KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA

Optimization of Enzyme-Assisted Hydrothermal Extraction of Shea Butter from Traditionally Pre-Treated Shea Kernels

By

Beauty Didia (BSc. Microbiology)

A Thesis submitted to the Department of Biochemistry and Biotechnology, Faculty of Biosciences, College of Science in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE IN BIOTECHNOLOGY

MAY, 2017

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI, GHANA

Optimization of Enzyme-Assisted Hydrothermal Extraction of Shea Butter from Traditionally Pre-Treated Shea Kernels

By

BEAUTY DIDIA (BSc. Microbiology)

A Thesis submitted to the Department of Biochemistry and Biotechnology, Faculty of Biosciences, College of Science in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE IN BIOTECHNOLOGY

MAY, 2017

DECLARATION

I, Beauty Didia, declare that this submission is my own work towards the MSc and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

Beauty Didia (PG2480714)	.....	18/05/2017
Student Name & ID	**Signature**	**Date**

Certified by:

Dr. Hilary Domakyaara Zakpaa	.....	18/05/2017
Supervisor's Name	**Signature**	**Date**

Certified by:

Dr. Hilary Domakyaara Zakpaa	.....	18/05/2017.
Head of Department	**Signature**	**Date**

ABSTRACT

Enzymes can be simultaneously used to selectively release targeted fats from pre-treated Shea nut kernels and improve extraction efficiency at optimized conditions. Three different pre-treated Shea nut substrates: raw kernels, roasted kernels and Shea nut paste from roasted kernels were subjected to treatment with three different industrial enzymes: lipases, pectinases and cellulases, separately and in combination, for Shea butter extraction. The process for enzyme mixtures was optimized at pH of 6, 3 % Enzyme-Substrate concentration, 2 hour hydrolysis time at a temperature of 60 ºC. The results showed that Shea nut paste yielding 48 % fat with pectinases (P), 52% with lipases (L) and 46% with cellulases (C). The amount of percentage fat increased to 52, 54 and 56 at 1:1 P+C, L+C and P+L enzyme combinations respectively. The highest extraction efficiency of 69.96% was recovered from 1:1:1 combination of all three industrial enzymes. The free fatty acids and the peroxide value of the Shea butter recovered ranged from 1.6 ± 0.5 – 2.7 ± 0.1 mgKOH/kg and 3.6 ± 0.6 – 5.4 ± 0.2 mEq/kg respectively. Thus enzyme assisted aqueous hydrolysis of Shea nut biomass for Shea butter production is a promising technology capable of eliminating the drudgery with the mechanical extraction, the safety concerns with the chemical methods and the arduous and cumbersome nature of the traditional village extraction methods. To make this process financially practical, further work is suggested to minimize cost with applications of modified mixes of crude enzymes.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

DEDICATION

This project is dedicated first and foremost to the Almighty God, the author and finisher of our faith, for starting and finishing with me. To my parents, Prof. Dr. and Mrs. Prince O. Didia for their moral and financial support and my siblings for their contribution.

ACKNOWLEDGEMENTS

My sincere and profound gratitude goes to the almighty God for being my help since ages past, giving me the strength to carry out this research successfully.

I am indeed grateful to my parents Prof. Dr. & Mrs. Prince Didia for their moral and financial support towards my education and also thankful to all my siblings (Emmanuel Didia, Unity Didia, Elizabeth Didia and Daniel Didia). God will continue to bless you all beyond measure. My gratitude also goes to my ever-supporting and understanding supervisor Dr. Zakpaa, H. for his suggestions, corrections and always being available to answers all my questions. My utmost gratitude also goes to Mr. Abdul-Mumeen Iddrisu, who has been a guardian carefully leading and encouraging me through every stage of my work. He has also been very understanding, always ready and willing to help whenever there was any need of his attention. Also to my post graduate course mates: Prosper Adzale, Samuel Katia, Fransica Appiah and Ali Baba Isa, you all have been wonderful.

