BIOCHEMICAL AND MICROBIOLOGICAL ANALYSIS OF SHEA NUT CAKE: A WASTE PRODUCT FROM SHEA BUTTER PROCESSING

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DECLARATION

I, Iddrisu Abdul-Mumeen, hereby declare that this submission is my own research work towards the award of the Master of Philosophy degree and that, to the best of my knowledge, it contains no materials previously published by another person nor material which has been accepted or concurrently been used for the award of any other degree in this University or elsewhere, except where acknowledgment has been duly cited in the text and in the references.

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ABSTRACT

In the quest for finding alternative ways of utilizing shea nut cake produced in large quantities as a by-product by both the local and well established industries, 24 samples of shea nut cake were obtained from six industries to investigate the presence of microorganisms, minerals, proximate and phytochemical constituents. The samples were examined for total viable count, total coliforms, faecal and *E. coli* contamination as well as other coliforms bacteria. The shea nut cake samples were also screened for bioactive (medicinal) potentials capable of managing microbial diseases. The means in log cfu/g of total viable count, total coliforms, faecal coliforms and *E.coli* were 4.98 ± 1.17, 1.95 ± 0.74, 0.82 ± 0.49, and 0.48 ± 0.42 respectively. Other microbes identified were *Brevibacilllus agri*, *Bacillus mycoides*, *Bacillus cereus* and *Staphylococcus epidermidis*. The phytochemical screening of extracts of the cake samples revealed that shea nut cake contains saponins, tannins, alkaloids, terpenoids and reducing sugar. The proximate results indicated that the shea nut cake has 13.03 ± 1.70% crude proteins, 59.37 ± 8.66% carbohydrates, 23.38 ± 10.15% crude fat, 4.25 ± 0.79% ash content, 5.29 ± 0.98% moisture and 8.71 ± 0.85% fibre. The Nitrogen, Potassium, and Magnesium contents of the cake were 2.96 ± 0.39, 4.05 ± 0.62, and 1.43 ± 0.65 mg/kg respectively. The rest of the minerals were Phosphorus 0.22 ± 0.04, Sodium 0.40 ± 0.05, Calcium 0.51 ± 0.09, Copper 0.09 ± 0.05, Mercury 0.10 ± 0.56 and Lead 0.13 ± 0.07 mg/kg. Lead exposures increase blood pressure in adults and have developmental and neurobehavioural effects on foetuses, infants and children. Mercury accumulates in the food chain when in organic form and it is toxic in the elemental form. This study highlights the potential applications of the shea nut cake in the animal feed, fertilizer and therapeutic industries.

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The urge to see through local belief and indigenous technology with a 'scientific eye' has always been integral part of me since infancy. This was the motivation when I first researched into the stability of herbal formulations in calabashes and clay pot containers often adopted by most Herbalist up north of Ghana for treatment of myriad of diseases. The challenge became more intense when it dawn on me the real situation on the ground after I was offered a place to pursue this course in this noble institution, KNUST. Fortunately for me this topic arose when I met Dr. Hillary D. Zakpaa of the Department of Biochemistry and Biotechnology, who suggested it to me. The success of this research could not have been possible without the support of my able supervisor Dr. Hillary D. Zakpaa, who suggested the topic and offered to supervise this research and whose immense support, contributions, advice and patience from the concept paper to the final write-up was evident. I am truly indebted to you.

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DEDICATION

I dedicate this dissertation to my wife, Niematu Abdul-Mumeen, to our son Abdul-Mumeen Abdul-Waaris and to my father Mallam Dawuda Iddrisu.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
CBE	Cocoa Butter Exchange
CF	Crude Fat
CFU	Colony Forming Unit
CP	Crude Protein
CRIG	Cocoa Research Institute of Ghana
DM	Dry Matter
EE	Ether Extract
FAO	Food and Agricultural Organization
FC	Faecal Coliforms
GNA	Ghana News Agency
GNL	Ghana Nuts Limited
ME	Metabolizable Energy
MPN	Most Probable Number
OM	Organic Matter
PLoS	Public Library of Science
SADA	Savanna Accelerated Development Authority
SD	Standard Deviation
SNC	Shea Nut Cake
TC	Total Coliforms
TVC	Total Viable Count

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The Northern Regions of Ghana have a long history of limited natural resources as compared to the southern sector with consequential economic hardships, a situation worsened by the continuous loss of soil fertility due to bush fires and overgrazing. The shea butter industry has been one of the major sources of redeeming the Northern part of Ghana but not without challenges such as the low extraction efficiencies. The shea tree (*Vitellaria paradoxa*) which occurs predominantly in the Northern, Upper East and Upper West Regions of Ghana and some parts of the Brong Ahafo Region serves as the source of raw material for the shea butter industry (FAO, 1988). The tree is perennial and deciduous and occurs mainly on dry open slopes (Yidana, 2004). It grows slowly from seeds, taking about 30 years to mature and can attain a height of about 6.1m and girth of 61cm in the wild where it is often ravaged by bushfires. However they can reach heights of about 15 m and 17 cm girths under protected conditions.

The tree has gained importance as an economic tree because of the heavy demand for its butter both locally and internationally. 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 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.

Recent studies suggest that the shea industry will continue expanding forever and may pick up speed over time (Moore, 2008). The shea industry has been projected to equalize the cocoa industry in future as shea butter gradually becomes the best substitute for cocoa butter (De Muelenaere, 1997 and Moore, 2008). Vigorous research has been conducted into the phenology of the shea tree, its usage and that of the shea butter extracted from the nuts of the shea fruit (Mahamadi *et al,* 2009, Yidana, 2004). However much cannot be said of the by-product, shea nut cake. Research into (Konlan 2010; Pousga *et al.,* 2007) development of animal feed rations in West Africa contradicts the perception that the shea cake is largely a waste produced from shea butter processing centres after recommending a percentage of its use in livestock rations.

1.2 Purpose

The research investigates the butter residue of both local and well established industries in the Northern and Brong Ahafo Regions of Ghana. This research intended to investigate the microbial load, the proximate composition, the mineral content and the bioactive ingredients inherent in the shea nut cake for purposes of filling the academic and information gap whilst concentrating on its economic potentials for the local woman in the industry especially and mother Ghana as a whole. Unfortunately, in Ghana no or very little research has been done aiming at examining the clinical analysis of the proximate, mineral and biochemical qualities of the cake that seek to expand its benefits by filling the academic gaps and unearthing the economic potentials of the shea nut cake.

1.3 Scope

This presentation reports the results of an analysis of shea cake from six industries of four Districts in two regions of Northern Ghana. The research was designed to pick samples of shea nut cake from the 'Wunni Song' women Group of Tolon, 'Gub-Danda' Women Group of Gumo both of the Tolon-Kumbungu District, 'Suglo N Bori Buni' Women Group of Kalariga, 'Tungteiya' Women Group of Giso-Naayili both of the Tamale Metropolis, Shebu Industries Limited of the Savelegu-Nantong District and Ghana Nuts Limited of the Techiman Municipality. These industries form an island of the shea butter producing communities.

The research in part observed the methodological approach to the extraction of shea butter at the Women Group level (Local Industries) and at the Industrial Level. The microbial load of the shea nut cake was delved into besides its proximate, mineral and medicinal properties using refined modern technologies as investigative tools in all the processes of analysis. Development of microbiological and biochemical protocol for the various shea cake and industries will ensure sustainable and cost effective shea butter production.

1.4 Problem statement

Northern Ghana is noted for the prevalence of harsh economic conditions. This persistent situation is normally attributed to myriad of problems: (i) low literacy rate of the inhabitants (ii) scarce natural resource such as gold, cocoa and timber and (iii) worn-out agricultural lands. This situation has devastating effects on children and the disadvantaged especially.

A quite remarkable opportunity is the occurrence of the shea tree (*Vitellaria paradoxa*) in the three Northern Regions of Ghana and parts of the Brong Ahafo

Region. The shea tree is the second most important economic tree in Ghana; next to the palm tree, in terms of oil production. Many believe it is a 'God sent' to the people of Ghana. The recognition of the shea butter as a cocoa butter substitute and in the pharmaceutical, cosmetic and other industries has led to the establishment of shea industries (both large and small scale) and women groups across the Northern Regions of Ghana.

Several methods have been used for oil extraction but three of them have been tried in the shea butter industry: the physical traditional method, the mechanical or screw press method and the mechanical coupled with chemical extraction method. To date there are few known uses of the shea nut cake with much of it inevitably thrown out as waste which as yet no appreciable use has been found for it. The quantity of residue thrown out after the butter is extracted by the local women groups incommensurate the input and it is not only unacceptable to the poor Northern woman but also throws a challenge to the scientific community in Ghana.

1.5 Justification

The notable industry that has the potential to engage the youth whilst arresting poverty is the shea butter industry. It offers many opportunities to the people of the North, women in particular and Ghana as a whole. The shea industry is energy driven industry and large quantities of fuel wood, water and physical exhaustion are highly expensed in the butter extraction processes. The benefits accumulating at the end of every butter extraction processing session are minimal and incommensurate with the level of effort and costs involved. In addition, a large amount of the by-product, known as shea nut cake, is discarded as a waste product. It is estimated that for every metric tonne nuts processed, 450–600 kg of shea nut cake is produced and

about 60, 000 metric tonnes of shea kernels are consumed locally in a year. Thus, about 30,300,000 kg shea nut cake is generated locally in a year.

It is expedient therefore to examine the qualities of the shea cake that would unearth its potentials in the animal feed, fertilizer and therapeutic industries. This would expand the supply chain and increase the benefits of the shea industry and thereby adding value to the industry.

1.6 Research Objectives

1.6.1 General Objective

This research seeks to analyse biochemically and microbiologically, shea nut cake: a waste product from shea butter processing.

1.6.2 Specific Objectives

The specific objectives of the research are to:

- determine the microbial load of Shea Nut Cake,
- identify coliforms bacteria and other microorganisms in the shea nut cake,
- investigate the proximate and mineral compositions of the shea nut cake,
- To screen the shea nut cake samples for phytochemicals.

1.7 Limitations

Much is said of the uses of the shea butter; however, there was very little documentation about the shea cake and its uses. This limited the amount of information a researcher needed to know about the shea cake and what it could be used for in the 21st century. The microbial community around the shea cake was also not known; indeed this was the first of its kind to document comprehensive microbial information about the shea cake in Ghana and the sub-region. With funds available, one could go the extra mile to document the specific uses of the cake to the Shea Butter Producing Industries, Research Institutions, the Government, Private Practitioners, Policy Directors and the Savannah Accelerated Development Authority (SADA) in particular.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Shea, Occurrence and Uses

The shea tree is known as "Taanga" in Dagbani and indeed among the Mole-Dagombas of the Northern Region and among the Dagaabas and Waalas of the Upper West Region of Ghana. The Shea tree is distributed across the towns and villages of the Northern Regions (Northern, Upper East and Upper West) and some parts of the Brong Ahafo Region of Ghana. The shea tree, *Vitellaria paradoxa,* belongs to the family of Sapotaceae (Caroline *et al.*, 2009).

The tree is an indigenous fruit tree distributed in the shea parklands of Africa. Olaniyan and Oje (2007) described the shea fruit as a green epicarp, a fleshy mesocarp (pulp) and a relatively hard shell (endocarp) which encloses the shea kernel (embryo). The shea fruit is an important source of food for rural communities especially at time of food shortages, hunger and other disasters in addition to providing enormous health benefit (vitamins A and C, etc) and income (Okullo *et al.,* 2010). The shea nuts can contain from 20% up to 50% edible fat. The Shea fruit according to Maranz *et al.,* (2004) and DFSC (2000) produces more solid shea butter as compared to other oil bearing fruits as it contains more stearic acid. The kernel, according to Axtell *et al.,* (1993) contains about 60% edible fat (shea butter) and the residual product from which the butter is extracted (shea cake), is an excellent ingredient for livestock feed production.

The early 1970s saw *Vitellaria* vegetable fat being announced as a cocoa butter exchange (CBE) followed by a marked increase in interest from the pharmaceutical

and cosmetics industries (Moore, 2008). In 1977, *Vitellaria* was included on the list of tree species constituting African forest genetic resource priorities for *in situ* conservation at the fourth session of the 294 Food and Agriculture Organisation (FAO) Panel of experts on Forest Genetic Resources (Moore, 2008). In the 1980s, this huge amount of interest and attention led to calls for the Cocoa Research Institute of Ghana (CRIG) to increase botanical and genetic exploration with research focusing on diversity, management and propagation of *Vitellaria* (CRIG, 2002). Almost 30 years have passed since these calls were first made and only limited amounts of information with regards to optimum growing conditions and improving the marketing of Shea products has been gained (Moore, 2008).

2.2 Ecology of Shea Tree

The shea tree also known as (*Vitellaria paradoxa* or *Butyrospermum parkii*) (Maranz *et al.,* 2004) grows wildly in the dry savannah belt of West Africa, from Senegal, in the west, to Sudan in the east. It occupies an estimated 1 million km^2 of land, where annual rainfall ranges from 500 to 1200 mm (Boffa, 1995). The species is found on various soil types on dry open slopes but avoids alluvial hollows or land subject to flooding. It is a fire-tolerant plant and therefore indigenous to the Guinea Savannah Woodland and usually grows to an average height of about 15 metres. In Ghana, it virtually covers about two-thirds of the country, mostly in the wild state (Abbiw, 1990).

