



Full Length Research Paper

An evaluation of the microbial contents of shea nut cake

Mathias Imram Ndayishimiye

Department of Biochemistry and Biotechnology, College of science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

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In the quest for finding ways of utilizing shea nut cake (SNC), 24 samples of SNC were obtained from six industries to investigate the presence of microbes, minerals, proximate and phytochemical constituents. The samples were examined for total coliforms, total viable, faecal and *Escherichia coli* contamination. Other microbes were isolated and identified with the aid of the API kit. The SNC samples were also screened for bioactive (medicinal) potentials. The proximate and mineral constituents of the waste product were studied as well. The means in log cfu/g of total coliforms, total viable, faecal and *Escherichia coli* were: 1.95 ± 0.74 , 4.98 ± 1.17 , 0.82 ± 0.49 , and 0.48 ± 0.42 , respectively. Other microbes identified were: *Brevibacillus agri*, *Bacillus mycoides*, *Bacillus cereus* and *Staphylococcus epidermidis*. The phytochemical screenings of extracts of the cake samples revealed that SNC contains saponins, tannins, alkaloids, terpenoids and reducing sugar. The proximate results indicated that the SNC has $13.03 \pm 1.70\%$ crude protein, $59.37 \pm 8.66\%$ carbohydrates, $23.38 \pm 10.15\%$ crude fat, $4.25 \pm 0.79\%$ ash content, $5.29 \pm 0.98\%$ moisture and $8.71 \pm 0.85\%$ fibre. The N, K, and Mg contents of the cake were respectively: 2.96 ± 0.39 mg/kg, 4.05 ± 0.62 mg/kg, and 1.43 ± 0.65 mg/kg. The rest of the minerals were: P (0.22 ± 0.04) mg/kg, Na (0.40 ± 0.05) mg/kg, Ca (0.51 ± 0.09) mg/kg, Cu (0.09 ± 0.05) mg/kg, Hg (0.10 ± 0.56) mg/kg and Pb (0.13 ± 0.07) mg/kg. This study highlights the potential applications of the SNC in the feed, animal, fertilizer and therapeutic industries.

Key words: Shea nut cake, microbiology, proximate-mineral composition, phytochemicals, Ghana.

INTRODUCTION

The shea tree (*Vitellaria paradoxa*) occurs predominantly in the Northern, Upper East and Upper West regions of Ghana and some parts of the Brong Ahafo region (FAO, 1988). The tree is perennial and deciduous and occurs mainly on dry open slopes (Yidana, 2004). The shea tree attains height of about 6.1 m and girths of 61 centimeters in the wild when it is often ravaged by bushfires. They can however reach heights of about 15 m and 17 cm girths under protected conditions. The trees grow slowly from seeds, taking about 30 years to maturity.

The tree has gained importance as an economic tree crop because of the heavy demand for its butter both locally and internationally. It has been reported that more than 2.5 million tons of shea kernel produced worldwide were used for the production of cosmetics, pharmaceuticals and confectionery and edible fats (Ghana News Agency, 2006). Shea butter is produced by women and women groups throughout the year in almost every community in the Northern regions of Ghana. The Tolon and Gumo communities of the Tolon-Kumbungu

*Corresponding author: E-mail: imram.mathi44@rediffmail.com

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District, Kalariga and Giso-Naayili of the Tamale Metropolis, Savelegu of the Savelegu-Nantong District and Techiman of the Techiman Municipality are some of the areas where shea butter is produced in large quantities.

The Northern regions of Ghana have a long history of limited natural resources with consequential economic hardships. The situation is worsened by the continuous loss of soil fertility to bush fires and overgrazing. The shea industry has contributed immensely to the economic growth of the Northern part of Ghana, but not without challenges. The task of finding a lasting solution to the challenges of the local shea industry was so great and so uncompromising that it demanded a practical approach. Recent discovery of biotechnology offered the opportunity and became the appropriate tool to consider in the approach to this problem. The applications of biotechnology in the agricultural sector are evident but yet to be felt openly in the industrial sector of Ghana in particular.

