea tree), an indigenous wild tree is one such plant of African savanna parkland [3]. Although the tree is mostly found in the wild, attempts to conserve it on farm have been initiated [3]. The eastern sub species *Vitellaria paradoxa sp. nilotica* occurs in Ethiopia, Southern Sudan and Uganda [4]. In Uganda, *Vitellaria paradoxa* are mostly distributed in northern, north-eastern and West Nile regions of the country [4]. *V. paradoxa* tree has been described as socially and economically important; and has been included in the priority list of African Genetic Resources by the FAO [5].

The *V. paradoxa* nuts/seeds are usually processed into shea oil that constitutes an important source of fat. The shea butter fat can also be used in soap making, cosmetic and traditional medicine in many rural areas [4, 6, 7]. Due to its richness in food nutrients, the shea oil has found market as baking fat, margarine and other fatty spreads, confectionery and chocolate industry in Europe and Asia [8]. Although use of shea butter alone for cosmetics in USA has been growing at an annual rate of 25%, differences between the physico-chemical composition and fatty acid profiles of shea oil have been reported for both West African and Ugandan shea butter [9]. In the case of West Africa, a variation even within neighbouring shea trees has been reported. These variations in physico-chemical composition of vegetable oils have often been attributed to environmental factors such as rainfall, soil fertility, maturation period, agronomic practices and genetic substitution [9, 10]. With the increasing global demand for shea oil, characterization of physico-chemical properties of shea oil originating from Uganda is essential. This study, therefore, investigated the physico-chemical composition of shea oil from the different shea districts of Uganda.

MATERIALS AND METHODS

Study area

Fresh shea fruits were collected between July and August 2007 from Pader, Lira, Katakwi and Arua districts, each representing the Acholi, Lango, Teso and West Nile shea zones, respectively in Uganda. Uganda is a landlocked nation in eastern Africa

that straddles the equator, west of Kenya, south of Sudan, with Rwanda and Tanzania to the south. Uganda's western border with the Democratic Republic of the Congo is defined by the Albertine Rift, the western branch of the Great Rift System of Africa. This Rift Valley boundary area includes two of the African Great Lakes (Lake Albert and Lake Edward), the snow-capped Rwenzori mountains (considered by many to be the legendary "Mountains of the Moon"), as well as many imperiled species, like the Mountain gorilla.

Sample collection and preparation

Five kilograms of shea fruits were randomly collected from under the different shea trees in each district. The fruits were then stored in a dark cool box at 4°C and transported to the laboratory for analysis. In the laboratory, the fruits were de-pulped and seeds/kernels dried (between 40 and 50°C) for 5 days. The dry kernels were dehusked manually using a metallic rod. The nuts were further dried for another 5 days at the same temperature, milled into powder using an electric grinder machine (Brooks Crompton series 2000, UK), packed in a low dense polyethylene (LDPE) bag and stored in a dry cupboard till oil extraction was complete. All chemical and reagents used were of analytical grade. For other specific preparations, distill water was used. Oil from the milled nuts was extracted with *n*-hexane solvent for 5 hours using Soxhlet apparatus at 60°C. The extracted oil was later concentrated using a rotary evaporator and dried by heating in a vacuum oven at 50°C for 60 minutes. The percentage yield of the crude oil was determined gravimetrically. The extracted oil samples were transferred into brown glass bottles and stored in a refrigerator at 5°C until all analyses were completed.

Laboratory analyses

Physical characteristics

Colour, refractive index and viscosity were determined using recommended methods [11]. A lovi bond apparatus (Tintometer model E, S. No.5064E England), a viscometer (BROOKFIELD DV-11+Pro, USA) at 34-35°C and a refractometer (Bellingham + Stanley (B⁺S), No. A86006, England) were used to determine colour, viscosity and refractive index, respectively

Chemical characteristics

Acid, saponification, peroxide and iodine values were determined using standard methods [11]. α -tocopherol was determined by High Performance Liquid Chromatography (HPLC), Perkin Elmer [12]. A calibration curve was prepared using a standard alpha tocopherol (CAS 59-02-9) from Sigma –Aldrich (USA). One ml of each working standard solutions (20ppm, 40ppm, 60ppm, 80ppm, 100ppm) was pipetted into separate conical flasks followed by methanol (30ml), 10% ascorbic acid solution (3ml) and 50% KOH (4ml). Contents were shaken and sonicated for 10 minutes. The solutions were saponified for 2 hours under reflux at a temperature of 40-50°C in the dark. The contents were then cooled to ambient temperature, transferred into a separating funnel and washed with 10ml of distilled water.