The tree grows slowly from seed, taking 12 to 15 years to bear fruit and about 30 years to mature (Adomako, 1985). It starts flowering in early November, with picking or gathering of fruits lasting from April to August every year. When the shea fruits ripen, they fall under their own weight to the ground and are gathered by

hand. The fruit contains protein and carbohydrates, and is very sweet (Maranz and Wiesman, 2004). The fruit pulp is a particularly rich source of ascorbic acid, iron and calcium (FAO, 1988). Within the fleshy pulp is the nut which in turn houses the kernel. Although shea nut is a major commodity, it is not a plantation crop (Olaniyan *et al.*, 2007). The nuts sold on the international market are harvested from village tree populations in several West African countries. The fat content of the kernel and fatty acid profile are, however, extremely variable across this zone (Maranz *et al.*, 2004). The fat obtained from the shea kernel is referred to as shea butter and it is the most valued product from the shea tree (Hall *et al.*, 1996).

Plate 1: The Shea tree

2.3 The processing of shea nut into butter and cake

2.3.1 Traditional method of shea butter extraction

Recent studies by Fleury (2000) list the equipment for primary processing of shea nut into butter and cake to include pan for boiling water, drying mat, mallets, pestles, winnowing basket, and clay pot. The pulps of the harvested berry are crushed under foot after fermentation. This berry (almond) sticks to the shell wall and to separate them, the nuts are immersed in boiling water and sun dried for a few days. During the drying stage, the berries become detached and the nuts can now be stored for months without deterioration.

Shelling is carried out using stone, mallets and pestles. Winnowing is achieved by holding basket filled with the mixture of nuts and shells at arm's length and gradually pouring the mixture into a pan. . If there is a strong wind, the piece of shell will be blown away, if not, then the operation is repeated many times. The day prior to oil extraction, the shelled almonds are dried again from a moisture content of 40 to 50% to 6 to 7% (Godwin and Spensley, 1971).

Fleury (2000) stipulates that there are two main methods for shea butter extraction: a traditional village process and a mechanical procedure. The traditional process **(Figure 1)** involves many time consuming stages. After drying, the kernels are crushed by simultaneous strokes in a mortar using a pestle. The paste that is gradually formed needs to be kept at a temperature of about 40°C. Shea butter tends to solidify between 34 to 38ºC. Once the paste becomes a fluid, it is strained and heated in a pan. A kneading process takes place to break up oil cell and ease oil extraction. The paste is then mixed with water to separate the remaining oil. The paste is rapidly mixed by hand until it starts to cover itself with a white emulsion of

fat. Once this is achieved, the paste is left to rest and the oil that floats to the surface is scooped off, and poured into a container filled with lukewarm water for decantation. During decantation, a white film form over the top of the surface, this is shea butter. It is separated and heated in a cauldron to evaporate remaining water and allow heavy impurities to settle at the bottom. The butter is left overnight to rest and solidify. Traditionally, it is then divided and hand-moulded into round shapes for selling or for storage. The butter will last for many years if kept away from light and heat as it is resistant to oxidative rancidity (Fleury, 2000).

Plate 2: Shea Butter

It should be noted that using a shea nut press does not only alleviates time consuming process but also improves the fat output. For example, using a shea press fat output will be between 40 to 45% whereas fat output using the traditional method will be about 25% (Niess, 1983). While Figures 3 and 4 depict the actual process involved, figures 1 and 2 show the flow charts for the processing of shea nut and how shea butter is extracted locally.

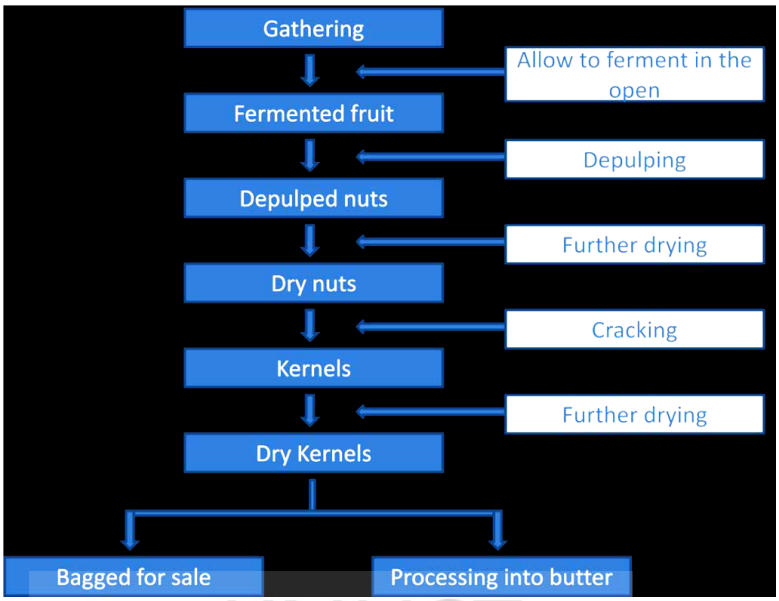


Figure 1: Flow Chart for Local Collection and Pre-treatment of Shea Nuts

(Source: Agyente *et al*, 2010)

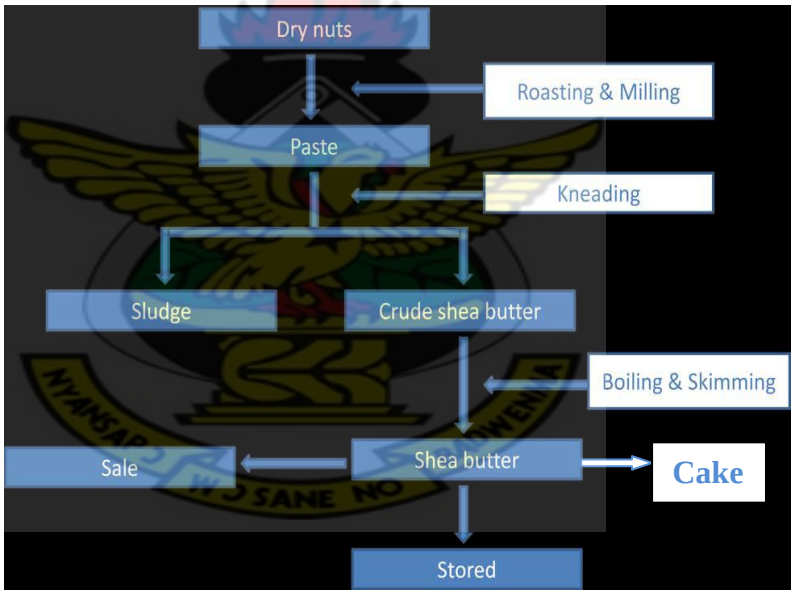


Figure 2: Flow Chart for Local Processing of Shea Butter

(Source: Agyente *et al*, 2010)

Shea-nut cake is a by-product of the indigenous technology for extraction of fat from the kernel of the shea tree (*Vitellaria paradoxa*). After gathering the fresh nuts from the bush, women will normally allow the fruits to ferment and processed as described in Figure 1 (the flow diagram). The fermented fruits (Figure 3A) are depulped to

expose the nuts (Figure 3B) which are boiled to temperatures ranging between 100ºC and 115ºC. This will deactivate all biological and enzymatic activities in the nut. The nuts are then exposed to the sun and air to dry for ease of removal of the shells to produce the kernels (Figure 3C). More heat is applied to the kernels much more effectively, to further deactivate enzymatic activities and reduce the moisture levels, after reducing the surface area of the kernels, most commonly, using the mortar and pestle (Figure 3D).

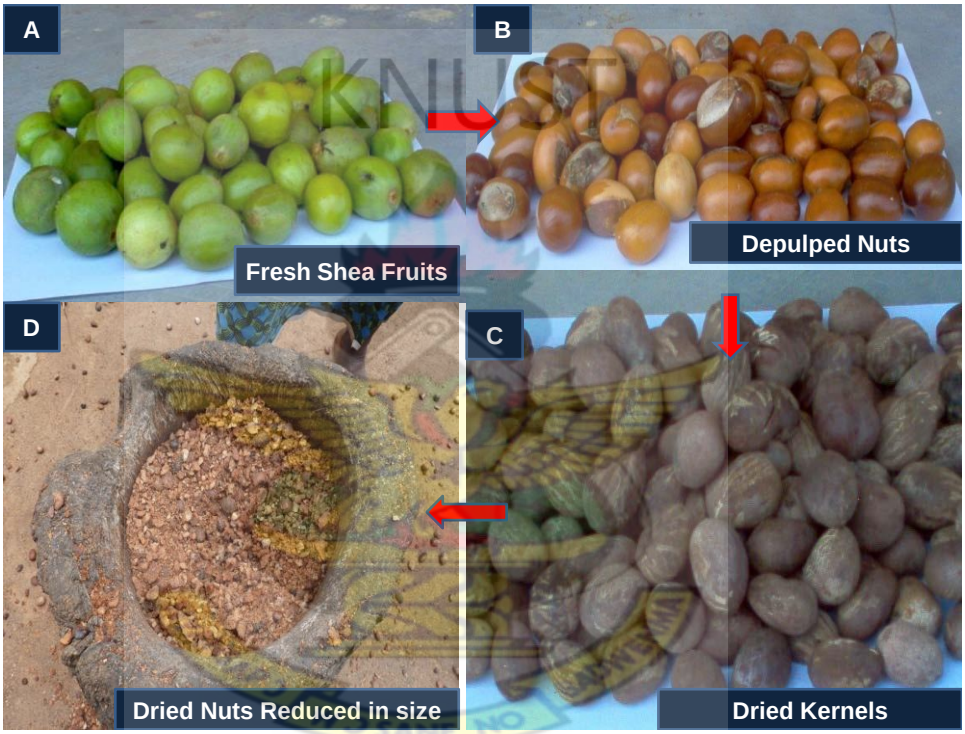


Figure 3: Shea butter processing; from fresh fruits to dried kernels

Dry frying of the pounded kernels goes with constant stirring to avoid losing much of the oil before the extraction and prevent the extractor from incurring losses. The crushed kernels are pounded into paste using the grinding mill so that women can take reasonable quantities to knead in metallic basins **(**Figures 4A, 4B and 4C**)**. Women take an average of 30 minutes to complete one kneading session. A kneading session involves taking a reasonable quantity of shea paste, adding an initial amount of about 3 litres of cold water, stirring slowly and then vigorously

later, with the hand, while whipping occasionally with the hand inside the paste until, eventually, the butter begins to rise in crude milky-white form.

At this stage, about 2 litres of warm water are added to complete the separation of the crude butter from the first extraction by-product. Thus, about 6-8 litres of cold water are added to the paste for the crude butter to float for ease of separation **(**Figure 4D**)**.

Figure 4: Shea butter processing; milled nuts to skimming

The crude shea butter is scooped from the first extraction waste and transferred into a previously washed and sterilized container **(**Figure 4E**)**. A cauldron is placed on well prepared fire that can provide stable and uniform heat for about an hour or more, inside which the crude shea butter is transferred. At about 105ºC the shea butter is well dried and float on top the of the second extraction cake. The liquid butter is gently skimmed off and or decanted **(**Figures 4F and 5A**)** into another container, usually a metallic basin previously put on fire to dehydrate all water molecules to

avoid hydrolysis of the butter that could lead to eventual spoilage of the butter after solidification.

Shea butter in liquid form takes about 8-12 hours to solidify and the solidification process requires constant stirring with locally made 'sterilized' stick (a dry stick that has been haphazardly exposed to heat). The continuous stirring of the shea butter prevents the formation of colloidal solids (Figure 5C).

Figure 5: Shea butter and shea cake

On just about becoming solid (between temperatures of 35-40ºC), the shea butter is transferred into calabash containers commonly of one of three recognized sizes: small, medium and large (Figure 6). The second extraction cake (Figures 5B and D) is thrown away or used as fuel cake to power the extraction of more shea butter.

Figure 6: Shea butter packaged for sale

2.4 Mechanical Extraction of Shea Butter

The supposedly tedious nature of the traditional shea butter extraction method, which yields about 25% (Niess, 1983) of oil, called for the application of scientific principles and methods into the butter extraction process. This mind shift led to the introduction of mechanical pressing methods (such as expellers, hydraulic presses, etc) into the shea butter industry. The mechanical extraction process is outlined in a flow chart below (Figure 7). The nuts are first heated to temperatures of between 15-20ºC and then directed into a crushing unit where they are reduced in size to increase the surface area for effective butter yield. The pulverized nuts are then pressed to release oil and the first extraction cake. The first extraction cake is directed into another expeller where it is pressed the second time to produce more oil and the second extraction cake (Figure 7). The Shebu Industry at Savulegu in the Northern Region and the Ghana Nuts Limited at Techiman in the Brong Ahafo Region of Ghana use the mechanical expeller for the extraction of shea butter. This has improved the extraction efficiencies by about 8%. About 30-33% of shea butter is

extracted from the shea nuts with the mechanical expeller (Personal communication with Aka Aristide, Quality Control Manager, GNL, 2012) as shown below.

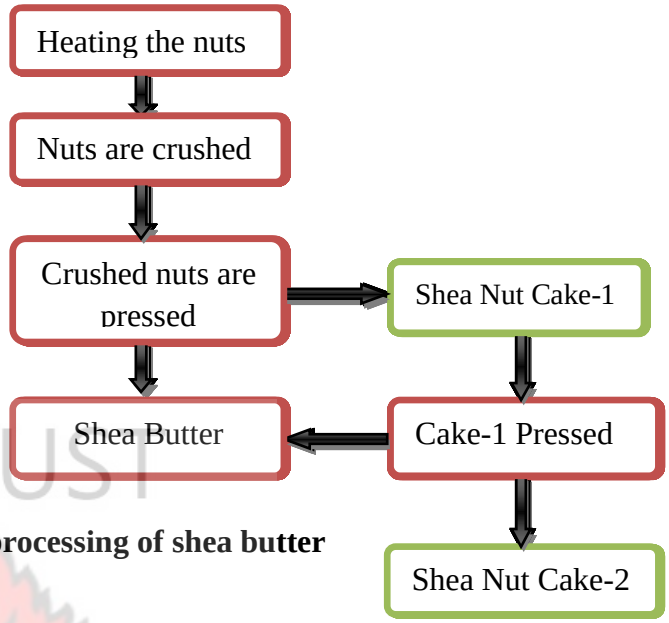


Figure 7: Flow chart for mechanical processing of shea butter

2.5 Chemical Extraction of Shea Butter

Chemical or solvent extraction methods are usually employed in extraction processes when other methods, such as the mechanical press, are probably not yielding the desired results. This is the situation at Ghana Nuts Limited (GNL) at the Techiman Municipality of the Brong-Ahafo region of Ghana. After the shea nut cake is pressed for the second time, the by-product, in this case the second shea nut cake is directed into the chemical plant for further extraction. For every tonne of shea nuts processed, 5 litres of hexane is diffused into the shea nut cake in the chemical plant for further shea oil extraction. A complete extraction cycle at the chemical plant takes four hours.