Recent studies suggest that the shea industry will continue expanding forever and may pick up speed over time (Moore, 2008). Vigorous research has been conducted into the phenology of the shea tree, its usage and that of the uses of the

shea butter extracted from the nuts of the shea fruit (Mahamadi et al., 2009; Yidana, 2004). The shea industry has been viewed to equalize the cocoa industry in the coming years as shea butter gradually becomes the best substitute for cocoa butter (De Muelenaere, 1997; Moore, 2008). Some researchers into animal feed development in West Africa have challenged the perception that the shea cake is largely a waste produced from shea butter processing centers in large quantities (Konlan, 2010; Pousga et al., 2007).

Unfortunately in Ghana no or very little research has been done aimed at expanding the benefits and adding value to the supply chain of the shea industry. This research is intended to investigate the microbial load of the shea nut cake, its proximate and mineral composition as well as its phyto-chemical constituents. This compositional study was intended to fill the academic and economic gaps created by inadequate research into the shea nut cake and to broaden its economic value.

MATERIALS AND METHODS

Sampling

Samples were taken monthly for four months (March to June 2011) with particular attention on the colour and texture of the shea nut cake (SNC) samples. They were collected from four local industries (women groups) and two well established industries in Ghana. The industries were all located in the Northern and Brong Ahafo regions of Ghana. Two of the local industries ('Suglo N bori buni' and 'Tungteiya Women groups') were found in Kalariga and GisoNaayili, respectively in the Tamale metropolis, the Northern Regional capital, 'Wunnisong' Women Group of Tolon, 24 km West of Tamale, 'Gub-Danda' shea women group of Gumo, 10 km

North-West of Tamale, Shebu Industries at Savulegu, 24 km North of Tamale and the Ghana Nuts Limited in the Techiman Municipality of the Brong Ahafo region of Ghana. In all, twenty four SNC samples (four from each industry), Table 1 were collected into aseptic containers. All samples were transported to the laboratory as early as practicable and stored at 4°C until needed.

Bacteria isolation

Total viable count

Total viable count was enumerated according to Tassew and Seifu (2011) with some modification. One gram of sample was aseptically transferred and mixed in 9 ml of sterile physiological saline. Ten fold serial dilutions were made and viability of microbes assessed in triplicates using the pour-plate method. The plates were incubated at 35°C for 24 hr after which all spots and spreads were counted and recorded as total viable count using the colony counter (Stuart Scientific Colony Counter, UK). Each colony was sub-cultured until pure cultures were obtained and stored as slant cultures at 5°C for further analysis.

Total and faecal coliforms

The most probable number (MPN) method was used to determine total and faecal coliforms in the samples. Serial dilutions (10^{-1} to 10^{-5}) were prepared by picking 1g of the sample into 9 ml physiological saline. One milliliter aliquots from each of the dilutions were transferred into 5 ml of MacConkey Broth with inverted Durham tubes and incubated at 35°C for total coliforms and 44°C for faecal coliforms for 18 to 24 h. Tubes showing colour change from purple to yellow and gas collected in the Durham tubes after 24 h were identified as positive for both total and faecal coliforms. Counts per 100 ml were calculated from most probable number (MPN) tables (Tassew and Seifu, 2011).

Isolation and identification of *Escherichia coli*

From each of the positive tubes identified, a drop was transferred into a 5 ml test tube of trypton water and incubated at 44°C for 24 hr. A drop of Kovac's reagent was then added to the tube of trypton water. All tubes that showed a red ring colour development after gentle agitation denoted the presence of indole and recorded as presumptive for thermotolerant coliforms (*Escherichia. coli*). Counts per gram were calculated from most probable number (MPN) tables (Hood et al., 1983).

Isolation and identification of other microbes

Colonies from the total viable count were sub-cultured until pure cultures were obtained. These were examined by their colonial and cell morphology, Gram reaction and other biochemical tests. Identification of species was carried out by assaying cultures in Analytical Profile Index (API) galleries; API 50CHL (bioMérieux, Marcy L'Etoile, France) and API 20E (Analytab Products, Inc., Plainview, N.Y.).

Chemical analysis of the shea-nut cake samples

Samples were analyzed for proximate composition using standard methods. Briefly the dry matter (DM) was determined after drying in a vacuum oven (Model VO 200, Germany) at 100°C and the

Table 1. Sample location and the number of samples collected.

District	Sampling site	Sample label	Number of samples
Tolon-Kumbungu	Tolon	A	4
Tamale Metro	Kalariga	B	4
Tolon-Kumbungu	Gumo	C	4
Tamale Metro	Gisonaayili	D	4
Savelugu-Nantong	Shebu	E	4
Techiman Municipal	Ghana Nuts Ltd	F	4

Table 2. Mean (\pm SD) log cfu/100ml of microbiological quality of Shea Nut Cake.