The saponified solution was extracted consecutively three times with 70ml, 40ml and 40ml, respectively using petroleum ether (40-60°C analytical grades) by shaking for 5 minutes. Ether extracts were combined, 100ml of distilled water added and shaken three times for 5 minutes until it was neutral to phenolphthalein indicator. The ether extract was then passed through anhydrous sodium sulphate and evaporated to dryness on a water bath at 70°C. The residue was dissolved in 3ml of distilled methanol and 20µl was injected into the HPLC. The samples were separated in a column (C8, Perkin Elmer, 250 mm x 4.0 mm) using methanol-water (85:15 v/v) solvent system (mobile phase).

Two to three grams of each shea oil sample type was treated the same way as the standard. Using the same standard preparation method for α -tocopherol, the (2-3g) of each shea oil sample type was separated using HPLC brownlee analytical C8 column, Perkin Elmer (250 mm x 4.0 mm) by employing Methanol-Water (85:15 v/v) solvent system (mobile phase) with a flow rate of 1.5 ml/min. The value of α -tocopherols in the standard was detected by the UV detector set at 284 nm. The α -tocopherols concentrations in the sample were computed using standard curve of α -tocopherols and reported in mg/100g [12]

Fatty acid profiles were assayed using trans-esterification method [13]. Shea butter sample (7.0 -10.0 mg) was transferred into a 15ml thick walled glass tubes, followed by the addition of 1ml anhydrous methanol containing 2M HCl. The contents were flushed with purified nitrogen gas and the tubes securely closed with teflon-lined screw caps. Each tube was then placed in an oven for 2 hours at 90°C for complete methanolysis. After cooling to room temperature, the tube was opened and the methanol evaporated down to 0.5 ml with a stream of nitrogen gas. To reduce solubility of methyl esters in the methanol phase, 0.5 ml distilled water was added to the methanolised lipid fraction. The cap of the tube was tightened and mixed for one minute followed by centrifugation to separate the phases. Using a pipette, the upper hexane layer containing fatty acid methyl esters was transferred carefully to a vial. The water-methanol phase was extracted twice using 1 ml n- hexane.

One microlitre (1µl) of the mixed hexane extract was injected splitless (the split opening after 4 ml) in Elmer 8500 gas chromatograph equipped with a flame – ionization detector (FID) on a 25 m x 0.25 mm (i.d.) column coated with polyethylene glycol (PEG) as a stationary phase of 0.2 µm thickness (CP-WAX 52CB Chrompack) and the mobile phase was hydrogenised at 20psi. The injector and detector temperatures were set at 260°C and 330°C respectively. The oven was programmed at 90°C for 4 minutes before cooling for the next run. The chromatographic peaks were identified by comparison with the standard chromatograph of the mixture of 20 fatty acid methyl esters, gas-liquid chromatography (GLC) reference standard 68D Nu-Check – Prep (Elysian , Minn., USA). The components eluting from the column were detected by FID whose output signal was captured and recorded in computer with Turbochrome 4 soft-ware data system.

To monitor the performance of the column in the gas chromatograph, the standard mixture of the fatty acid methyl esters were chromatographed at regular intervals for tenth sample running. The amount of each fatty acid in the sample was expressed as % of the sum of all fatty acids in the sample as indicated below:

$$\% .fatty.acid = \left(\frac{Fatty.acid.peak.area}{\sum total.fatty.ac.id.peak.areas} \right) \times 100$$

RESULTS

Physicochemical composition of shea oil

Oil yield from the shea kernels samples in the different shea districts ranged between 41 and 53.56%. Katakwi district in the Teso shea zones exhibited the highest oil yield (53.56%) compared to Pader, Lira and Arua districts. Analysis of the physical characteristic showed that there was a significant variation in oil colour but not refractive index and viscosity ($P \leq 0.05$). The shea oil samples' colour ranged from orange to orange–yellow, refractive index was between 1.67-1.69 while viscosity was in the range of 2.4 to 2.8 cP (Table 1).