Eventually, a solution of shea oil in solvent (hexane) is formed. It is directed thereafter into a distillation chamber where the mixture is heated to 67ºC, the boiling point of hexane. This temperature allows the hexane to evaporate and be retrieved by condensation with the production of non-contaminated shea oil. The choice of

hexane over other solvents is informed by several factors: the physical properties of the solvent, the commercial economics of the product and the edibility of shea oil from the extraction. The shea nut cake produced at this stage is used to generate heat to produce steam and thus power the plant (Personal communication with Aka Aristide, GNL). He further revealed that the shea nut kernel is estimated to contain about 52% oil. A combination of the chemical and mechanical methods at the GNL yields 98% extraction efficiency. The company targets only 1.5% of oil left in the shea cake after extractions.

2.6 The uses of shea and shea products

Moore (2008) reported that the shea tree produces fruit which has multiple uses at the local level; it is highly nutritious and is also a valuable commodity on the local, national and international markets, making it the ideal candidate to research and invest in. According to a study, the shea tree is the second most important oil crop in Africa after oil palm as was reported by Poulsen (1981), but as it grows in areas unsuitable for palm growth, it takes on primary importance in West Africa. The importance of the shea tree to Ghana's economy became even more significant in the early 1970s when it was announced that it was one of only six plant species whose vegetable fat can be used in the production of cocoa butter equivalents (CBEs) in chocolate as well as being a prized ingredient in the pharmaceutical and cosmetics industries (OJEC, 2000). Ofosu *et al.* (2009) asserts that Shea nuts contain 40–55 % fat and it is estimated that for every metric tonne of nuts processed, 450–600 kg of cake is produced. This amount of cake produced is substantial. The CRIG research station is yet to unearth the potentials of the shea nut cake produced in substantial amount from the local as well as well established industries. As a result, huge

amount of information with respect to the limited uses of the shea nut cake across Ghana and the sub-region is yet to be gathered. Such information would not only benefit the local communities and women groups of northern Ghana but the well established industries and mother Ghana as a whole. Besides, this would commensurate for the time and efforts spent on the methods of processing of shea nuts into butter that are currently employed.

2.7 Proximate composition of shea nut cake

The proximate composition of the shea nut cake has not been investigated in recent times in Ghana with the innovations and improvements in the methodologies of extraction of shea butter, both traditionally and industrially. Pousga *et al*. (2007) evaluated the nutritive value for chicken of shea nut (*Vitellaria paradoxa*) cake. In all, a total of 36 shea nut cake samples were collected from Sapone and Bobo-dioulasso in Burkina Faso between October and December 2006. The study employed standard methods (AOAC, 1997) and reported that the proximate composition of the shea nut cake collected from the two locations had no significant difference as presented in Table 1.

Table 1: Chemical composition of traditional shea-nut cake from two climatic zones of Burkina Faso (% of DM) (n = 36)

Item	Bobo-Dioulasso*	Sapone**	Mean	Max	Min	SEM	P-value
Dry matter	95.1	96.1	95.6	98.0	87.3	1.03	0.47
Organic matter	94.5	93.3	93.9	97.3	89.1	0.76	0.30
Ash	5.4	6.7	6.1	10.3	2.6	0.76	0.30
Crude protein	7.4	5.9	6.7	10.3	2.5	0.89	0.29
Ether Extract	6.6	8.0	7.3	9.6	5.3	0.37	0.29
Crude fibre	10.3	11.5	10.9	11.0	7.8	0.33	0.98
ME (MJ/kg)***	13.3	12.9	13.1	14.1	12.4	0.15	0.65

*Sub-humid zone, **Soudano-Sahelian zone (Source: Pousga *et al.*, 2007)

The Mean Dry Matter (DM), Crude protein (CP), Ether Extract (EE), Crude Fat (CF) and Metabolizable Energy (ME) contents were 95.6, 6.7, 7.3, 10.9% and 13.1 MJ/kg, respectively. No significant difference was found in proximate composition between the two locations. However, Organic Matter (OM), Crude Protein (CP) and Metabolizable Energy (ME) contents were numerically higher in Bobo-Dioulasso (94.5 and 7.4% and 13.3 MJ/kg, respectively) compared to Sapone (93.3 and 5.9% and 12.9 MJ/kg, respectively), while Ash, CF and EE were higher in Sapone (6.7, 11.5 and 8.0%, respectively) compared to Bobo-Dioulasso (5.4, 10.3 and 6.6%, respectively) ($p>0.05$). Mean calcium and phosphorus contents were 0.40 and 0.19%, respectively. The study concluded that for shea nut cake to be used as poultry feed, feeding trials were necessary to evaluate the effect of different inclusion levels in the diet on growth and egg production performance (Pousga *et al.*, 2007).

2.8 The state of shea nut cake in Ghana

The use of the shea nut cake has not changed in Ghana over the last decade. It has been reported that the residual meal (the first extraction cake), as in the case with shea butter, is also used as a waterproofing agent to repair and mend cracks in the exterior walls of mud huts, windows, doors and traditional beehives. The sticky black residue (the second extraction cake), which remains after the clarification of the butter, is used for filling cracks in hut walls (Marchand, 1988) and as a substitute for kerosene when lighting firewood (Wallace-Bruce, 1995) and plastering of walls for protection from harsh weather conditions.

Ofosu (2009), claims that the shea waste, the shea nut cake, is a biodegradable organic material with high volatile solids content and has shown to have the potential to produce biogas with high methane content. The study indicated that co-fermentation of the shea waste with cattle manure was found to be the feasible anaerobic digestion option in the generation of up to 61.4% of methane and the treatment of shea waste prior to disposal in an effort to reduce its pollution potential on the environment. The study concluded that the production of biogas from anaerobic digestion of shea waste provides an important energy potential, which should be of value in improving the economics of shea processing.

Konlan (2010), in his search for an alternative feedstuff for the Djallonke sheep indicated that the inclusion of shea nut cake from 11.5% to 23% (230 g/kg DM) in the supplemental diet of growing Djallonke sheep fed crop residues (rice straw and groundnut haulms) led to improved growth performance of the sheep. An increasing inclusion level from 11.5% to 23.0% of shea nut cake in the concentrate diet of Djallonke rams led to depression in the straw intake but increased dry matter digestibility and so did affect positively the average daily growth rate in the study.

Shea nut cake inclusion levels from 11.5% to 23% in the supplementary diet of Djallonke rams did not cause any negative effect in the haematology and serum metabolites of the Djallonke rams, the study concluded. It is therefore important to further research into the beneficial properties of the shea nut cake.

2.9 The microbial Quality of shea nut cake

The microbial load and presence of bacterial pathogens in any given substance give an indication of the substance's quality and the potential health risk it poses to consumers (Adu-Gyamfi, 2012). Total viable count for instance is indicative of the populations of spoilage microorganisms and act as an index of hygienic quality. However, the concern mainly is the levels at which the organisms occur. Generally the microbial quality of most products including the shea nut cake depends on factors such as the quality of the raw materials and other substances (such as water) added during processing operations, the sanitation surrounding the processing environment, the personnel involved in the operations, the equipment, apparatus and containers used in the processing. The shea nut cake is often treated as waste product and so little attention is paid to many of the factors mentioned above especially at the local industry level.

2.10 Methods of Bacterial Identification

2.10.1 Analytical Profile Index (API)

API 50CH is a standardized system made up of 50 biochemical tests that allow the identification of bacteria taking into account the type of metabolism of carbohydrates adopted by that bacteria. API 50 CHB/E medium is a solution made up of 2 g ammonium sulphate; 0.5 g yeast extract; 1 g tryptone; 3.22 g disodic

phosphate; 0.12 g monopotassic phosphate; 10 mL oligoelement solution; 0.17 g phenol red; demineralized water; pH 7.4-7.8 with a known cloudy pattern of 1 x 10^6 CFU mL^{-1} called "MacFarland". Colonies of pure bacteria from the dishes are transferred to a tube containing 2 ml sterile distilled water (SDW), forming a bacterial suspension until reaching cloudiness equal to the MacFarland pattern. Then, API 50 CHB/E is inoculated with the bacterial suspension under study and is incubated at 24 °C for 24 h. During this period, fermentation will lead to a change of colour from red to yellow in the medium, interpreted as a (+) reaction; when no change of colour is observed, or is very weak, a (-) reaction is recorded. Results are analyzed with the V 3.0 identification program (bioMérieux, Marcy L'Etoile, France).

The API 20E kit is an identification system for Enterobacteriaceae and other non-fastidious Gram-negative rods, which uses 21 standardized and miniaturized biochemical tests and a database. It consists of 21 microtubes containing dehydrated substrates. These tubes are inoculated with a bacterial suspension which reconstituted the media. During incubation, bacteria metabolism produces colour changes that are either spontaneous or revealed by the addition of reagents. The reactions are read according to the table provided and the identification is done using the software provided by the manufacturer on the Internet, the API web. A seven digit profile is obtained. API 20E ratings are based on three parameters, including the likelihood of a match between the unknown organism's profile and the computer profile, the relative value between the likelihood of the first and the likelihood of the second choice, and the number of tests against the first choice (Gacitua *et al.*, 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sampling

Samples were taken monthly for four months (March to June 2011) with particular attention to the colour, texture and smell of the shea nut cake samples. The first extraction cake is the by-product obtained after the extraction of the crude butter by the women groups and after the first press by the mechanical expeller at the industry while the second cake is the by-product after the crude butter is heated and skimmed off by the women groups and the second extraction by the mechanical expeller at the industries. The samples 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 Giso-Naayili respectively in the Tamale metropolis of 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.

Three samples each of the first and second extraction cakes were taken from each of the six industries. However, a representative sample was developed out of the three of the first extraction cake and used in the analysis together with the three samples of the second extraction cake. In all, twenty-four shea-nut cake samples (four from each industry) were collected into sterile containers. All samples were transported to

the laboratory as early as practicable and stored at 4°C until needed. Microbiological, physico-chemical and phyto-chemical analyses were carried out on the samples.

Table 2: Sample collection sites and number of samples collected

District/Metropolis	Sampling Site	Sample Label	Number of Samples	
			1st Extraction Cake	2nd Extraction Cake
Tolon-Kumbungu	Tolon	A	1	3
Tamale Metro	Kalariga	B	1	3
Tolon-Kumbungu	Gumo	C	1	3
Tamale Metro	Gisonaayili	D	1	3
Savelugu-Nantong	Shebu	E	1	3
Techiman	GNL	F	1	3

3.2 Isolation and Enumeration of Bacteria

3.2.1 Total viable count

Total Viable Count was determined according to Tassew and Seifu (2011) with some modifications. One gram of sample was aseptically transferred and mixed in 9 ml of sterile physiological saline solution. Ten fold serial dilutions were made and viability of microbes assessed using the pour-plate method in triplicates. The plates were incubated at 37ºC for 24 hours 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^{o}C for further analysis.

3.2.2 Total and faecal coliforms

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

3.2.3 Identification of *E. coli*

From each of the positive tubes identified above, a drop was transferred into a 5 ml test tube of trypton water and incubated at 44^{o}C for 24 hours. 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 (*E. coli*). Counts per 1 ml were calculated from Most Probable Number (MPN) tables (Hood *et al*, 1983).

3.2.4 Identification of other Microorganisms

Colonies from the total viable count were subcultured 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 and API 20E (BioMérieux, Marcy L'Etoile, France).

3.3 Chemical analysis of the shea-nut cake

The shea nut cakes or test samples were subjected to proximate analysis by the methods outlined by AOAC (1997) and Pousga et al., (2007). The Atomic Absorption Spectrophotometer (AAS) was used to determine the mineral content of the by-product.

3.3.1 Chemicals and reagents

All reagents and chemicals used in the analysis were of analytical grade. Petroleum ether spectrophotometric grade was purchased from Merck. Sodium hydroxide, tetraoxosulphate (VI) acid, boric acid and Sodium sulphate were sourced from Fisher scientific, US.

3.3.2 Ash content

The ash represents the inorganic component (minerals) of the sample after all moisture has been removed as well as the organic material. The ash content was determined according to Pousga *et al.,* (2007). The method was based on the decomposition of all organic matter such that the mineral elements would not be lost in the process. Approximately 1g of finely ground sample was weighed into porcelain crucible which had been ignited. The crucible was placed in a muffled furnace and heated at 500ºC for four hours, removed and cooled. The ignited residue was moistened with 2 ml distilled water and slowly and carefully 5 ml of 8 N HCl (2

parts of conc. HCl was mixed with one part of water). It was transferred again into the cool muffle furnace and the temperature was increased step wise to 550 ± 5^{o}C. The temperature was maintained for 8 hours until white ash was obtained.