Parameter	Log of cfu/100 ml
Total coliforms	1.95 \pm 0.74
Faecal count	0.82 \pm 0.49
Total viable count	4.98 \pm 1.17
<i>Escherichia.coli</i>	0.48 \pm 0.42

Each value is a mean of six means of four determinations \pm SD.

Kjeldahl method (AOAC, 1997) was used for determination of crude protein (CP) after determining the nitrogen content and multiplying by 6.25 factor. Crude fat was determined according to AOAC (1997) whiles Fibre content was determined according to Pousga et al. (2007) with some modification. The ether extract (EE) was determined after acid hydrolysis with the total ash determined by burning at $550 \pm 5^\circ\text{C}$ for 4 h. The Atomic Absorption Spectrophotometer (210 VGP, US) was used to determine the mineral content of the by-product.

Phytochemical screening of the shea nut cake

Saponins, cynogenic glycosides, reducing sugars, tannins, flavonoids, terpenoids, anthraquinones and alkaloids were determined according to Adebayo and Abdul-Mumeen (2012) with some modifications. Coumarins were determined according to Savithramma et al. (2011).

Statistical analysis

Data were subjected to the ANOVA procedure using the Minitab 15 (2000 version) software. Pair-wise comparisons were made where differences were significant using the Fisher's LSD to compare the effect of location and method of butter extraction on the chemical composition of the by-product (the shea-nut cake).

RESULTS AND DISCUSSION

The shea nut industry holds a potential for the development of northern Ghana. The total value of an industry lies in the individual value of the various chains of production of goods and services. Apart from the international recognition of the butter extracted from the shea fruit, the shea tree has enormous contribution to the herbal medicine folklore. The proximate, mineral, phytochemical and microbial analyses of the shea

nut cake reveal value addition to the supply chain of shea products.

Microbiological quality of shea nut cake

Table 2 shows the microbiological quality of shea nut cake (SNC) from six shea butter extraction centres of Northern Ghana. The mean total viable count (TVC) for the six industries ranged from 3.55 ± 0.60 to 6.63 ± 0.22 log cfu/g, respectively. The TVC values were significantly different ($p \leq 0.05$) across the shea industries. The highest figures of total coliforms (3.14 ± 1.84 log cfu/g), faecal coliforms (1.64 ± 1.23 log cfu/g) and *E. coli* (1.15 ± 0.78 log cfu/g) were observed in samples from the Kalariga women group. The samples from Shebu Industry of Savulegu recorded the least values in terms of coliforms.

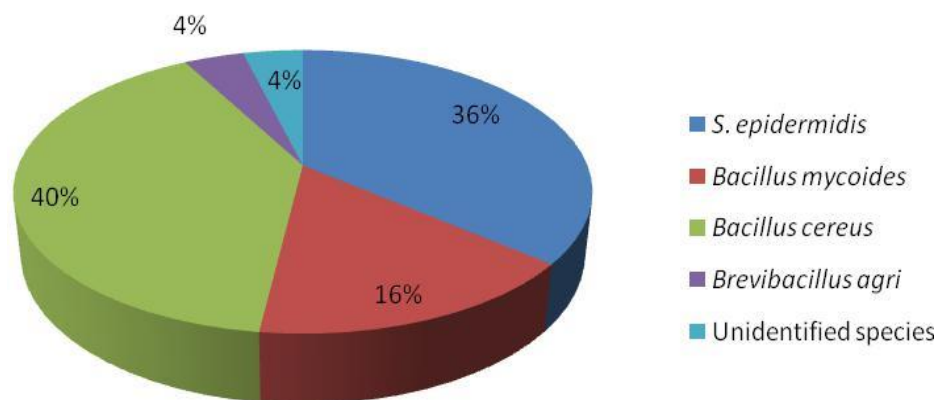
It is possible that the presence of *E. coli* in the samples was as a result of contamination from the water or containers used in the processing or even from the personnel involved in the processes and sometimes from the processing environment. The total coliforms, faecal coliforms and *E. coli* values across the shea industries, both the local and well established, were not significantly different. Differences in the TVC across the shea industries were significant. It was highest in samples from Gumo and least in the GisoNaayili samples. Microbial information about the shea nut cake has not been documented until now. The average shea qualities were expressed in Table 3.