I would also not forget to thank Mr. Teddy Blutse and Mr. Nathaniel Ato Yason in charge of the laboratories, as they have greatly assisted me with laboratory guidance. The Savannah Accelerated Development Authority (SADA) is acknowledged for funding this research. Furthermore, am grateful to the entire lecturers and staff in the department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology for their immeasurable support. The Lord Almighty will reward you all.

Thank you.

GLOSSARY

Abbreviations	Meaning
ANOVA	Analysis of Variance
CAC	Codex Alimentairus Commission
CBEs	Cocoa Butter Equivalents
CRIG	Cocoa Research Institute of Ghana
EAHE	Enzyme-Assisted Hydrothermal Extraction
EUCD	European Union Chocolate Directive
GNL	Ghana Nuts Limited
GSA	Global Shea Alliance
HWF	Hot Water Floatation method
IMC	Intermediate Moisture Content Method
ITTU	Integrated Technology Transfer Unit
OJEC	Official Journal of the European Communities
PHC	Population and Housing Census
RASK	Raw Shea Kernels
ROSK	Roasted Shea Kernels
SKEP	Shea Kernel Paste
SNV	Netherlands Development Organization
UEMOA	Union Economique Monétaire Ouest Africaine

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Shea butter is a natural creamy-coloured fatty product made from the nut products of Shea tree (*Butyrospermum parkii* or "butter seed") and has a large number of applications. The Shea tree grows normally in the wild Savannah belt over 20 nations across West Africa (Addaquaye, 2004). Often referred to as women's gold, the extraction of Shea butter is produced by many young women especially in the Northern regions of Ghana. Evidence of the utilization of Shea butter, and the Shea tree itself, dates back about 4000 years to ancient Egypt. The Shea tree was first documented as a high-value commodity in regional trade across West Africa as early as 1354, by the Moroccan traveler Ibn Battuta, then again in 1799 - more than four centuries later - by Mungo Park (Addaquaye, 2004).

Shea butter is produced by female groups' all-season-long in almost every community in the Northern parts of Ghana. The Tolon and Gumo towns of the Tolon-Kumbungu District, Savelegu of the Savelegu-Nantong District, Techiman of the Brong Ahofo Region, Kalariga and Giso-Naayili of the Tamale Municipal Assembly are some of the locations Shea butter is produced in bulk. The extraction of Shea fat encompasses two broad extraction techniques generally described as conventional or modern (Tano-Debrah and Ohta, 1994).

The importance of Shea tree is considered second to the palm tree (Paulsen, 1981) because of the usefulness of Shea butter benefits industries both locally and

internationally (Soladoye *et al*., 1989; Russo and Etherington, 2001; Chaffin, 2004; Ndukwe *et al*., 2007; Ogbonnaya and Adgidizi, 2008; Akihisa *et al*., 2010). The traditional method of extraction is considered the preferred choice of most Shea butter industries in Ghana (Addaquaye, 2004). This method of Shea butter production encompasses many manual unit operations. The processing stages apparently have some problems which render the whole process tedious and laborious.

Alternative to the traditional aqueous extraction methods used in extraction of Shea butter (Southwell and Harris, 1992; Al-hassan, 2012; Warra, 2011; Alenyorege *et al*., 2015; Kaviani *et al*., 2015), other modern methods involves expellers or hydraulic presses, ghanis or mechanical rig (Olaniyan and Oje, 2007) and the use of chemical extraction method (Abdul-Mumeen *et al*., 2013; Chen and Diosady, 2003) or enzyme assisted aqueous extraction (Tano-Debrah and Ohta, 1995; Rosenthal *et al*., 1996; Apea and Larbi, 2013; Otu *et al*., 2015).