It was then brought out and allowed to cool in a desiccator and weighed again. Percentage weight was calculated as weight of ash multiplied by 100 over original weight of the samples used. The formula used is presented as:

$$Ash\ content = \frac{weight\ of\ ash\ (g)}{weight\ of\ original\ sample} \times 100\%$$

3.3.3 Determination of lipid content

The method employed was the Soxhlet extraction technique adopted by Pousga *et al.* (2007). Twenty grams (20g) of the samples were weighed and carefully placed inside a fat free thimble. This was covered with cotton wool to avoid loss of the sample. The loaded thimble was put in the Soxhlet extractor and about 200 ml of petroleum ether poured into a weighed fat free soxhlet flask with the flask attached to the extractor. The flask was placed on a heating mantle such that the petroleum ether in the flask refluxed. Cooling was achieved by a running tap connected to the extractor for at least 6 hours after which the solvent was completely siphoned into the flask. Rotary vacuum evaporator was used to evaporate the solvent leaving behind the extracted lipids in the soxhlet. The flask was removed from the evaporator and dried to a constant weight in the oven at 60°C. The flask was then cooled in a desiccator and weighed. Each determination was done in triplicate. The amount of fat extracted was calculated by the formula presented below:

$$Ether\ Extract\ (EE)\% = \frac{weight\ of\ extracted\ lipids(g)}{weight\ of\ dry\ sample(g)} \times 100\%$$

3.3.4 Crude Protein determination

Total protein was determined by the Kjeldahl method as modified by Pousga *et al,* (2007). The analysis of protein content in a compound by Kjeldahl method is based upon the determination of the amount of reduced nitrogen present. Thirty grams (30 g) of each sample was weighed into a filter paper and put into a Kjedahl flask, 10 tablets of Na_2SO_4 were added with 1 g of $CuSO_4$ respectively. Twenty millilitres (20 ml) of concentrated H_2SO_4 were added and then digested in a fume cupboard until the solution became colourless. It was cooled overnight and transferred into a 500 ml flat bottom flask with 200 ml of distilled water. This was then cooled with the aid of packs of ice block. About 60 to 70 ml of 40% of NaOH were poured into the conical flask which was used as the receiver with 50 ml of 4% boric acid using methyl red as indicator. The ammonia gas was then distilled into the receiver until the whole gas evaporated. Titration was done in the receiver with 0.1 N H_2SO_4 until the solution became colourless. The formula used was presented as:

$$Crude\ Protein\% = \frac{Vs-Vb \times 0.01401 \times N\ acid(6.25)}{Original\ weight\ of\ sample\ used} \times 100\%$$

Where Vs = Volume (ml) of acid required to titrate sample; Vb = Volume (ml) of acid required to titrate blank; N acid = normality of acid.

3.3.5 Crude fibre

Crude fibre content was determined according to methods adopted by Pousga *et al.,* (2007). Twenty grams (20 g) of each shea nut cake (SNC) samples were defatted separately with Diethyl ether for 8 hours and boiled under reflux for exactly 30 min with 200 ml of 1.25% H_2SO_4. It was then filtered through cheese cloth on a fluted funnel. This was later washed with boiling water to completely remove the acid. The residue was then boiled in a round bottomed flask with 200 ml of 1.25% Sodium

hydroxide (NaOH) for another 30 min and filtered through previously weighed couch crucible. The crucible was then dried with samples in an oven at 100°C, left to cool in a desiccator and later weighed. This was later incinerated in a muffle furnace at 600°C for 2 to 3 hours and later allowed to cool in a desiccator and weighed. The formula used is presented as:

$$Crude\ Fibre\ (\%) = \frac{weight\ of\ fibre(g)}{weight\ of\ original\ sample(g)} \times 100\%$$

3.3.6 Determination of moisture content

This method was based on moisture evaporation used by Pousga *et al.* (2007). In this method, aluminium dishes were washed dried in oven and desiccators for cooling. The weight of each dish was taken. Hundred grammes (100 g) of each sample of shea nut cake were weighed into a sterile aluminium dish; weight of the dish and weight of sun dried sample (in triplicate) were taken. This was transferred into an oven and set at 100ºC and less than 100 mm Hg for approximately 5 hours after which the dish was removed from the oven, covered, cooled in desiccator, and weighed. Then the weight was measured using a measuring scale balance. It was transferred back into the oven for another one hour and then reweighed. The process continued until a constant weight was obtained. The difference in weight between the initial weight and the constant weight gained was taken as the moisture content. The loss in weight multiplied by 100 over the original weight is percentage moisture content. The formula used is presented below:

$$Moisture\ content\ (g/100g) = \frac{lost\ in\ weight\ (w2 - w3)(g)}{Original\ weight\ of\ sample\ (w2 - w1)(g)} \times 100\ \%$$

Where W1= initial weight of empty crucible, W2 = weight of crucible + SNC before drying, W3 = final weight of crucible + SNC after drying.

% Total solid (Dry matter) (%) = 100 - moisture (%)

3.3.7 Carbohydrates

Carbohydrates were calculated using the formula:

$$Carbohydrates\ (\%) = (100 - x)\%$$

Where $x = \{CP + CF + EE + MC + AC\}$

CP is crude protein, CF is crude fat, EE is ether extract, MC is moist content, and AC is ash content.

3.3.8 Mineral Analysis

The AOAC (1990) procedure was adopted to determine the mineral contents in different shea nut cake samples. Each sample of the shea nut cake was digested in a di-acid mixture (HNO_3:$HClO_4$) in the ratio (7:3) on hot plate at a temperature of 180^{o}C for 2 h. The contents were diluted to volume of 100 ml with double distilled deionised water. The mineral contents (P, K, Na, Mg, Ca and Cu) in the digested samples were estimated by using atomic absorption spectrophotometer (Model Varian Spectra AA 250 plus).

3.3.9 Heavy Metals

The analyses of heavy metals were performed by adopting the AOAC (2000) methods of analysis of heavy metals. One gram each sample of shea nut cake was taken in a conical flask. The samples were first digested with 10 ml HNO_3 at a temperature range of 60-70^{o}C for 20 minutes and then redigested with 5 ml $HClO_4$ at

a temperature of 60-70ºC for 20 minutes and subsequently raising the temperature to 195^{o}C till the volume was near to dry or until a clear solution was obtained. The digested samples were transferred to 100 ml volumetric flask and volume was made with distilled deionized water and then filtered and stored in air tight bottles for analysis of heavy metals with the help of atomic absorption spectrophotometer (Model Varian Spectra AA 250 plus) at wavelengths: 283.3 nm for lead, and 254 nm for mercury.

3.4 Phytochemical Screening of the Shea Nut Cake

Saponins, cynogenic glycosides, reducing sugars, tannins, flavonoids, terpenoids, anthraquinones and alkaloids were determined according to Standard methods. Coumarins were determined according to Savithramma *et al.* (2011). Twenty-five grams of each sample of Shea Nut Cake was cold extracted in a combined solution of ethanol (95%) and distilled water (1:1) for four days with occasional shaking. The extracts were filtered through Whatman's No. 1 filter paper and the filtrates were concentrated to dryness *in vacuo* using a rotary evaporator to remove the solvents. The extract was ready for screening for bioactive constituents.

3.4.1 Test for alkaloids

About 1g of the extract was stirred with few drops of 1% HCl on a steam bath. The solution obtained was filtered and 1 ml of the filtrate was treated with 1 drop of Mayer's reagent. Turbidity of the extract filtrate on the addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extract (Adebayo *et al.*, 2012).

3.4.2 Test for saponins

About 2 ml of the shea nut cake sample extract was measured into a test tube and shaken vigorously. Test becomes positive if characteristic honeycomb froth persists for at least 30 minutes (Oseni and Alphonse, 2011).

3.4.3 Test for cynogenic glycosides

About 2 ml of the shea nut cake sample extract was measured into a test tube and 1ml of chloroform added to it. A piece of picric acid paper was then inserted into the test tube just above the extract and folded over the rim of the tube. The test tubes were then stoppered and warmed at about 35ºC in a water bath for about 30 minutes. A change in colour of the yellow picric paper to various shades of red indicates the presence of cynogenic glycosides (Adebayo *et al.*, 2012).

3.4.4 Test for reducing sugars

About 0.5 ml each of Fehling's solutions A and B were measured into a test tube. About 0.5ml of the shea nut cake sample extract was added to the solution and heated in a water bath. A brick-red precipitate denoted the presence of reducing sugars (Oseni and Akwetey, 2012).

3.4.5 Test for flavonoids

About 3 drops of dilute NaOH was added to 1 ml of the shea nut cake sample extract. An intense yellow colour is produced in the sample extract which becomes colourless on addition of few drops of dilute HCl indicating the presence of flavonoids (Adebayo *et al.*, 2012).

3.4.6 Test for tannins

About 0.5 ml of the shea nut cake sample extract was heated in a steam bath for about 5 minutes. About 2 drops of 5% $FeCl_3$ was then added. Presence of greenish precipitate indicated the presence of tannins (Oseni and Akwetey, 2012).

3.4.7 Test for Terpenoids

Two millilitres (2 ml) of the shea nut cake extract was added to 2 ml of acetic anhydride and concentration of H_2SO_4. Formations of blue green rings indicated the presence of terpenoids (Oseni and Alphonse, 2011).

3.4.8 Test for coumarins

Three millilitres (3 ml) of 10% NaOH was added to 2 ml of the shea nut cake sample extract and the formation of yellow colour indicated the presence of coumarins Savithramma *et al.* (2011).

3.5 Statistical analysis

Data from the laboratory analysis 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 Least Significant Difference (LSD) to compare the effect of location and method of butter extraction on the chemical composition of the by-product (the shea-nut cake). Graphs were drawn using the Microsoft Office Excel (2007) version.

CHAPTER FOUR

4.0 RESULTS

The data collected after the analysis of the shea nut cake were processed and presented in graphs and tables to explain the results of the experiments. Microbiological, mineral, physico-chemical, phytochemical, and proximate analysis of the shea nut cake samples from the six industries of Northern Ghana were unlike what has been perceived of the shea cake as merely a waste product.

4.1 Visual examination of the shea nut cakes

The colour, texture and smell were keenly monitored from sample collection to analysis. There were occasional differences in colour among samples from the women groups. However there were marked differences in colour between samples from the women groups and the mechanized industries. The colour of all samples in general was brownish with the samples from the women groups being dark brown. The first extraction cake was light brown and lighter in weight (Plate 4**)** for samples from the local industries (first four from right). The texture, structure and smell of the cake samples did not change during the period between sampling and analysis. Samples from the Ghana Nuts Limited (GNL) in particular were pale brown and fairly lighter than all other samples (Plate 3- first row from left to right).

Plate 3: Shea Nut Cake samples

Plate 4: Shea Nut Cake Samples showing different colours

4.2 Microbiological analysis

4.2.1 Total Viable Count (TVC)

Table 3 shows the analysis of the microbiological quality of 24 shea nut cake from six shea butter extraction centres of Northern Ghana. The mean Total Viable Count (TVC) for the six industries in Tolon, Kalariga, Gumo, GisoNaayili, Savulegu and Techiman were 5.18 ± 0.65, 5.88 ± 1.13, 6.63 ± 0.22, 3.55 ± 0.60, 4.80 ± 2.02 and 3.86 ± 0.94 log cfu/g, respectively. It is obvious from the results that the TVC was significantly different ($p \leq 0.05$) across the shea industries. The TVC was highest at

Gumo (6.63 ± 0.22 log cfu/g) and least at GisoNaayili (3.55 ± 0.60 log cfu/g), both being local industries.

Table 3: Microbiological quality (log cfu/g) of shea nut cake from six industries of Northern Ghana

Location	Sample Symbol	No. of Samples	Total Viable	Total Coliforms	Faecal Coliforms	*E. Coli*
Tolon	A	4	5.18 ± 0.65	1.91 ± 1.38	0.81 ± 1.24	0.34 ± 0.68
Kalariga	B	4	5.88 ± 1.13	3.14 ± 1.84	1.64 ± 1.23	1.15 ± 0.78
Gumo	C	4	6.63 ± 0.22	1.89 ± 0.72	0.64 ± 0.79	0.49 ± 0.65
Giso-N.	D	4	3.55 ± 0.60	1.41 ± 0.42	0.54 ± 0.39	0.15 ± 0.30
Savulegu	E	4	4.80 ± 2.02	1.03 ± 0.64	0.24 ± 0.48	0.00 ± 0.00
Techiman	F	4	3.86 ± 0.94	2.34 ± 2.06	1.08 ± 1.26	0.75 ± 0.87
P-Value			0.007	0.256	0.443	0.161

4.2.2 Total coliform count

The analysis of total coliforms of different shea cake samples presented in Table 3 showed that the total coliforms were not significantly ($p \geq 0.05$) different across the shea industries. The highest record of total coliforms (3.14 ± 1.84 log cfu/g) was observed in samples from the Kalariga women group. The least count of 1.03 ± 0.64 log cfu/g was observed in samples from Shebu Industry of Savulegu. The total coliforms across the shea industries, both local and well established industries, were not significantly ($p \geq 0.05$) different. Total coliforms did not show differences between first and second extraction cakes.