Microbial identification

The isolation of other microbes was carried out using

Table 3. Identified bacterial species in shea nut cake.

Bacteria Identified	No. of isolates
<i>Staphylococcus epidermidis</i>	18
<i>Bacillus mycoides</i>	8
<i>Bacillus cereus</i>	20
<i>Brevibacillus agri</i>	2
Unidentified species	2
Total microbes	50

**Figure 1.** Percentage distribution of bacterial isolates from shea nut cake.

dilution plate method. Pure colonies were obtained by repeated streaking. Fifty isolates (Table 3) were obtained in all. The isolates were a mix of both Gram positive (29 isolates) and Gram negative (21 isolates), mostly rod shaped microorganisms. Almost all the isolates were motile and possessed endospores at the centre.

Based on morphological characteristics, biochemical, cultural and the combination of API 20E and API 50CHL, 36% Gram (+), mostly rods, were identified as *Staphylococcus epidermidis*, 16% Gram (+), mostly rods were identified as *Bacillus mycoides*, 40% Gram (+) rods as *B. cereus* and 4% Gram (+) rods as *Brevibacillus agri*. 4% isolates showed no reaction on the API 20E and 50CH (Table 3 and Figure 1).

It is reported that research efforts are geared towards the scanning of the microbial diversity of various environments (and many agricultural waste substances) and deciphering their genetic information aimed at isolating micro-organisms that could be used in the manufacture of drugs, enzymes and a wide range of bioactive compounds, as well as in bioremediation processes (Sasson, 2005). The biotechnological approach is multifaceted in nature capable of transforming the chemical industry through biotechnology innovations, research and the supporting policies.

Tallent et al. (2012) reported that *Bacillus cereus* is a group of ubiquitous facultative anaerobic spore-forming Gram (+ve) rods commonly found in soils. *B. cereus*

is widely distributed in nature: rice, soil, growing plants, intestinal tract of insects and mammals, oriental dishes, ingredients, peas, beans, cereals, milk, infant foods, most Chinese 'take out', honey samples, dairy products, rabbit erythrocytes, seafood (Wong et al., 1988). The present study has also identified *B. cereus* in shea nut cake, the first of its kind to be reported.

The use of *B. cereus* in the industrial sector has been evident. Romanczyk et al. (1995) reveals that the aroma associated with 'pop corn, corn chip' is as a result of 2-Acetyl-1-pyrroline produced by *B. cereus*. Optimum production of protease, a useful enzyme in the leather industry, is achieved at Ph = 8 and 35°C extracted from *B. cereus* (Uyar et al., 2011). The results presuppose that shea nut cake could be source of useful enzymes for the food and leather industries. *Brevibacillus agri* is a spore-forming Gram (+ve) bacterium. *B. agri* has been studied and known to achieve optimum growth at pH 7 and temperature of 45°C with specific growth rate of $0.102 \pm 0.015 \text{ h}^{-1}$ (Kongpol et al., 2009). The *B. agri* has shown high level tolerance for toxic organic solvents such as vinyl acetate, n-tetradecane, cyclohexane, n-pentanol, n-butanol, iso-butanol, butyl acetate, ethyl acetate and fairly towards toluene (Kongpol et al., 2009; Mayorga et al., 2010). Thus, 4% of the isolates could be useful microorganisms for transforming xenobiotics for bioremediation of polluted environments. Kittikun et al. (2008) have showed that *B. agri* has lipase activity of 1.8

Table 4. Proximate qualities of various shea nut cake samples from six shea industries of Northern Ghana.

Location	Total ash %	Moisture %	Crude protein %	Crude fat %	Fibre %	Carbohydrate %
Tolon	4.88	4.55	10.37	36.50	9.19	48.26
Kalariga	4.50	5.20	11.85	27.88	8.41	55.61
Gumo	3.75	4.90	12.87	24.63	7.11	59.06
GisoNaayili	3.50	6.50	13.88	26.13	8.96	56.50
Shebu Ind.	3.50	6.45	14.99	18.88	9.35	62.64
GNL*	5.38	4.15	14.22	6.25	9.26	74.16
P-Value	0.125	0.006	0.038	0.007	0.311	0.016

*GNL – Ghana Nuts Limited.