Research and development of the Shea resource began nearly a century ago, in the former French and British colonies (Addaquaye, 2004). Over the past four decades, village-level technologies for improved Shea processing have been developed, which have proven highly successful in Eastern and Central Africa. Enzymes are catalysts that accelerate the rates of biological reactions (Garrett and Grisham, 1995). To address the problem of low efficiency and yield, studies have alternatively recognized the application of enzymes in generating oil from oilseeds which is a natural strategy for conquering these difficulties (Barrios *et al.,* 1990). The use of enzymes has emerged as an effective novel means to improve the oil yield in cold pressing and aqueous extraction techniques

(Tano-Debrah and Ohta, 1994) and it has been reported by many researchers (Cheah *et al*., 1990; Rosenthal *et al*., 1996; Hernandez *et al*., 2000; Chen and Diosady, 2003; Abdulkarim *et al*., 2006; Huyanh *et al*., 2013).

1.2 PROBLEM STATEMENT

In Western Africa, the manual process employed by non-urban women in Shea butter extraction involves: beating the kernel with pestle in mortar to break the seed into grits, cooking the kernel to accomplish easy removal, grinding the grits into paste, kneading the paste in water to capture the fat into an emulsion, steaming the combination to separate the fat and skimming off the fat (Addaquaye, 2004). The final cooling procedure leads to "unrefined Shea Butter". This aqueous extraction method is tedious, time intensive, energy sapping, environmentally unfavourable and generally gives low oil yield with poor quality (Olaniyan and Oje, 2011). The ineffectiveness of the handling methods decreases the amount of Shea butter available in the industry. According to Carette *et al.* (2009), Shea butter handling in West Africa includes "minimum technical input, drudgery as well as high usage of fire wood, and all these have effect on the quality of Shea butter." Despite the huge and wide usage, the conventional methods of Shea butter prepared in Ghana are recognized by low qualities.

The low quality of Shea butter is another issue, as it generally falls below international standards. According to Ademola *et al. (*2012), continual demand is reducing and the possibilities of Shea butter in treating non-urban hardship is diminishing, requiring an evaluation of the handling methods. The hydrothermal

extraction method is another way of solving the problems associated with the extraction of oils from oilseeds. It is basically an advanced stage of the traditional extraction procedure in which water and heat are used at different stages and at different levels of combination. The equipment required such as mortar and pestle, kneading and boiling pans, seed roasters among others are less costly and are easily obtainable.

Despite the hydrothermal extraction method being considered as low yielding, tedious and time consuming by some researchers, it proves to be comparatively high yielding, environmentally friendly, efficient, less expensive and as such employs no toxic chemicals. Therefore, there is need for continuous development of Shea oil extraction methods that are effective, efficient, and easy to operate. This necessitated the present study in the use of commercial enzymes to eliminate technological limitations of traditional hydrothermal extraction method.

1.3 RESEARCH QUESTIONS

This study sought to answer the following questions:

a. What pH, temperature, hydrolysis time, and enzyme concentration will give the best butter yield?
b. What are the comparisons in extraction yield among samples: raw ground Shea nuts, roasted ground Shea nuts and Shea nuts processed to paste form?
c. Will the chemical characteristics of enzyme extracted Shea butter have similar qualities as from other technologies?

1.4 RESEARCH OBJECTIVES

1.4.1 General Objective and hypothesis

This study evaluated the optimization of the enzyme-assisted hydrothermal extraction of Shea butter from traditionally pre-treated Shea kernels. The hypothesis underlying this objective was that energy intensive steps such as roasting can be eliminated and that Shea butters will share similar chemical properties regardless of the processing method, be it unroasted, roasted or further milled to paste.

1.4.2 Specific Objectives

The specific goals of the study included:

a. To determine the optimization of extraction parameters (pH, temperature, and enzyme concentration) for improved Shea butter recovery and Shea butter quality.
b. To compare extraction yields of raw ground Shea nuts, roasted ground Shea nuts and Shea nuts processed to paste through the use of commercial enzymes.
c. To characterize the Shea butter extracted through the use of commercial enzymes.