The colour of oil from Katakwi, Lira and Arua samples were yellow-orange while the Pader sample was orange. The colour of shea oil from Arua and Lira districts was more yellow-orange than Katakwi district sample (Table 1). The chemical characteristics of shea oil such as acid, peroxide, saponification and iodine values ranged between 2.3 and 12.59 mgKOH/kg, 2.10 and 2.50 meq/kg, 160 and 184 mgKOH/g and 39.21 and 41.37 I₂/100, respectively (Table 1).

The acid values of the samples from Pader, Lira and Katakwi districts were significantly different from that of Arua district ($P \leq 0.05$). There was, however, no significant difference in peroxide, saponification and iodine values in the samples from the different shea districts of Uganda ($P \leq 0.05$). The α -tocopherols values in the shea oil samples from the different districts of Uganda ranged between 0.40 and 26.3mg/100g. The α - tocopherols content of shea oil samples from Pader was significantly lower than shea oil samples from Lira, Arua and Katakwi districts (Table 1).

Fatty acid profile of shea oil

The fatty acid composition of shea oil is presented in Table 2. The values of the five major fatty acids in shea oil: palmitic, stearic, oleic, linoleic and arachidic fatty acids in Uganda ranged between 6.52 and 8.12%, 28.65 and 30.94%, 54.99 and 57.72%, 6.18 and 7.79% and 0.65 and 0.90%, respectively. Although oleic and stearic fatty acids were the dominant fatty acids in the shea oil, there was no significant variation ($P \leq 0.05$) in the values of these fatty acids in the different shea districts of Uganda (Table 2).

DISCUSSION

Physicochemical composition of shea oil

With the emerging shea oil market in the world, its oil yield or fat content is the most important characteristic to be considered. The oil yield from shea kernels in the different districts of Uganda varied from 41 to 53%. The variation in shea kernel oil content exhibited in the different shea districts of Uganda has previously been reported [14]. Even if the mean shea oil kernel content for samples from Katakwi district (53.56%) is quite distinct from the rest, the shea oil yield content of above 40% obtained for the different districts of Uganda is good. This is an indication that shea oil can easily be extracted from the seeds for use as vegetable oil.

Although Uganda's shea kernel oil content is the highest in Africa, the variation in the shea oil content of samples from the different shea zones could be attributed to environmental influence, geographical location, agronomic factors and genetic variation [14, 15]. Katakwi and Lira districts with the highest shea oil content are characterized by bi-modal rains, which are usually experienced between April to August with hot and dry season occurring from November to February which is the fruiting season for shea trees.

High shea oil content in the samples from Katakwi and Lira districts could be due to their early fruiting during the dry season between December and February where temperatures range between 31-35°C. High elevation and cool temperatures are also associated with high levels of shea kernel oil content [15, 16]. In Uganda, different shea districts have elevations of between 1,100-1,350 m. Katakwi and Lira shea districts with high shea oil content fall within altitudes of between 900-1,000 m and with temperatures of between 30-35°C during the dry season. On the other hand, Pader and Arua districts with low shea oil contents have elevations of between 1,200m and 1,350m and experience temperatures ranging from 35-40°C. The high shea oil content from the samples in Uganda makes it a potential source of shea oil supply.

In commercial setting such as manufacturing and trade, evaluation of physico-chemical characteristics of shea oil quality is very significant. The shea oil acid value obtained in this study is representative of samples from many trees. Because of the recalcitrant nature of shea fruits, early germination may increase the free fatty acid of shea oil. Free fatty acid of shea oil (1 and 20%) with peroxide value of less than 10, obtained in this study, is the characteristic of the majority of many edible vegetable oils [17, 18].

Saponification and iodine values of shea oil exhibited in this study are also lower than saponification and iodine values for most vegetable oils [17, 18, 19]. The peroxide value (2.1-2.5 meq/kg), saponification value (160-192 mgKOH/g) and iodine value (39-41 I₂/100g) are, however, similar to those reported previously for the shea oil. The colour of shea oil obtained in this study is similar to reported findings in the previous studies [9, 14, 20]. The yellow-orange colour of shea oil samples may be an

indication of the presence in shea oil of β -carotene pigments which are nutritionally important.

The viscosity values obtained for shea oil samples from the shea districts of Uganda fall within the category of most fluids while their refractive index does not differ so much from refractive indices of sun flower, soya bean and palm oil [17, 18]. The chemical composition of shea oil obtained in this study also conforms to the proposed regional standards for shea butter [21]. This means that shea oil can be commercialized both locally and internationally.