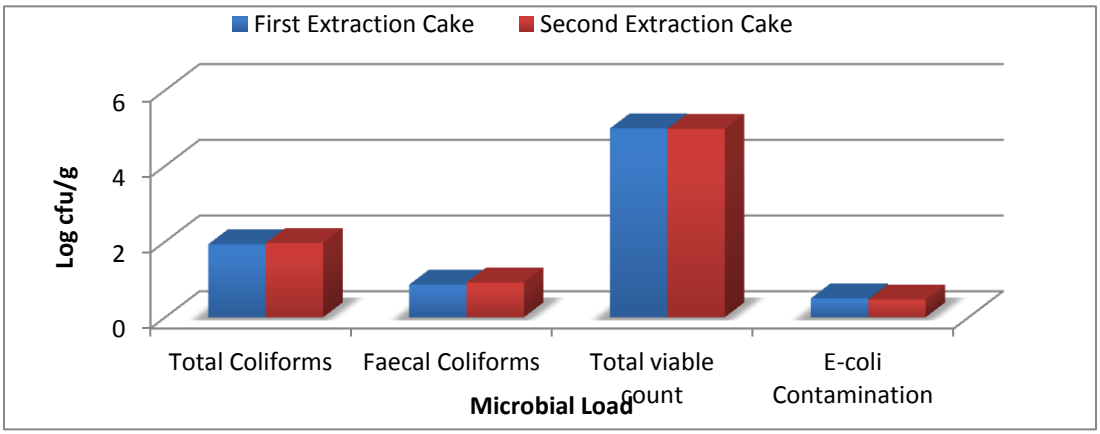


Figure 8: Comparison of Microbial load obtained from first and second shea nut cakes

The TVC showed non-significant difference between samples of the first extraction and the second extraction cake (Figure 8). The TVC in general was higher at the local industry than at the mechanized industry.

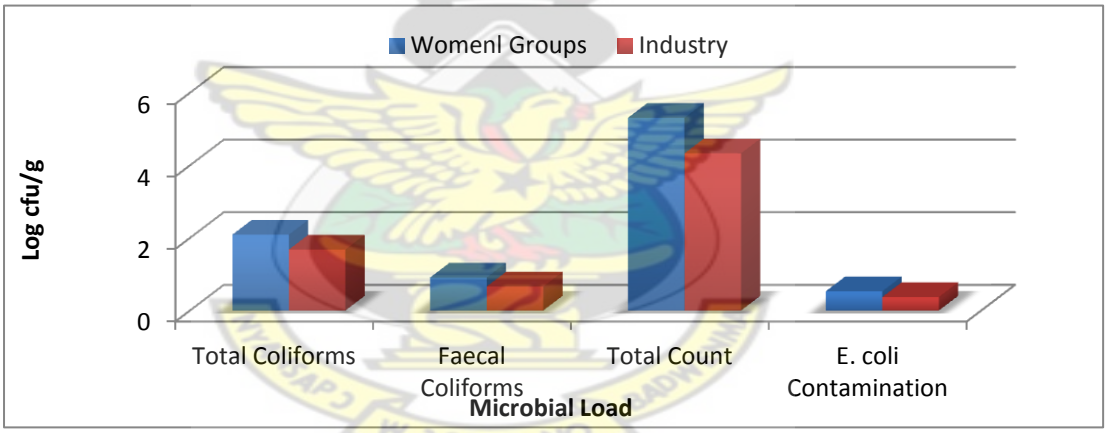


Figure 9: Comparison of microbial load of shea nut cake obtained from women groups and mechanized industry

Table 4: Mean (± SD) log cfu/g of microbiological quality of Shea Nut Cake

Parameter	Shea Nut Cake	P-Value
Total coliforms	1.95 ± 0.74	0.256
Faecal count	0.82 ± 0.49	0.443
Total viable count (TVC)	4.98 ± 1.17	0.007
E.coli	0.48 ± 0.42	0.161

Each value is a mean of twenty-four determinations ± SD

4.2.3 *E.* coli contamination

The results revealed that *E.* coli contamination was not significantly ($p \geq 0.05$) different across the shea industries of the shea zones of Ghana (Table 3). The *E. coli* contamination did not also show differences between first and second extraction cakes. The *E.* coli contamination levels ranged between 0.00 ± 0.00 log cfu/g at the Shebu industry of Savelugu to 1.15 ± 0.78 log cfu/g at Kalariga (Table 3). The average *E. coli* contamination was 0.48 ± 0.42 log cfu/g **(**Table 4**)**.

4.2.4 Faecal contamination

The analysis for faecal contamination of shea cake samples across the six industries of the shea zones of Ghana are presented in Table 3. It is very clear from the results that this contamination was similar across the industries (P = 0.05). While the contamination (0.24 ± 0.48 log cfu/g) at Shebu, Savelugu, was the least, contamination (1.64 ± 1.23 log cfu/g) at Kalariga was the highest. Slight differences between the first and second cakes were noticed in the faecal contamination of the shea nut cake. Count of faecal coliforms was higher (0.92 ± 0.51 cfu/g) in the second extraction cake (Fig. 8) but not statistically ($p \geq 0.05$) different. On the bases

of local and mechanized industry, count of faecal contamination was higher (0.91 ± 0.50 cfu/g) at the local industry (Figure 9), however, not statistically ($p \geq 0.05$) different from that of the mechanized industry.

Table 5: Microorganisms isolated from shea nut cake across six industries of Northern Ghana

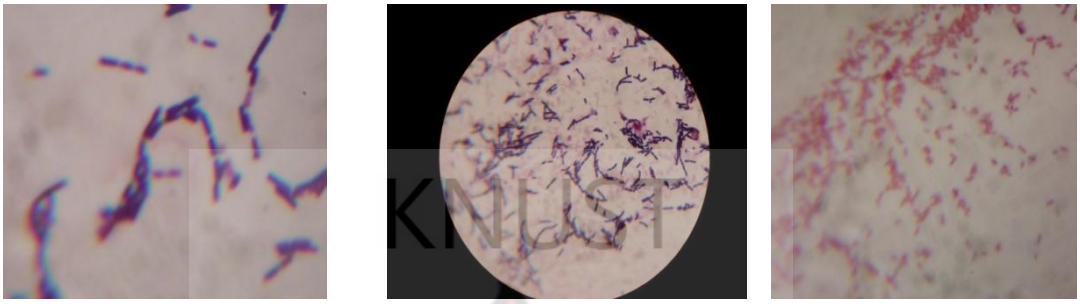
Industry /Location	Organism Isolated	Number of Isolates	Total number of Isolates
Tolon	*Bacillus mycoides*	2	
	Brevibacilllus agri	1	
	Bacillus cereus	3	11
	S. epidermidis	4	
	Unidentified species	1	
Kalariga	*Bacillus mycoides*	1	
	Brevibacilllus agri	1	
	Bacillus cereus	5	11
	S. epidermidis	3	
	Unidentified species	1	
Gumo	*Bacillus mycoides*	1	
	Bacillus cereus	6	9
	S. epidermidis	2	
GisoNaayili	*Bacillus mycoides*	2	
	Bacillus cereus	3	9
	S. epidermidis	4	
Shebu Industry	*Bacillus mycoides*	1	
	Bacillus cereus	3	8
	S. epidermidis	4	
Ghana Nuts Limited	*Bacillus mycoides*	1	
	S. epidermidis	1	2

4.2.5 Microbial Identification

The isolation of other microbes was carried out using dilution plate method. Pure colonies were obtained by repeated streaking. The results were presented in Table 5. *Bacillus mycoides* and *S. epidermidis* were present in all the industries. All bacterial species that were isolated from the cake were found at Tolon and Kalariga, with two unidentified species suspected to be fungus or mould. In all 40 isolates were

associated with the local industries with 10 isolates from the mechanized industries (Table 5).

Fifty isolates of microorganisms (Table 6) were obtained in all of the shea nut cake samples. The isolates were composed of both Gram-positive (Plate 5A and B) and Gram-negative **(**Plate 5C**),** rod shaped microorganisms.



A **B** **C**

Plate 5: Microscopic view of stained slides of bacteria

Table 6: Summary of bacterial species identified in the shea nut cake

Bacteria Identified	No. of Isolates
S. epidermidis	18
Bacillus mycoides	8
Bacillus cereus	20
Brevibacillus agri	2
Unidentified species	2
Total microbes	**50**

Based on morphological characteristics, biochemical, cultural and the combination of API 20E and API 50CHL, 36% Gram (+), mostly rods, with some few cocci and staphylococci were identified as *Staphylococcus epidermidis*, 16% Gram (+), mostly rods were identified as *Bacillus mycoides*, 40% Gram (+) rods as *Bacillus cereus* and

4% Gram (+) rods as *Brevibacillus agri*. Four percent Gram negative isolates showed no reaction on the API 20E and 50CH (Table 6 and Fig. 10).

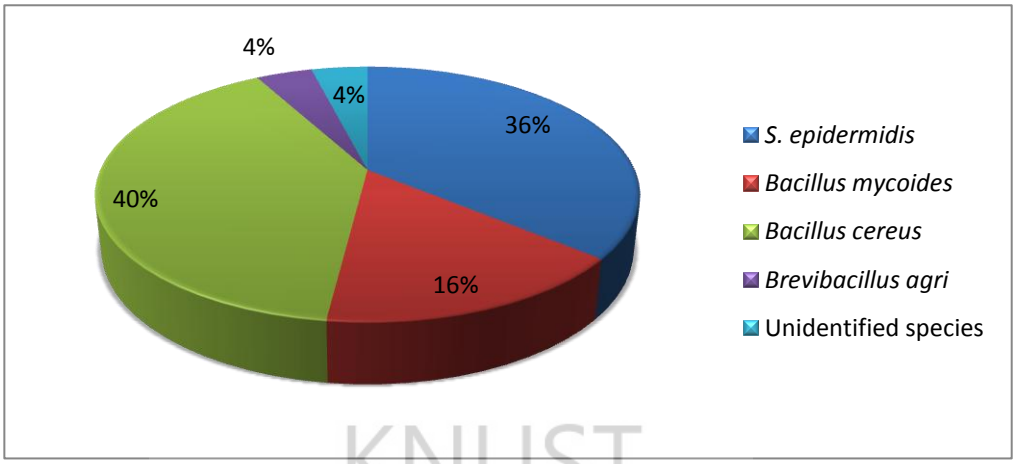


Figure 10: Percentage Bacterial Isolates from shea nut cake

Table 7: Proximate qualities of various shea nut cakes from six 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

Each value is a mean of four determinations

4.3 Proximate Analysis

The results of proximate analysis carried out on shea nut cake from the six industries are presented in Table 7. The values of crude fat ranged from 6.25 to 36.50% with an average of 23.38 ± 10.15%. The fat content was least at GNL-Techiman and highest at Tolon.

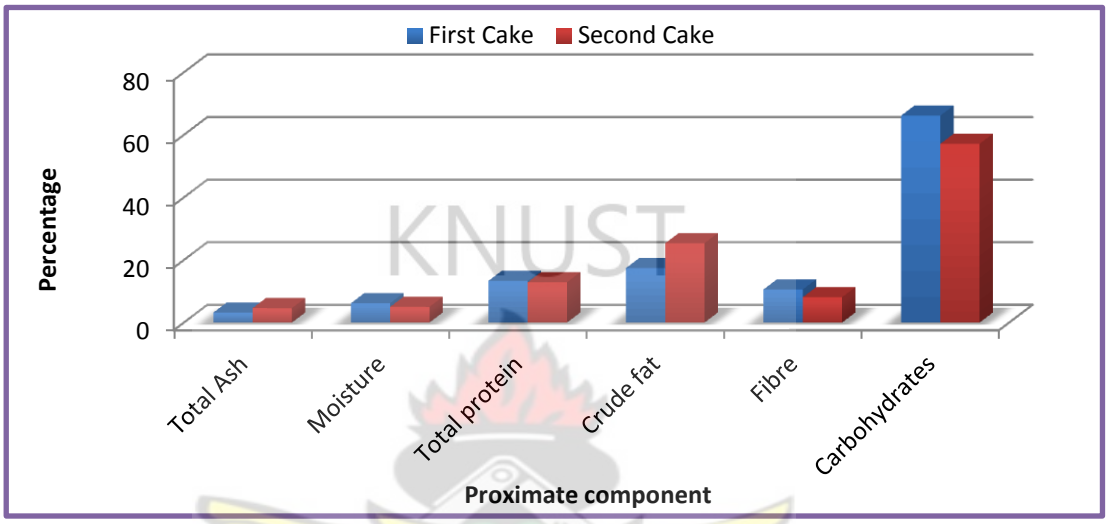


Figure 11: Comparison of proximate traits of shea nut cake derived from first extraction and second extraction

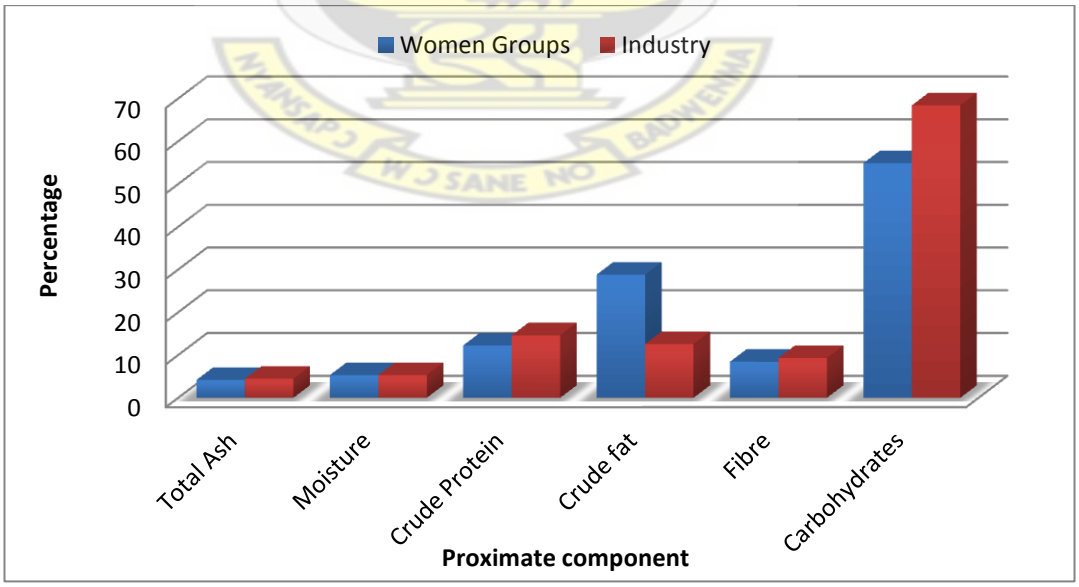


Figure 12: Comparison of proximate traits of shea nut cake derived from traditional and industrial processing methods

Table 8: Mean (±SD) proximate composition of Shea Nut Cake samples

Composition	%(Dry Matter)	P-Value
Crude Protein	13.03 ± 1.70	0.04
Moisture content	5.29 ± 0.98	0.01
Crude Fat	23.38 ± 10.15	0.01
Ash Content	4.25 ± 0.79	0.13
Crude Fibre	8.71 ± 0.85	0.31
Carbohydrates	59.37 ± 8.66	0.02

Each value represents the average of twenty-four determinations ± SD.