1.5 JUSTIFICATION FOR THE STUDY

The removal of the Shea nut fat gives butter highly valued by the non-urban communities and the industrialists (Dodiomon *et al*., 2011). According to Aboyella (2002), Shea butter extraction and trading are significant successful actions that offer career to non-urban women. In Aboyella's perspective, Shea butter extraction constantly plays an important part in hardship relief and food security, thus getting the interest of

government which has led to the creation of a department of the Cocoa Research Institute at Bole in the North Region of Ghana to improve cultivable different species of the Shea nut tree.

The study builds upon the works of George *et al*. (2010), who studied the effect of unroasted Shea nuts for Shea butter extraction and Otu *et al.* (2015), who employed the use of commercial enzymes on Shea butter extraction. The study also seeks to evaluate the performance of the hydrothermal extraction of oil extraction in terms of its extraction yield as it is used by rural folks in extracting oil from the oilseeds. Alternative enzyme assisted extraction of Shea butter is the technology that has a scope in the future. Effective growth and development of enzyme-based processes are activated by technology advancement seen in the oil removal industry such as cost benefits, safety issues, and nourishment issues (Rosenthal *et al*., 1996).

1.6 SCOPE AND LIMITATION OF THE STUDY

Sample collection was limited to Tolon District from the Northern Region of Ghana and the use of three commercial enzymes: pectinase, cellulase and lipase in the extraction of Shea butter. The study was also limited to using the Hydrothermal Extraction, Solvent extraction (using *n*-hexane) and Enzyme-assisted hydrothermal extraction method. The study focused on physico-chemical characteristics assessed; moisture content, acid value, peroxide value, saponification value, iodine value, and free fatty acids.The study was laboratory based.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 INTRODUCTION

This chapter is an overview of the Shea butter industry. The industry is examined from its historical evolution to its current situation. It covers literature on benefits of the Shea butter industry, the extraction technologies and the quality standards of Shea butter.

2.2 OVERVIEW OF SHEA BUTTER

2.2.1 The Botany of shea trees

Gaertner Karl (1807), who was a German botanist first named the Shea tree as *Vitellaria paradoxa* and it belongs to the Sapotaceae family (Caroline *et al.,* 2009). In 1961, it was relabeled as *Butyrospermum parkii* (Maranz *et al*., 2003), with the "*parkii*" making reference to Mungo Park (1771-1805), "who was the truly amazing Scottish traveler presenting Shea butter to Europe" (Goreja, 2004). Some journals use *Butyrospermum parkii,* while many others use *V. paradoxa* which is the currently approved name for the African Shea trees. The Western Africa trees could be the subspecies "*paradoxa*" and the Eastern Africa one as "*nilotica*" (Mbaiguinam *et al*., 2007).

Masters *et al*. (2004), reported Shea trees growing across a 5000 km wide belt of savanna such as from Senegal (West) to Sudan, Ethiopia, Uganda (East) and across the African Region. The Shea tree develops normally in the dry Savannah belt and semi-arid areas (Masters *et al*., 2004). The 19 countries in which Shea is found across the continent

of Africa are Benin, Ghana, Mali, Niger, Nigeria, Senegal, Chad, Burkina Faso, Cameroon, Central African Republic, Ethiopia, Guinea Bissau, Cote D'Iviore, Sierra Leone, Sudan, Togo, Uganda, Democratic Republic of Congo and Guinea (CRIG, 2002).

This savanna belt is generally known as "Shea belt" among investors (Ferris *et al*., 2001). The Shea tree usually develops to a normal size of about 15 m (height) with excessive branches and a dense wax-like and greatly fissured bark that makes it fire resistant. The West African Sub-Region where majority of Shea plants are found and where Shea products can be found are shown in Figure 2.1. Amongst these nations, Ghana and Burkina Faso are considered the main Shea nut exporters (Walter *et al*., 2003).