U/ml in medium containing 1.5% lard as sole carbon source at 40°C for 48 h. *S. epidermidis* is commonly found in the epithelial surfaces of human being and at the surfaces of indwelling medical devices e. g. catheters (Cheung et al., 2010). Many researchers have assessed the industrial importance of *S. Epidermidis*, *S. epidermidis* has, since 1974, been identified as source of extracellular lipases extracted at pH range of 7 to 9. The lipases are said to be very effective for triglyceride hydrolysis at the α -positions to produce monoglycerides more rapidly than at the β -positions (Pablo et al., 1974). Thus lipases from *S. epidermidis* do not produce free fatty acids (FFA's).

B. mycoides is a Gram-positive spore-forming species of the family Bacillaceae (Goodwin et al., 1994). It has evolved since 1886 as species to *B. cereus* var. *mycoides* in 1946 and was reclassified in 1986 *B. mycoides*. Unlike *B. cereus*, there has not been any report of toxin production and disease caused by *B. mycoides*. On the contrary, *B. mycoides* has been reported to produce bacteriocin capable of fighting serious food pathogens (Sharma and Gautum, 2008). According to the findings of that research, the antibacterial substance was purified by the salt saturation method with partially purified bacteriocin withstanding temperatures of up to 100°C, and was pretty active at pH range of 4 to 11 (Sharma and Gautam, 2008). Therefore *B. mycoides*, found in shea nut cake could be important source of bacteriocin extraction that could be used for food preservation in the food industry.

Proximate analysis

The results of proximate analysis carried out on shea nut cake from six industries are presented in Table 4. The values of crude fat ranged from 6.25 to 36.50% with an average figure of $23.38 \pm 10.15\%$. The fat content was least at GNL-Techiman and highest at Tolon. There were significant differences ($P \leq 0.05$) in all the proximate constituents including the fat content except crude fibre and total ash of the shea nut cake across the industries. This gross phenomenal disparity in fat content in

particular could be attributed to the shea butter extraction method adopted by each industry. There are about twice as much crude fat in the cake from the women groups as compared to that from the industries (Table 5). The GNL employs both mechanical and chemical methods to achieve maximum (98%) butter extraction from the shea kernel (personal communication with Aka Aristide, GNL Techiman) whiles the local industry at Tolon adopts the traditional method of shea butter extraction which achieves about 30% of butter extraction (Ofosu, 2009). Although the Tolon, Kalariga, Gumo and Giso-Naayili all adopted the traditional method of butter extraction, there were remarkable differences in the crude fat content of the cake across the local industries. This undoubtedly exposes the arbitrary nature of the methods used at the various local setups. It is possible that there is the involvement of *S. epidermidis* commonly found on the epithelial surfaces of human including the hands. *S. epidermidis* has been reported to have high lipase activity. The women believe that there are some women whose hands are best in causing the butter rise during kneading. Some are better and others are good; the good ones are the ordinary people. The best ones possibly have high numbers of *S. epidermidis* on the epithelial surfaces of their hands.

The crude protein and fibre contents were highest at Shebu followed by GNL industries (Table 4). The local groups in general produced lower protein content as compared to the industries (Table 5). It is possible that the shea nut cake which goes through three different heating processes (boiling of the shells, dry frying of the pulverized kernels and intense heating of the crude butter to dryness) in the hands of the women groups results in the lost of some of the protein molecules. The level of protein content in the SNC makes it suitable for animal feed and this is supported by the results obtained by Oddoye et al. (2012) which showed that SNC generated in shea nut processing industries in Ghana is a potential feed ingredient due to its high values of protein. The various heating conditions could also lead to breakage of glycosidic linkages in the dietary fibre polysaccharides in the shea nut cake. The total Ash and fibre contents between the local groups and industries (Table 5) were

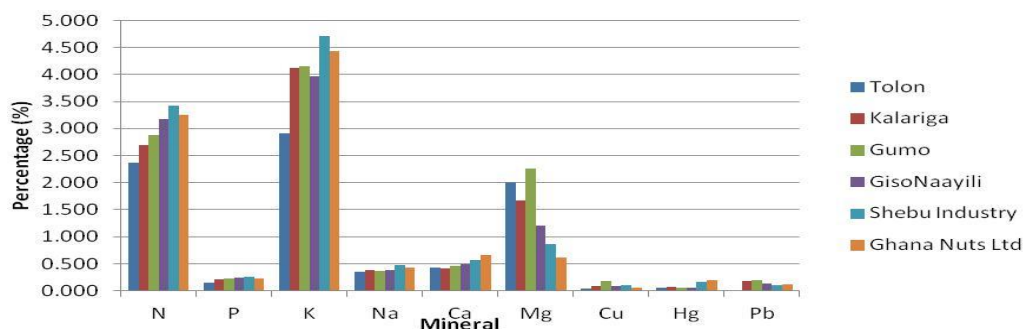
Table 5. Proximate composition (%DM) of Shea Nut Cake according to industry.