4.3.1 Total Ash Content

The total ash content presented in Table 7 did not vary significantly among samples across the industries. The total ash content ranged from a minimum of 3.50% at Giso-Naayili and Shebu Industries and a maximum value of 5.38% at the GNL. The total ash content was statistically ($p \geq 0.05$) not different between the local groups and mechanized industries **(**Figure 12**)** although it was higher at the mechanized industry. The second extraction cake contained more total ash than the first extraction cake (Figure 11). The average total ash content for shea nut cake was 4.25 ± 0.79%.

4.3.2 Moisture Content

The moisture content is presented in Table 7 alongside other proximate traits. The moisture content was significantly ($P \leq 0.05$) different across samples from the six shea butter processing industries. The least of 4.15% was recorded at the GNL and the highest of 6.50% at Giso-Naayili local industry. Moisture content was higher in

the first extraction cake (Figure 11**)** but was not statistically different from the second extraction cake**.** The moisture content was also not statistically ($p \geq 0.05$) different between the local groups and mechanized industries **(**Figure 12). The average moisture content for the shea nut cake in general was 5.29 ± 0.98%.

4.3.3 Crude Protein

Crude protein was significantly ($P \leq 0.05$) different from one industry to the other as elaborated in Table 7. The crude protein content for the two mechanized industries were closer (14.22 and 14.99%) but higher than (10.37 to 13.88%) records at the local industries (Table 7). The crude protein content ranged significantly from 10.37 to 14.99% across the industries. The Women Groups in general produced lower protein content as compared to the industries (Figure 12). Crude protein content between first and second extraction cake was fairly the same (Figure 11). Shea nut cake presented in Table 8 contains 13.03 ± 1.70 percent of crude protein on the average.

4.3.4 Crude Fat

There were significant ($P \leq 0.05$) differences in crude fat content of shea nut cake across the six industries (Table 7 and Figure 12**).** The difference in the crude fat content between first and second extraction cakes was also evident **(**Figure 11**)**. There was about twice as much crude fat in the cake from the women Groups as there were in the mechanized industries (Figure 12). Shea nut cake from the results presented in Table 8 contains 23.38 ± 10.15 % of crude fat on the average.

4.3.5 Crude Fibre

The statistical analysis of the crude fibre content indicated that it was not different ($P \geq 0.05$) across the various industries. The crude fibre content ranged from 7.11 at Gumo, a local industry to 9.35% at Shebu, a mechanized industry. Crude fibre content was higher in the first extraction cake (Figure 11). The crude fibre content was statistically not different ($p \geq 0.05$) between the local groups and mechanized industries **(**Figure 12). Shea nut cake from the results presented in Table 8 contains 8.71 ± 0.85 percent of crude fibre on the average.

4.3.6 Carbohydrates

Carbohydrates were high in the SNC obtained from mechanized industries than the local industries and as a result it was significantly ($P \leq 0.05$) different across the shea industries (Table 7). The carbohydrates content ranged from the least of 48.26% at Tolon and a maximum of 74.16% at the GNL. Analysis based on first and second extraction cake showed that there were more carbohydrates in the former than there were in the latter (Figure 11). Further analysis of the shea cake samples indicated that there were more carbohydrates in shea cake samples from the mechanized industries than there were in the local industry (Figure 12).

4.4 Mineral Analysis of shea nut cake

Table 9: Mean mineral composition of shea cake (%DM ± SD) mg/kg from six industries of Northern Ghana

Sample	Tolon	Kalariga	Gumo	GisoNaayili	Shebu In	GNL
Nitrogen	2.370 ± 0.649	2.693 ± 0.437	2.873 ± 0.141	3.173 ± 0.450	3.425 ± 0.375	3.250 ± 0.513
Phosphorus	0.155 ± 0.010	0.205 ± 0.044	0.225 ± 0.019	0.240 ± 0.035	0.260 ± 0.020	0.230 ± 0.037
Potassium	2.915 ± 1.143	4.115 ± 0.864	4.160 ± 0.852	3.973 ± 0.906	4.710 ± 0.390	4.428 ± 0.888
Sodium	0.345 ± 0.158	0.380 ± 0.103	0.363 ± 0.076	0.385 ± 0.096	0.468 ± 0.062	0.430 ± 0.079
Calcium	0.435 ± 0.130	0.420 ± 0.102	0.455 ± 0.124	0.490 ± 0.140	0.575 ± 0.136	0.665 ± 0.141
Magnesium	1.995 ± 0.544	1.668 ± 0.468	2.253 ± 1.330	1.208 ± 0.342	0.860 ± 0.534	0.618 ± 0.413
Copper	0.038 ± 0.010	0.093 ± 0.037	0.183 ± 0.117	0.088 ± 0.035	0.103 ± 0.015	0.058 ± 0.026
Mercury	0.060 ± 0.091	0.076 ± 0.052	0.049 ± 0.078	0.060 ± 0.100	0.170 ± 0.075	0.196 ± 0.012
Lead	0.010 ± 0.00	0.188 ± 0.210	0.200 ± 0.077	0.133 ± 0.075	0.108 ± 0.015	0.123 ± 0.050

DM –dry matter, SD – standard deviation

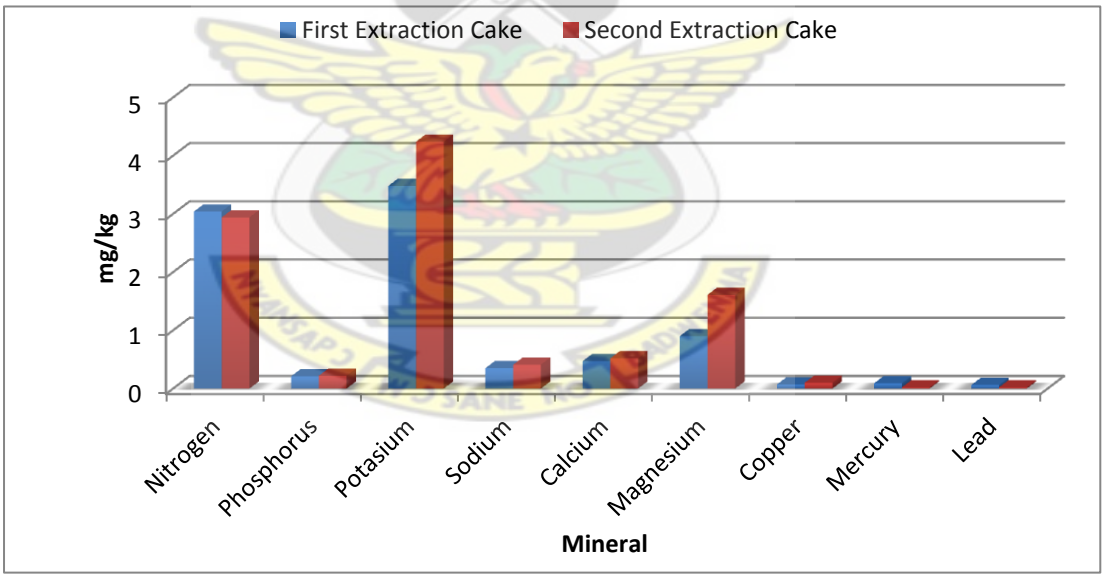


Figure 13: Comparison of mineral composition of shea nut cake derived from first and second extractions

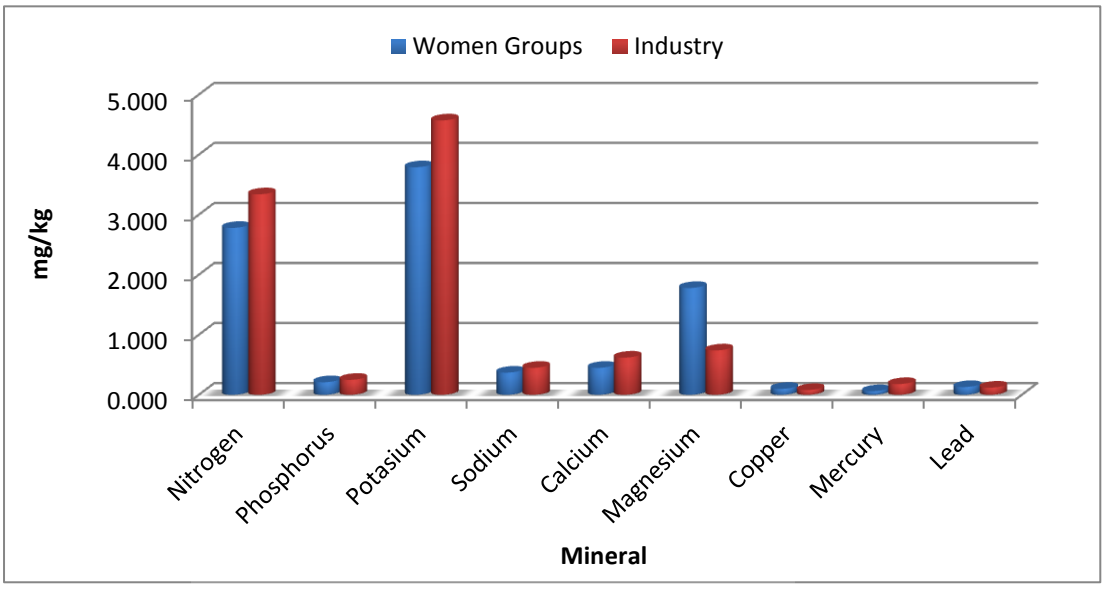


Figure 14: Comparison of mineral composition of shea nut cake derived from traditional and mechanized processing

Table 10: Mean (±SD) mg/kg mineral composition of shea nut cake

Mineral	Mean ± SD(mg/kg)	P-Value
Nitrogen	2.96 ± 0.39	0.038
Phosphorus	0.22 ± 0.04	0.001
Potassium	4.05 ± 0.62	0.128
Sodium	0.40 ± 0.05	0.552
Calcium	0.51 ± 0.09	0.472
Magnesium	1.43 ± 0.65	0.022
Copper	0.09 ± 0.05	0.020
Mercury	0.10 ± 0.56	0.056
Lead	0.13 ± 0.07	0.533

Each value is a mean of twenty-four determinations ± SD.

4.4.1 Nitrogen content (N)

The analysis of variance for nitrogen content in shea nut cake from different shea industries was significantly ($P \leq 0.05$) different across the industries as indicated in Table 10. The minimum recorded amount for Nitrogen was 2.370 ± 0.649 mg/kg at Tolon compared to a maximum value of 3.425 ± 0.375 mg/kg (Table 9) at Shebu industries. The analysis of nitrogen content based on type of industry indicated that it was higher at the mechanized industry (Figure 14) but on the basis of type of sample it was higher in the first extraction cake (Figure 13). The nitrogen content (3.425 ± 0.375 mg/kg) was highest at Shebu Industry and was closely followed (3.250 ± 0.513 mg/kg) by the GNL (Table 9) – the two mechanized industries. The average nitrogen content was 2.96 ± 0.39 mg/kg.

4.4.2 Phosphorus content (P)

Phosphorus was slightly higher at the mechanized industries as compared to the local industries (Figure 14) and as a result it was significantly different ($P \leq 0.05$) across the shea industries (Table 9). The phosphorus content ranged from the least of 0.155 ± 0.010 mg/kg at Tolon and a maximum of 0.260 ± 0.020 mg/kg at the Shebu industry. There was similar amount of Phosphorus in the first and second extraction cakes (Figure 13). The phosphorus (0.26 ± 0.02 mg/kg) content was highest at Shebu Industry and closely followed by GNL (Table 9**)**.

4.4.3 Potassium content (K)

The potassium content presented in Table 9 did not vary significantly among samples across the industries. The potassium content ranged from a minimum of 2.915 ±

1.143 mg/kg at Tolon and a maximum value of 4.710 ± 0.390 mg/kg at Shebu industry. The Potassium content was statistically not different ($p \geq 0.05$) between the local groups and mechanized industries **(**Figure 14**)** although it was higher at the mechanized industry. The second extraction cake contained more Potassium than the first extraction cake (Figure 13). The average Potassium content for shea nut cake was 4.05 ± 0.62 mg/kg.

4.4.4 Sodium content (Na)

The analysis of variance for sodium content in shea nut cake from different shea industries was not different ($P \geq 0.05$) as indicated in Table 10. The minimum recorded amount for sodium was 0.345 ± 0.158 mg/kg at Tolon which rose to a maximum value of 0.468 ± 0.062 mg/kg at Shebu industries. The results for Mineral analysis indicated that the sodium content of the cake were not different ($P \geq 0.05$) across the six shea industries (Tables 9 and 10). The Sodium (0.468 ± 0.062 mg/kg) content was highest at Shebu Industry and closely (0.430 ± 0.079 mg/kg) followed by GNL (Table 9).

4.4.5 Calcium content (Ca)

The statistical analysis of the calcium content indicated that it was not different ($P \geq 0.05$) across the various industries. The calcium content ranged from 0.420 ± 0.102 mg/kg at Kalariga, a local industry to 0.665 ± 0.141 mg/kg at the GNL, a mechanized industry. Calcium content was lower in the first extraction cake (Figure 13). The calcium content was statistically not different ($p \geq 0.05$) between the local groups and mechanized industries **(**Figure 14**)**. Shea nut cake from the results presented in Table 10 contains 0.51 ± 0.09 mg/kg of calcium on the average.