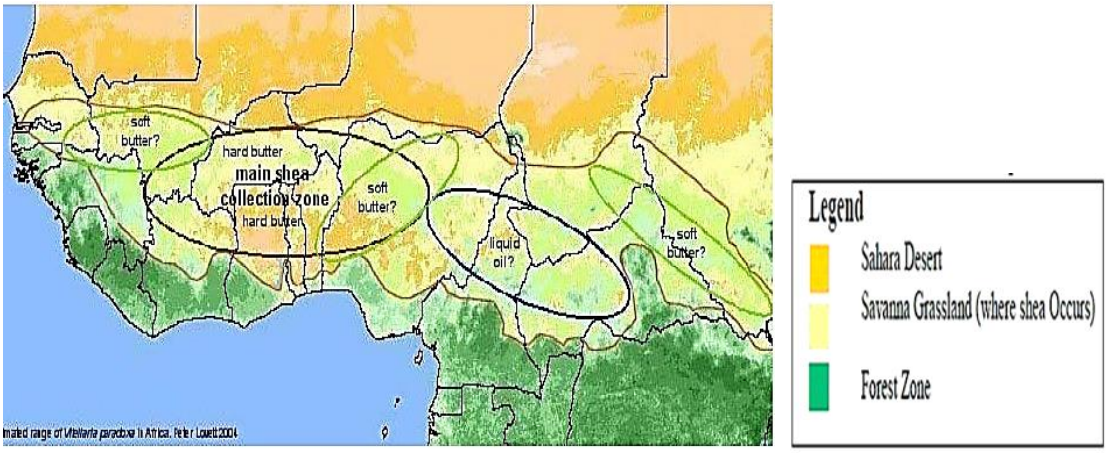


Figure 2.1: Cross-cutting Shea producing countries in Africa ranging from Senegal across to Sudan and Ethiopia.
Source: Lovett (2004), WATH Technical Report No. 2.

The map zeros-in on the West Africa Sub-Region where the bulk of shea trees occur and where Shea products can be found. The high shea production countries are Ghana, Nigeria, Mali, Togo., Burkina Faso, Benin and, Cote d'Ivoire. Northern Ghana is singled out as being one of the main sources of high quality Shea.

Plate 2.1: Shea tree

Plate 2.2: Ripe Shea fruits

Plate 2.3: Fresh Shea nuts

Plate 2.4: Dry Shea Nuts

2.2.2 Benefits of the Shea Industry: The uses of Shea and Shea products.

According to Paulsen (1981), the value of Shea tree follows the value of palm oil in Africa. The Shea tree is the second most important oil plant and as it develops in areas unsuitable for palm tree growth, it takes on primary importance in West Africa. In the early Nineteen seventies, the value of the Shea to the economic system of Ghana increased extremely when it was stated that, “it was one of only six plant varieties whose

vegetable fat can be used in the production of Cocoa Butter Equivalents (CBEs), in candy as well as being a valued component in the drug and beauty products industries" (OJEC, 2000).

2.2.2.1 Traditional use of Shea butter and Shea tree parts.

The Shea butter together with the oil palm represents significant resources of edible oil for many houses in many areas of the Sahel Africa (Ndukwe *et al*., 2007; Chaffin, 2004). Typically unprocessed prepared Shea butter is purchased from "loaves" in market places and in cases where the Shea butter are prepared properly and wrapped in leaves, they are resistant to oxidative rancidity and can be kept for years if not exposed to heat and air (Chaffin, 2004). The Shea butter is also used as raw material for the manufacture of margarine, detergent and candlestick (Russo and Etherington, 2001).

Different enteric attacks such as dysentery, diarrhoea, helminthes and other digestive system attacks, skin illnesses and injury attacks may perhaps be treatable by different parts of the Shea tree such as leaves, roots, seeds, and others (Soladoye *et al*., 1989). Local healers use Shea butter as a treatment for rheumatism, swelling of the nose, nose blockage, leprosy, coughing, and minimal bone tissue dislocation (Tella, 1979; Badifu, 1989; Goreja, 2004; Olaniyan and Oje, 2007). When Shea butters are produced and are of low quality, they are smeared on earthen surfaces of homes which provides as water resistant to secure surfaces during rain (Fluery, 1981). After circumcision of new born male child, treatment can be multiplied using Shea butter and it can also be used to prevent stretch-marks in African expectant mothers and as a bug resilient, offering protection against *Simulium* disease (Goreja, 2004).