Composition	Local groups	Industry
Total ash	4.16	4.44
Moisture	5.29	5.30
Crude protein	12.24	14.61
Crude fat	28.79	12.57
Fiber	8.42	9.31
Carbohydrates	54.86	68.40

DM-Dry matter.

Table 6. Mineral composition of shea nut cake (mg/kg) from the local Groups and Industry.

Composition	Local groups	Industries
Nitrogen	2.780	3.34
Phosphorus	0.210	0.25
Potassium	3.790	4.57
Sodium	0.370	0.45
Calcium	0.450	0.62
Magnesium	1.780	0.74
Copper	0.100	0.08
Mercury	0.060	0.18
Lead	0.130	0.12

**Figure 2.** Mean Percentage Mineral Composition of Shea Nut Cake from six shea production centres.

statistically not significant.

Mineral analysis of shea nut cake

Mineral analysis was also carried out. The results indicated that potassium, sodium, calcium and lead content of the cake were not different ($P \geq 0.05$) across the six shea industries (Table 6). Mercury, copper, magnesium, phosphorus and nitrogen were all different ($P \leq 0.05$) across the industries. The N, P, K and Na contents were highest at Shebu Industry and closely followed by GNL (Figure 2). The two industries however showed lower magnesium contents relative to the local industries. In addition to other qualities, the load of heavy metal of the cake was fairly reasonable for use in

compost development for agricultural purposes. William (2000) reports that the recommended metal limits for heavy metal use of compost is 75, 50 and 0.5 mg/kg for lead (Pb), copper (Cu) and mercury (Hg), respectively (Bodsch, 1998). Shea nut cake has 0.127 mg/kg, 0.094 mg/kg and 0.102 mg/kg for Pb, Cu and Hg, respectively according to the findings of this research.

Phytochemical constituents of shea nut cake

The medicinal value of plants and plant products lies in some chemical substances that produce a definite physiological action on human body (Adebayo and Abdul-Mumeen 2012).

The value of herbal medicinal substances is embedded

Table 7. Phytochemical constituents of shea nut cake from six shea industries of Northern Ghana.

Sample source	Saponins	Tannins	Reducing sugars	Cyanogenic glycosides	Anthraquinones	Alkaloids	Flavonoids	Terpenoids	Coumarins
Tolon	+	+	+	-	-	+	-	+	-
Kalariga	+	+	+	-	-	+	-	+	-
Gumo	+	+	+	-	-	+	-	+	-
GisoNaayili	+	+	+	-	-	+	-	+	-
Shebu Industry	+	+	+	-	-	+	-	+	-
GNL	+	+	+	-	-	+	-	+	-

+ = present, - = absent

in the phytochemicals present in that substance. Phytochemical are the basic source for the establishment of several pharmaceutical Industries (Savithramma et al., 2011). The limited knowledge about the shea nut cake medicinal potential, hitherto, has limited its use in the therapeutic industry. Saponins, tannins, alkaloids, terpenoids and reducing sugar were all present in the shea nut cake (Table 7).

Conclusion

Shea nut cake (SNC) has much more nutritional quality than previously thought since the protein, carbohydrates, fibre, potassium and magnesium contents observed in this study were quite high. Although there were the presence of some bacterial pathogens such as *B. cereus* and some heavy metals in the shea cake samples, their contents did not exceed the permissible safety limits. The SNC is also a host of useful microorganisms that can be exploited for the production of enzymes, bacteriocins, probiotics and for bioremediation processes. Forty percent of microbes isolated from the SNC are capable of producing the enzyme lipase which can influence shea butter extraction when extracted. The high nitrogen, potassium and magnesium contents also

make SNC ideal for use as manure and fertilizer production. Also the presence of essential phytochemicals in the SNC widens its application to therapeutic remedies.

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