4.4.6 Magnesium content (Mg)

Magnesium was significantly different ($P \leq 0.05$) from one industry to the other as was presented in Table 10. The Magnesium content for the two mechanized industries each was less than one (0.860 and 0.618 mg/kg) but lower than values at the local industries (Table 9). The Magnesium content ranged significantly from 0.618 ± 0.413 to 2.253 ± 1.330 mg/kg across the industries. The Women Groups in general produced higher Magnesium content as compared to the industries (Figure 14). Magnesium content was also higher in the second than the first extraction cake (Figure 13). Shea nut cake from the results presented in Table 10 contains 1.43 ± 0.65 mg/kg of Magnesium on the average.

4.4.7 Copper content (Cu)

The statistical results for all minerals including that of copper content were presented in Tables 9 and 10. The results obtained for copper content across the industries indicated that it was significantly ($P \leq 0.05$) different. Shea nut cake had an average of 0.094 mg/kg copper content **(**Table 10**)**. The copper content ranged from 0.038 ± 0.010 mg/kg at Tolon to 0.183 ± 0.177 mg/kg at Gumo **(**Table 9). The concentration of copper in the first extraction cake was not different from that of the second extraction cake as shown in Figure 13. In a similar manner, the copper content was not different between local and mechanized industries **(**Figure 14**)**.

4.4.8 Mercury content (Hg)

Values obtained for Mercury across the industries were all different ($P \leq 0.05$) as in Table 9 and 10. The mercury content ranged from 0.049 ± 0.078 at Gumo to 0.196 ± 0.012 mg/kg at GNL (Table 9). The concentration of mercury in the first extraction cake was higher than that of the second extraction cake (Figure 13). In a similar manner, the copper content was higher in the mechanized industries compared to the local industry **(**Figure 14**)**. Shea nut cake had an average of 0.102 mg/kg mercury content (Table 10).

4.4.9 Lead content (Pb)

The statistical examination of lead content is presented in Tables 9 and 10 alongside other minerals. The results obtained for lead content across the industries indicated that it was not significantly different ($P \geq 0.05$). Shea nut cake had an average of 0.13 ± 0.07 mg/kg Lead content (Table 10). The Lead content ranged from 0.010 ± 0.00 mg/kg at Tolon to 0.200 ± 0.077 mg/kg at Gumo (Table 9). The concentration

of lead in the first extraction cake was higher but not statistically different from that of the second extraction cake as shown in Figure 13. The Lead content was not different statistically between local and mechanized industries (Figure 14).

4.5 Phytochemical constituents of shea nut cake

Phytochemical screening of samples from all the industries revealed that saponins, tannins, alkaloids, terpenoids and reducing sugars were present in the shea nut cake extracts (Table 11). Coumarins, flavonoids, anthraquinones and cynogenic glycosides were absent in the by-product.

Table 11: Phytochemical constituents of shea nut cake from six shea industries of Northern Ghana

Sample Location	Tolon	Kalariga	Gumo	GisoNaayili	Shebu Industry	Ghana Nuts Limited
Saponins	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Reducing Sugars	+	+	+	+	+	+
Cynogenic Glycosides	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-
Alkaloids	+	+	+	+	+	+
Flavonoids	-	-	-	-	-	-
Terpenoids	+	+	+	+	+	+
Coumarins	-	-	-	-	-	-

+ = present, − = absent

CHAPTER FIVE

5.0 DISCUSSION

Shea nut cake (SNC) is a brown amorphous substance released as waste during shea butter processing and has not been used extensively for any form of benefit. The substance is apparently thrown out as waste. The by-product is composed of microorganisms, proximate constituents, minerals, and phyto-chemical components. The quality of the shea nut cake is determined by the levels of constituents of each of these four parameters.

The microbial load analysis of shea nut cake from both the local and mechanized industries especially for total coliforms bacteria, faecal indicator bacteria and *E. coli,* were not significantly different ($p \geq 0.05$). The total viable count across the industries was significantly different and highest levels were recorded at Gumo, one of the local industrial establishments (Table 3). The high prevalence of coliforms in samples from the local industry could be attributed to, amongst other factors, contaminated water used during the pre-treatment and processing of the nut and during the actual extraction process leading to cross-contamination. Handlers not practising proper sanitation during processing and the use of unsterilized containers were other possible sources of contamination. Similar reasons could explain the presence of total and faecal coliforms at the GNL in particular which recorded second highest values for both parameters (Table 3). In addition to this, the water used for the cleaning of machine parts directly involved in the cutting of the nuts to size could also be another source of contamination.

All microbiological parameters: total viable count, total coliforms, faecal coliforms and *E. coli*, considered in this study were not significantly different in both first and

second extraction cakes (Figure 8). Such relatively similar microbial load values in the two types of cakes could imply that irrespective of which cycle the raw material goes through, similar sources of contamination exist.

The local industries or women groups had soaring values for microbial contamination (Figure 9) and the results indicated that the values and dynamics of microbial loads could have been influenced by the conditions surrounding the method of extraction adopted at the local industries. The isolation of high numbers of total and faecal coliforms and *E. coli* from shea nut cake at the local industry (Appendix Table A-1**)** was not unexpected since water used in the extraction process was not treated and could therefore contain coliforms and other enteric bacteria. The high prevalence and counts of coliforms found in the local industries could also be explained by the fact that the women do not wash the shea nuts before processing and some form of contamination could have also come from the soil where the fruits were picked. Visits to the industries revealed that hygienic conditions in the mechanized industries were generally better than those in the local industries and this could account for the reason why the mean total viable counts observed were significantly different ($P \leq 0.05$) between the local industry and the mechanize industries (Table 4 and 3).

SNC samples were screened for microbial information with *Bacillus cereus, Brevibacillus agri, Staphylococcus epidermidis* and *Bacillus mycoides* being isolated. *Bacillus mycoides* and *Staphylococcus epidermidis* were associated with all samples from the industries; *Brevibacillus agri*, however, were less frequent (Table 5). *Bacillus mycoides*, although detected across the shea industries, it was the second least detected in terms of numbers. The least detected bacteria were *Brevibacillus agri*, which was found only at local industries in Tolon and Kalariga. The second

higher bacterial detected was *S. epidermidis*. *Bacillus cereus* was found in five industries with the modal frequency of 20. Goodwin et al. (1994) affirms that *Bacillus mycoides* is a Gram-positive spore-forming species of the family *Bacillaceae* which has evolved since 1886 as species to *B. cereus var. mycoides* in 1946 and was reclassified in 1986 as *B. mycoides*. Unlike *B. cereus*, there has not been any report of disease caused by *B. mycoides*. On the contrary, *B. mycoides* has been reported as an important probiotic (Gumbo 2006) which produces bacteriocin capable of fighting serious food pathogens (Sharma and Gautum, 2008). Sharma and Gautam (2008) reported that the antibacterial substance, the purified bacteriocin, can withstand temperatures of up to 100ºC, and that it is very active at pH range of 4-11. Therefore, *B. mycoides*, found in shea nut cake may not be considered a contaminant but could be important source of bacteriocin and probiotics that could be useful to the food industry.

Staphylococcus epidermidis is commonly found in the epithelial surfaces of human beings and at the surfaces of indwelling medical devices e. g. catheters (Cheung *et al.*, 2010). The bacteria colonize such indwelling devices posing serious medical threat to patients. It is noted as one of the commonest contaminants and has been linked to the presence of some infections during clinical operations. Conversely, other researchers have assessed the industrial importance of *S. epidermidis*. *Staphylococcus epidermidis* has, since 1974, been identified as source of extracellular lipases extracted between pH range of 7-9. The lipases are said to be very effective in 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 (FFAs). *Brevibacillus agri* and *Staphylococcus epidermidis* present in the shea nut cake (Table 5) largely as

contaminants may on the contrary help the local shea industry maximize the production of shea butter; thus, they could be harnessed for lipase production. It is possible that there is the involvement of *S. epidermidis* in the extraction of shea butter at the local industry since it is commonly found on the epithelial surfaces of human including the hands which is directly used in kneading. *S. epidermidis* has been reported to have high lipase activity. Most women into shea butter extraction believe that there are some of them whose hands are best at causing the crude butter to rise easily and faster 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 the hands.

Tallent *et al.,* (2012), reported that *Bacillus cereus* is a group of ubiquitous facultative anaerobic spore-forming Gram (+) rods bacteria. *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 (Table 5). Forty percent of the bacteria found in shea nut cake was *Bacillus cereus.* The *Bacillus cereus*es are soil-dwelling saprophytes which cause a wide range of diseases in humans, including food poisoning and systemic infections (Didelot *et al.*, 2009). The use of *B. cereus* in the industrial sector however has been evident. Romanczyk *et al.* (1995) revealed 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 reported to be achieved at pH 8 and 35ºC extracted from *Bacillus cereus* (Uyar *et al.*, 2011). The results (Table 5) revealed that shea nut cake could be

source of useful enzymes for the food and leather industries but could be very dangerous for human consumption if not treated well.

Brevibacillus agri is a spore-forming Gram (+) bacterium and has been studied and known to achieve optimum growth at pH 7 and temperature of 45ºC with specific growth rate of 0.102 ± 0.015 h^{-1} (Kongpol *et al*., 2009). The *Brevibacillus 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) unearthed that *Brevibacillus agri* has also shown lipase activity of 1.8 U/ml in medium containing 1.5% lard as sole carbon source at 40ºC for 48h. They can therefore aid the shea butter extraction with the help of the lipase they produce. However the *B. agri* has been linked to some human infections (Gumbo, 2006).

Shea nut cake contained a host of useful chemical substances and this is evident across the industries. The total ash content (Table 7) which predicts the mineral composition of the shea nut cake was not significantly different ($P \geq 0.05$) across the industries but appear higher in the second extraction cake as compared to the first. What this could mean is that, especially for the local industry, the number of low density metal oxides in the cake was high since the crude shea butter from which the second cake was obtained, floated on the water.

Across the local and mechanized industries (Figure 12) the ash contents were similar and this suggests that the mineral contents are not different statistically. The average ash content of shea nut cake according to this research was 4.25 ± 0.79 %. This

value is lower than what was recorded for shea nut (6.1%) by Pousga *et al.* (2007). However this value is similar to the ash content of 'Kuli kuli'-groundnut cake and 'eri'-by-product of soyamilk as was investigated by Ogbonnaya and Bosede (2011).

Crude fat was higher in the second extraction cake (Figure 11); a phenomenon difficult to explain by the methods employed in the mechanized industries but could easily be explained by the traditional method of butter extraction (Figure 4A-F). The gross phenomenal disparity in crude fat content (Table 7) across the industries therefore could be attributed to the shea butter extraction method adopted by each industry. It was observed in the present study that the traditional method left as much as five (5) times crude fat (28.79% i. e. mean value of crude fat from local industries) in the shea nut cake as compared to the combined methods of mechanical press with chemical methods (6.25%) employed at the Ghana Nuts Limited (GNL) (Table 7). The local industry at Tolon adopts the local traditional method of shea butter extraction which achieves about 25% of butter extraction (Ofosu, 2009) while the mechanical with chemical methods have achieved about 98% extraction efficiencies (personal communication with Aka Aristide, GNL Techiman). 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.

The first extraction cake had high moisture, protein, fibre and carbohydrates content possibly due to the fact that it received lesser heat (Figure 11). The protein, the fibre and carbohydrates contents were also high at the mechanized industries where the nuts had not been exposed to extreme heat (15-20)ºC unlike processes adopted by the women groups. This supports why the same constituents were high with the first

extraction cake (Figure 11) which had not been exposed to extensive heat in the extraction process. These parameters: the protein, the fibre and carbohydrates contents were lower in the second extraction cake samples of the local industry because they had undergone four different heating sessions: sun drying, parboiling, dry frying and boiling to skim the oil. The protein molecules some of which might have been lost and the glycosidic linkages in the dietary fibre polysaccharides could have also been broken and consequently the low contents recorded for each of them.

The proximate composition of the shea nut cake is comparable to works of other researchers. Differences in fat content of shea cake across the industries do not agree with findings by Pousga *et al.,* (2007) who found fat content to vary across two climatic zones of Burkina Faso. The present findings of crude fat levels (6.25% – 36.5%) were much higher than 5.3% – 9.6% recorded by Pouasga *et al.,* (2007). The differences in fat levels could be attributed to the differences in approach (the pre-treatment, the care and attention at every stage of the process) of oil extraction and the level of efficiencies reached. This was also likely indicative of the differences in fat levels of the shea trees due to their genetic disparities or probably due to their different geographical and ecological zones (Pousga *et al*., 2007). Ugese *et al.* (2010) also reported similar low figures (2.8% – 4.0%) of crude fat levels for shea nut cake. It presupposes therefore that if the crude fat levels could reach values as high as 36.5% at the local industry, then their extraction efficiency do not commensurate of their efforts.

The range of values for protein (10.37 – 14.99%) from our discovery of the shea cake in Ghana is different from what was reported by Pousga *et al.* (2007), (2.5 – 10.3%) and Ugese *et al.* (2010) (7.6 – 10.1%). However, their carbohydrate values

were (89.1 – 97.3%) and (58.4 – 71.9%) respectively and the present findings (48.26 – 74.16%) fell within the same range (58.4 – 71.9%) obtained by Ugese *et al*. (2007).