Although tocopherols represent an important class of antioxidants and shea oil contains α -tocopherols, a low value of between 26.3mg/100g and 44.4mg/100g) with significant variation ($P \leq 0.05$) has been exhibited. Several factors linked to environment factors, storage period of the oil and genetic influence have been reported to cause variation in α -tocopherols [9, 22]. Low values of α -tocopherol obtained in this study could have resulted from the prolonged storage of shea oil samples before analysis since some tocopherols might have been used-up in chemical quenching of oxidants (free radicals) during long periods of storage.

Earlier studies indicate that Uganda's shea butter from Pader district had a mean value of 29 $\mu\text{g/g}$ (mg/100g) compared to a mean value of 220 $\mu\text{g/g}$ for the shea oil samples from the different countries in West Africa [9]. It has been reported that α -tocopherol always increases with temperature during seed maturation and also drought [23]. The high α -tocopherol values (44.4mg/100g) obtained in Katakwi compared to other shea districts in Uganda could, therefore, be due to the high temperatures above 30°C always experienced in the area .

Characterization of shea oil for nutritional, pharmaceutical and cosmetic purposes is, therefore, very important since changes in α -tocopherol can be an aspect for monitoring of the shea oil quality. Even if shea oil has been used as vegetable fat, cosmetic as well as medicine for centuries, the cosmetic industries, for example, require oils with unique fatty acid profile such as oil with very high oleic fatty acid which makes a soft base for the creams [7]. Acid value, peroxide value, saponification value and iodine value are also indicators of edible oils that are suitable for food, cosmetic, soap making and lubricants since increase in these values can be associated with rancidity of oils due to oxidation. The changes in acid, peroxide and iodine values can, therefore, be used in monitoring deterioration of shea butter.

In general, the chemical composition of shea oil indicates that it can be used as edible vegetable oil, cosmetic, lubricant and for soap making. Anti-oxidants such as α -tocopherol can be responsible for reducing degenerative diseases and also for mopping up free radicals responsible for oxidative damage of cell membranes, skin and causing of cancer [24]. Since α -tocopherol is one of the groups of fat soluble vitamin E compounds that cannot be synthesized by animal cells, it must be obtained from plant sources through diet [23]. Because of their vital role in nutrition and

cosmetic industry, the presence of α -tocopherol in shea oil makes it an important oil especially in cosmetic applications, human diet, nutrition and health.

Fatty acid profile of shea oil

Although shea oil is characterized by 16 saturated and unsaturated fatty acids, the five fatty acids (oleic, stearic, palmitic, linoleic and arachidic) are the most dominant in shea oil with values greater than 0.01%. The five fatty acid composition of shea oil in the Pader, Lira, Katakwi and Arua shea districts of Uganda exhibited no significant variation ($P \leq 0.05$). Although Uganda's shea oil oleic fatty acid values has been reported to range between 37% and 55% [9], the differences in shea oil fatty acid reported in this study could probably be due to variation in the harvesting season, geographical locations, method of laboratory analysis and genetic variability.

Variation in fatty acid composition between Ugandan and West African shea oil exists. In these reports [6, 9], Uganda's shea oil had 57% oleic and 30% stearic while the West African had 45% oleic and 34% stearic. Linoleic acid is an essential fatty acid that is vital in nutrition because of its un-saturation. Shea oil samples from the shea districts of Uganda had linoleic acid in the range of 6% to 8% which is lower than in passion fruit seeds with values of 67% to 74% [25]. Sunflower oil and soya bean oil were also found to have values of 74% and 53% of linoleic acid, respectively [26]. The linoleic acid value content of 6-8% makes shea oil a moderate source of essential fatty acids in the human diet. In addition, linoleic acid can be used to synthesize arachidonic acid and other biologically important compounds in most mammals including humans [26].

Chemical composition and dietary profile of the fat is an important aspect of human nutrition since fats serve as a source of dietary energy. The high level of un-saturated fats in Uganda's shea oil makes it better edible oil. This is because food containing high levels of un-saturated fats can: improve digestibility, easily infiltrate the bile salt and bind to low weight proteins. The fact that the oleic fatty acid values in shea oil are higher than in soya bean oil (25%) and palm oil (36%) makes shea oil a good source of poly-unsaturated fatty acids too. Linoleic acid content of shea oil from Uganda of 7% when compared with that of soya bean oil (53%) and palm oil (10%) is moderately lower in essential fatty acids [26, 27, 28].