2.2.2.2 Use of Shea butter in the international market.

Shea butter enjoys its use as an element of aesthetic remedies (Akihisa *et al*., 2010) and as an alternative for cocoa butter in chocolate sectors (Ogbonnaya and Adgidizi, 2008) although distinction in flavour has been noticed (Fold, 2000). In the chocolate market, Shea butter is enhanced and deodorized to be used usually as Cocoa Butter Equivalents (CBEs) since 1960s with its likeness in actual qualities to a more costly cocoa butter (Alander, 2004). CBEs are plant fat which have identical and substance qualities to cocoa butter containing no lauric acidity, and are mixable with cocoa butter in every amount without changing the qualities of cocoa butter (Hee *et al*., 2013). Under the European Union Chocolate Directive (EUCD), a 5% non-cocoa is permitted in the produce of chocolate. However, in the U.S, products that contain CBEs are not permitted to be called "chocolate" (Lovett, 2004).

The drug market has taken advantage of the effectiveness of Shea butter as it is used as an element in the treatment of inflammatory related illnesses due to its anti-inflammatory action (Masters *et al*., 2004). It has also been used to create solution to herpes lesions, joint disease, acne, and to reduce blood cholesterol stages by a drug company, BSP Pharmaceutical (Masters *et al*., 2004). High stages of unsponifiable matters have been revealed in Shea butter as in comparison to other vegetable fats and oils (Alander, 2004). This results in more possibilities to create Shea butter products showcasing "Shea butters' therapeutic qualities such as anti-oxidant, anti-inflammatory and other supposed activities" (Maranz *et al*., 2004).

2.3 PRETREATMENT OF SHEA NUTS PRIOR TO EXTRACTION PROCESS

The first stage of pre-treatment involves the removal of unwanted materials. The Shea fruits are then left to ferment in the open for 3 - 5 days after which they are de-pulped to separate the fruit pulp (mesocarp and epicarp) from the nuts. The removal of the fleshy pulp is facilitated by fermentation. Moisture content is reduced by sun-drying the nuts for 5 - 10 days. The nut, which is made up of a hard outer shell with the kernel inside, is pounded in a mortar with a pestle, cracked between two stones or trampled upon with the feet to liberate the kernel (Salunkhe *et al.,* 1992). The kernels are then thoroughly dried for 10 - 20 days, depending on weather conditions, bagged and stored for sale or processed to obtain the butter (Figure 2.2).