The fibre content (7.11 – 9.35%) of Shea nut cake as reported in the present study (Table 7) appeared to be within the range (7.8 – 11.0)% to what was reported (Table 1) by Pousga *et al. (2007)* but was lower than values (9.9 – 19.3%) recorded by Ugese *et al*. (2010). The International Centre for Research in Agro-forestry (ICRAF) (2000) reported that although shea nut cake was already being used as a livestock and poultry feed, one key disadvantage of its use was the presence of tannins which exhibit a bitter taste even though the levels have not been quantified. Umali and Nikiema, (2002) have also reported the presence of anti-nutritional factors and low digestibility on shea nut cake and noted them as setbacks that need to be overcome for it to be fully used as feed. In addition to these findings, the presence of *B. cereus and S. epidermidis* in the shea nut cake calls for a second look at its use as feed although *B. mycoides* which is also present in the cake may counteract their effect as a probiotic. The present findings unearthed excellent proximate traits of Ghanaian shea nut cake and support their potential significance in the animal feed industry in Ghana.

The mineral content of the shea cake is promising. Nitrogen, potassium and magnesium were high across the industries (Table 8) and they were followed by phosphorus, sodium and calcium. These are all useful minerals for supporting the life of plants in the agricultural sector. In addition to other qualities, the heavy metal load of the cake was fairly reasonable for use in compost development for agricultural purposes. William (2000) reported 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). This present study found 0.09 mg/kg, 0.10 mg/kg and 0.13 mg/kg for Cu, Hg and Pb respectively (Table 9).

Sodium which was present in the shea nut cake samples facilitates and maintains the fluid balance of the body and promotes the normal functioning of the body nerves and muscles (Mohammad, 2009). The sodium content (0.46 mg/kg) found in this present research is higher than what (0.05 mg/kg) was reported by Pousga *et al.* (2007) for shea nut cake. Among other by-products, the sodium content of shea nut cake compares well with that of cotton seed cake (0.35 mg/kg) but higher than that of sorghum beer residue (0.06 mg/kg) (Pousga *et al.*, 2007).

The potassium content in shea nut cake across the industries was generally high and comparatively higher (4.05 mg/kg) than the mean value (0.54 mg/kg) determined by Pousga *et al.* (2007). The present findings also indicate that shea nut cake has higher potassium content than both cotton seed (1.8 mg/kg) and sorghum beer residue (0.11 mg/kg), (Pousga *et al.*, 2007). Potassium plays a significant role in protein synthesis in addition to its involvement in the functioning of cell organelles (Muhammad, 2009). Potassium is also noted for the life supporting role it plays for plants.

Magnesium is generally required for healthy bones and muscles and the functioning of many enzymes in living systems (Holistic, 2007). The present study reveals that shea nut cake has high magnesium content (1.43 ± 0.65 mg/kg) as compared to (0.15 mg/kg) observed by Pousga *et al.* (2007). The Magnesium content which was lower in the Mechanized industries suggests that the source of Magnesium in the shea nut cake was different for the local and mechanized industries.

The calcium and phosphorus contents of shea nut cake according to the findings of the present research (0.51 mg/kg and 0.22 mg/kg respectively) are concordant with

the findings of Pousga *et al.* (2007). Whereas the calcium content was comparatively high compared to values for both cotton seed cake (0.34 mg/kg) and sorghum beer residue (0.14 mg/kg), phosphorus in the shea nut cake was lower than cotton seed cake (1.3 mg/kg) and sorghum beer residue (0.25 mg/kg).

Heavy metals when ingested into humans and other animals have detrimental effects on health. Some heavy metals such as lead and mercury have been distinguished as likely toxic elements especially within some specific restraining values, and are known as latent risk for human diet (IOCCC, 1996).

The lead content of the shea nut cake across the industries is very uniform which suggests that the metal is coming from a common source. The highest occurrence of the metal was 0.200 ± 0.077 mg/kg and the average lead content of shea nut cake was 0.13 ± 0.07 mg/kg. This value is below the EU permissible level of lead concentration (0.2 mg/kg) for most plant products and cereals (Muhammad, 2009) (Table 9).

Mercury content was high in the mechanized industries and this presupposes that the source of mercury contamination of the shea nut cake at the mechanized centres was different from the local industries. The sources of mercury are marine, fruit nuts, farm animals, cereals and dairy products (Brenner and Snyder, 1980; Davies *et al.*, 1974). The average mercury content of shea nut cake observed was 0.10 ± 0.56 mg/kg.

The copper content was generally inconsistent across the industries, and across the first and second extraction cakes. The mean copper content of shea nut cake was 0.09 ± 0.05 mg/kg. There has not been any documented standard set for copper content of shea nut cake. The tolerance level of copper in edible oil at Taiwan, for

instance, is set at 0.4 mg/kg according to the standard of edible oils announced by the Department of Health in 1993 (Chen *et al.*, 1999). Copper is an essential element for the maintenance of body functions and not a hazardous metal since it does not induce adverse symptoms on human when consumed in trace amounts (Chen *et al.*, 1999). The harmful effect only occurs when it is overdosed.

The SNC has five of nine phytochemicals (Table 11) that were screened for their presence in this study. Saponins, tannins, reducing sugars, alkaloids and terpenoids were present in the SNC. The medicinal value of plants and plant products lies in some chemical substances that produce a definite physiological action on the human body (Adebayo *et al.*, 2012). Phytochemicals are the basic source for the establishment of several pharmaceutical industries (Savithramma *et al.*, 2011).

Alkaloids were present in the shea nut cake across the industries (Table 11) and alkaloids have been reported to have higher anticancer activities (Nobori *et al.*, 1994). Alkaloids are also known to have anti-microbial, antifungal and anti-inflammatory effects (Okwu and Okwu, 2004). Sofowora (1993) indicated in a study that alkaloids are anti-hypertensive agent. Terpenoids and triterpenoids in particular have demonstrated antibacterial activities (Roshila *et al.*, 2011). Tannins were also present in the shea nut cake and Dharmananda (2003) reported that tannins are extensively used for the treatment of intestinal disorders such as diarrhoea and dysentery. The treatment of sore throat, haemorrhage and wound healing has been linked to tannins (Okwu and Okwu, 2004). Saponins were observed in all the samples across the shea industries. They are known to possess anti carcinogenic properties and other health benefits (Gbadamosi *et al.*, 2011). Saponins could also play an essential role in the treatment of malaria (Adesokan and Akanji, 2010). The phytochemical analysis also revealed the presence of reducing sugars in all the

extracts confirming the presence of carbohydrate. Sugars are mostly monosaccharide and disaccharides and reducing sugars in particular play an important role in energy generation.

CHAPTER SIX

6.0 Conclusion and Recommendation

6.1 Conclusion

Shea nut cake (SNC) has much more nutritional quality than previously thought since the protein, carbohydrates, fibre, potassium and magnesium observed in this study were quite high. Although there were the presence of some bacterial pathogens such as *Bacillus cereus, Bacillus mycoides, Brevibacillus agri* and *Staphylococcus epidermidis* and some heavy metals in the shea cake samples, their contents did not exceed the permissible safety limits. However, the detection of *E. coli* in the present study poses serious threat to the use of the SNC for consumption.

The shea nut cake also contains 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 shea nut cake could be capable of producing the enzyme lipase which could influence shea butter extraction. The high nitrogen (2.96 mg/kg), potassium (4.05 mg/kg) and magnesium (1.43 mg/kg) contents also make shea nut cake ideal for use as manure and for fertilizer production. Also the presence of essential phytochemicals: Saponin, tannins, reducing sugars, alkaloids and terpenoids in the SNC widens its application in therapeutic remedies.

6.2 Recommendations

- In the context of this research, an interesting study would be to carry out enzyme activity tests including the possibility of extracting enzymes from the isolated microorganisms.
- It is recommendable to carry out further research on the two unidentified microorganisms associated with the SNC.
- Future research could also investigate the protein profile (amino acid content) to unearth the specific nutrient value of the SNC as well as its effect on the growth rate of plants.
- Further research could also quantify the phytochemical constituents of the cake for its specific usage in the treatment of certain diseases.
- The research notes the possibility of different sources of contamination of the shea cake in the various industries. Given the diversity of sources of contamination especially at the local industry level, it seems appropriate to question which techniques best promote pure cake productivity that will meet standards for full use in the animal industry.
- It is hoped that with these stunning revelations about the shea nut cake, Research Institutions (KNUST for example), the local industries, women and women groups, the mechanized industries into shea butter production, the Government, NGO's, and the Savana Accelerated Development Authority in particular could lead in the education of these findings and further investigations about the shea nut cake.

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APPENDICES

APPENDIX A

Table A-1: Mean (± SD) Microbial Load (Log cfu/g) of shea nut cake of Local Groups versus Industries

Parameter	Local Group	Industry
Total Count	2.09 ± 0.74^{a}	1.68 ± 0.93^{b}
Faecal Count	0.91 ± 0.50^{a}	0.66 ± 0.60^{b}
Total Count	5.31 ± 1.31^{a}	4.33 ± 0.66^{b}
E. coli	0.53 ± 0.43^{a}	0.37 ± 0.53^{b}

Similar superscript across rows did not show significant differences; different superscripts across rows were significant

Table A-2: Mean (± SD) Microbial Load (Log cfu/g) of First and Second Extraction Cakes

Parameter	First Extraction Cake	Second Extraction Cake
Total coliforms	1.93 ± 1.26^{a}	1.96 ± 0.84^{a}
Faecal count	0.86 ± 1.15^{a}	0.92 ± 0.51^{b}
Total viable count	4.99 ± 1.58^{a}	4.98 ± 1.11^{a}
E-coli	0.50 ± 0.77^{a}	0.47 ± 0.47^{a}

Rows with similar superscript are not significantly different; the vice-versa holds.

Table A-3: Percentage Mean values (± SD) of proximate composition of first and second extraction shea nut cakes

Composition	First Extraction Cake	Second Extraction Cake
Total Ash	3.17 ± 1.13	4.61 ± 0.92
Moisture	6.17 ± 1.05	5.00 ± 1.10
Crude Protein	13.32 ± 1.56	12.87 ± 2.50
Crude fat	17.42 ± 5.66	25.36 ± 14.62
Fibre	10.57 ± 1.46	8.09 ± 0.89
Carbohydrates	66.13 ± 5.83	57.13 ± 12.50

Table A-4: Proximate composition (%DM) of Shea Nut Cake according to Industry

Composition	Women Groups	Industry
Total Ash	4.16 ± 0.56	4.44 ± 1.33
Moisture	5.29 ± 0.74	5.30 ± 1.63
Crude Protein	12.24 ± 1.30	14.61 ± 0.54
Crude Fat	28.79 ± 4.60	12.57 ± 8.93
Fibre	8.42 ± 0.81	9.31 ± 0.06
Carbohydrates	54.86 ± 4.01	68.40 ± 8.15

DM-Dry matter

Table A-5: Mineral composition of shea cake (mg/kg) of the local Groups versus Industry

Composition	Women 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

Table A-6: Mineral composition of SNC; First versus Second Extraction Cakes

Composition	First Extraction Cake	Second Extraction Cake
Nitrogen	3.04 ± 0.35	2.94 ± 0.57
Phosphorus	0.21 ± 0.05	0.22 ± 0.04
Potassium	3.48 ± 1.46	4.24 ± 0.52
Sodium	0.35 ± 0.16	0.41 ± 0.04
Calcium	0.47 ± 0.13	0.52 ± 0.13
Magnesium	0.90 ± 0.43	1.61 ± 0.76
Copper	0.07 ± 0.03	0.10 ± 0.07
Mercury	0.09 ± 0.09	0.02 ± 0.02
Lead	0.06 ± 0.03	0.02 ± 0.01

APPENDIX B

MEDIA, STAINS AND INDICATORS

B-1 MacConkey Broth

Ingredients	Grams/Litre
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Bromocresol purple	0.01
Final pH	7.4 ± 0.2 at 25°C

B-2 Solutions for Gram Staining

B-2.1 Crystal Violet Staining Reagent

B-2.1.1 Solution A

Crystal violet	2 g
Ethanol (95%)	20 ml

B-2.1.2 Solution B

Ammonium oxalate	0.8 g
Distilled water	80 ml

Solution A and B were mixed to obtain crystal violet staining reagent.

B-2.2 Iodine Solution

Iodine	1 g
Potassium iodide	2 g

Distilled water	300 ml

Iodine and potassium iodide were grinded. Water was added slowly and the solution was stirred until the iodine had dissolved. The solution was stored in amber bottle for use.

B-2.3 Safranin Solution

Safranin (2,5% in 95% alcohol) 10ml / Distilled water 100ml

B-3 Methyl Red Indicator

Composition:

Methyl red	0.1 g
Ethanol 95%	300 ml
Distilled water	200 ml

B-4 Trytone Water

Composition

Sodium Chloride	5.0 g/L
Tryptone (tryptic hydrolysate of casein	10.0 g/L

APPENDIX C

CHEMICALS

PET Ether

NaOH

H_2SO_4

H_3BO_3

Na_2SO_4

HCl

$CuSO_4$

HNO_3

$HClO_4$

Ethanol

Meyer's Reagent

Chloroform

Picric acid

Fehling's Solution

$FeCl_3$

Acetic anhydride

APPENDIX D

DEFINITION OF TERMS

Industry	Mechanized industry in shea butter extraction
Local Industry	Women Groups
Shea Nut Cake	A by-product of shea butter extraction process
P-Value	Probability value
Tolon	"WunniSong" Shea Women Group
Kalariga	"Suglo N Bori Buni" Shea Women Group
Gumo	"Gub-Danda" Shea Women Group
Giso-Naayili	"Tunteiya" Shea Women Group
Shebu	Shebu Induustries Limited, Savelugu
GNL	Ghana Nuts Limited, Techiman
First Cake	By-Product from first extraction of shea butter
Second Cake	By-Product from second extraction of shea butter

APPENDIX E

PICTURES OF FIELD OBSERVATION OF SHEA OIL EXTRACTION AND ANALYSIS OF SHEA NUT CAKE