Consumption of monounsaturated fatty acids such as oleic acid (>55% in shea oil) is believed to be beneficial in reducing blood levels of low-density lipoprotein (LDL) cholesterol ("bad" cholesterol), hence lowering the risk of coronary heart diseases now on the increase among urban populations in developing countries such as Uganda. Shea oil could, therefore, offer an alternative source for dietary fat especially at this time when food prices are increasing. The implication of this is that shea oil would need to be promoted both locally and internationally.

Because cocoa butter has high level of stearic and palmitic fatty acids just like shea oil, the European Union has recommended shea butter to be blended into chocolate as an equivalent to cocoa butter or cocoa butter improver which is a more affordable

substitute [29]. This increase in the demand of the shea butter in the cocoa industry could lead to promotion and improvement in household income for the shea parklands' communities. Since certification of shea kernels and shea butter is going to become important in the European markets, the demand for consistency in quality of shea products exported to such highly regulated markets would benefit from findings of this study. Besides, the information that has been generated through analysis of data on physico-chemical characterization of shea oil could also be used to guide traceability of geographical origin and certification of shea products from Uganda.

CONCLUSION

Although the results show that different components (chemical composition and fatty acid profile) of shea oil from different shea districts in Uganda are not significantly different ($P \leq 0.05$) from each other, the differences observed in acid value, colour and α -tocopherols, require further investigation associated with post-harvest handling of shea oil. The characteristics exhibited by the physico-chemical composition and fatty acid profile of shea oil from Uganda make it a possible and potential raw material for cosmetics, soap, food processing (as edible vegetable oil) and in the bakery/confectionery sector. These values are of high significance in the development of standards for Uganda's shea oil and improving its commercialization and tradability both locally and internationally.

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Table 1: The physico-chemical properties of shea butter oil from different districts of Uganda

Physico-chemical properties	District				
	Pader	Lira	Katakwi	Arua	Soya bean (<i>Glycine max</i>) Oil [30]**
**Colour (Degree of colour mixtures)	Orange	Yel-Or	Yel-Or	Yel-Or	Brown
Refractive index	1.467 ± 0.001	1.468 ± 0.001	1.468 ± 0.000	1.469 ± 0.001	1.467
Viscosity (cP)	2.4 ± 0.2	2.6 ± 0.2	2.8 ± 0.4	2.6 ± 0.2	-
Oil yield content (%)	41.11±1.02	50.83±1.26	53.56±1.26	45.11±0.19	20.1
Acid value (mgKOH/kg)	3.00±0.53	3.18±0.27	2.30±0.66	12.59±0.17	1.0
Peroxide value (mEq/kg)	2.25±0.35	2.20±0.42	2.10±0.14	2.50±0.71	0.2
Saponification value (mgKOH/g)	177.32±1.89	192.15±1.99	160.35±1.21	184.14±1.85	193
Iodine value (I ₂ /100g)	39.34±1.07	36.60±1.15	41.37±6.10	39.21±0.54	126
α-tocopherols content (mg/100g)	26.30±4.29	36.6±0.02	44.4± 0.29	40.0±0.17	-

*The short abbreviations are represented as follows: Yel-Or ≡ Yellow-Orange colour, Yel-Gr ≡ Yellow-Green colour, Red-Or ≡ Red-Orange. All results in (Table 1) are means of triplicate analysis and are compared with ** Properties of Soya bean (Glycine max) oil*

Table 2: The fatty acid profile of shea butter oil from different districts of Uganda

Fatty acid properties (%)	District				
	Pader	Lira	Katakwi	Arua	Soya bean (<i>Glycine max</i>) oil [30]**
Palmitic acid (C _{16:0})	8.04±1.23	6.52±0.18	7.14±.54	8.12±0.55	10.60
Stearic acid (C _{18:0})	28.65±2.23	29.43±0.49	30.94±1.67	28.76±0.89	04.00
Oleic acid (C _{18:1n9})	55.54±0.87	57.63±0.77	56.72±0.98	54.99±1.05	23.30
Linoleic acid (C _{18:2n6})	6.86±0.44	6.42±0.12	6.18±0.11	7.79±0.92	53.70
Arachidic acid (C _{20:0})	0.67±0.11	0.78±0.01	0.90±0.18	0.65±0.09	00.30

*All results in Table 2 are means of triplicate analysis and are compared with ** Properties of Soya bean (Glycine max) oil*

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