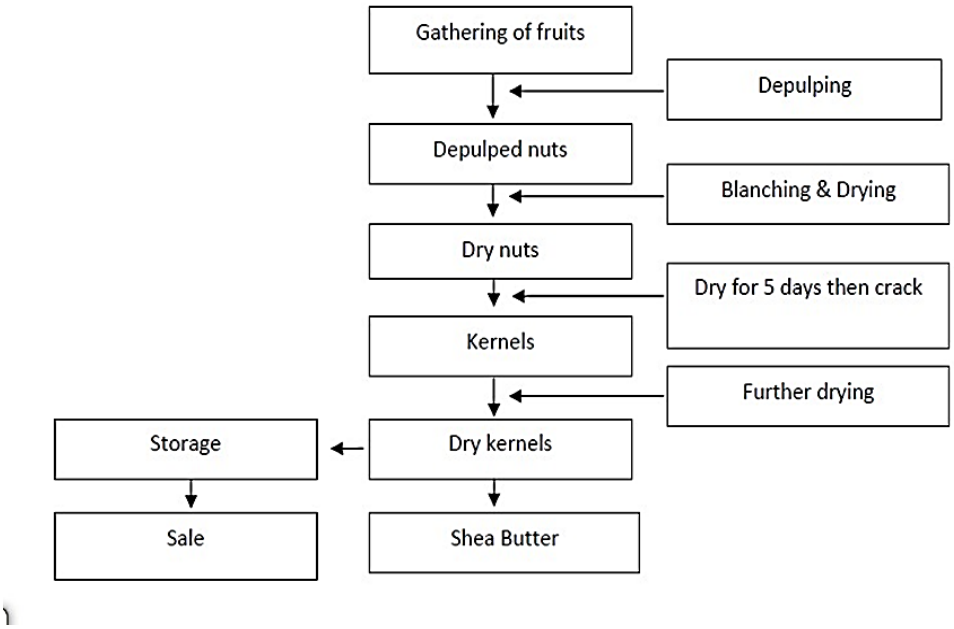


Figure 2.2. Flow Chart for Local Collection and Pre-treatment of Shea Nuts

(Source: Agyente-Badu *et al*., 2010).

2.4 PROCESSES FOR SHEA BUTTER EXTRACTION

There are three different main techniques for the manufacture of Shea butter: conventional manual processing, semi-mechanized (using hydraulic/mechanical presses), and completely mechanized techniques (Addaquaye, 2004). In Ghana, the traditional manual processing predominates. The other two methods are hardly used, due mainly to the infant nature of the commercialization of the activity. Processing groups are largely household family units, micro and small scale producers being currently organized in cooperatives through the efforts of the Shea Alliance / Shea Network under the initiative of Stichting Nederlandse Virjwilligers (SNV) Ghana and its partners. The semi and fully industrialized processing methods give higher yields per unit input of the raw material than the traditional method (Addaquaye, 2004).

2.4.1 Traditional Manual Extraction of Shea Butter

The traditional method predominates in West Africa. A report by the Netherlands Development Organization (SNV) (2006), gives an estimation of about 60% of all the crude butter extracted using the traditional method at an extraction rate of about 20% - 31%. The conventional procedure is commonly described as less efficient because it generates low quality butter and low income. Traditional extraction has been usually done by hot water and scooping off the oil while commercial ones are carried out by pressing or solvent removal with further improving and deodorizing of Shea butter (Alander, 2004). The modern technique, which is an enhanced type, uses appropriate technological innovation to mechanize some of the device functions of the guide conventional system. For example, a nut crusher, a kneader or a hydraulic/screw media

frequently enhances the guide procedure and cuts down on drudgery associated with the conventional technique.

However, with the higher interest in naturally produced products, natural Shea butter manufacturing is recommended and thus initiatives have been made to industrially generate Shea butter by following the conventional extraction techniques (Figure 2.3). Al-hassan (2012), reviews that about 35% of females use the contemporary technique of handling Shea butter with the remaining still depending on the conventional technique in the Northern region of Ghana. The Shea butter obtained from the traditional extraction procedure not including a refining stage is called “unrefined Shea butter”.

The Shea nuts are either roasted or boiled in West Africa, while in East Africa the nuts are sun-dried. The boiling process done in the West Africa method is aimed at killing the embryo thereby preventing germination of seeds. Hydrolytic degradation of the Shea butter which may occur as a result of the enzyme lipase is inactivated, which gives this method its additional advantage. However, it has been stipulated that high temperatures involved in boiling can cause high peroxide values and oxidation can be accelerated by water (Bail *et al.,* 2009). Once boiling is done, the nuts are further dried under the sun.

According to Moharram *et al.* (2006), when Shea nuts are sun-dried during rainy season, it may result in mold contamination and thus change the quality of the Shea butter. After the boiling and sun-drying steps, the Shea nuts are cracked to remove shells from the dried nuts and then kernels are further dried by roasting or sun-drying (Moharram *et al.,* 2006). In addition, the West African oven method which involves

roasting or smoking in ovens has a disadvantage. The roasting or smoking in the oven can cause high amounts of polycyclic aromatic hydrocarbons (PAHs) known to be carcinogenic (Hee *et al*., 2013).

The East African method involves no heating step, instead the nuts are directly sun-dried, de-husked, and sun-dried again (Lovett, 2004). The nuts are re-dried occasionally after the stored nuts are dried. In this method there is a lesser chance of the lipases being deactivated as they are not subjected to high temperatures. According to Lovett (2004), these high temperatures are usually linked with high levels of free fatty acids. A paste is then formed by wet milling the dried kernels to which the paste is then emulsified by kneading (Moharram *et al.*, 2006). Separation of the fat from the Shea nut cake is achieved by boiling the paste and the resultant butter is scooped up. This is then filtered through a filter cloth and placed to solidify in a cool place (Plate 2.5).

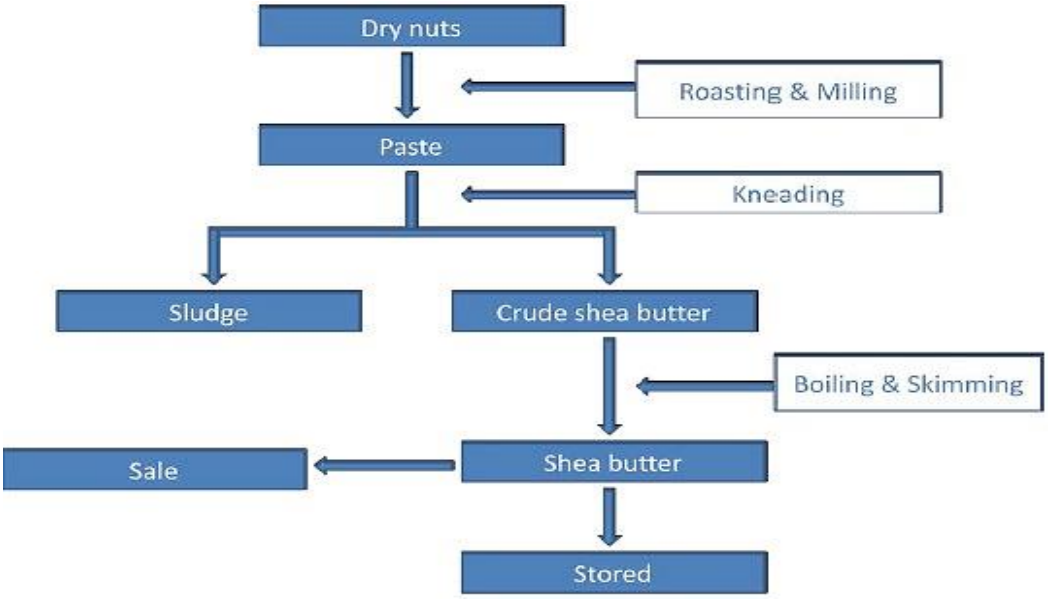


Figure 2.3: Flow Chart for Local Processing of Shea Butter

(Source: Agyente-Badu *et al*., 2010).

Plate 2.5: Shea butter processing; milled nuts to skimming.

(Source: Abdul-Mumeen *et al*., 2013).

2.4.2 Mechanical Extraction of Shea Butter

Mechanical pressing methods (such as expellers, hydraulic presses, and others) were introduced into the Shea butter industry. The nuts are heated and then transferred into a crushing unit where they are reduced in size to get better oil yield. The heated (pulverized) nuts are then pressed to release oil and the first extraction cake (Abdul-Mumeen *et al*., 2013). The first extraction cake is directed into another expeller where it is pressed for the second time to produce more oil and the second extraction cake.

The Shebu Industry at Savulegu in the Northern Region and the Ghana Nuts Limited (GNL) at Techiman in the Brong Ahafo Region of Ghana use the mechanical expeller for the extraction of Shea butter. The use of these mechanical expellers has improved the extraction efficiencies of Shea butter by about 8%. Abdul-Mumeen *et al*. (2013), reported that about 30 - 33% of Shea butter is extracted from the Shea nuts with the mechanical expeller.

2.4.3 Chemical Extraction of Shea Butter

Chemical or solvent extraction is the modern way of processing vegetable oil using solvent extracts such as petroleum-derived hexane, which produces higher oil yields and is quicker and less tedious. The extraction of industrial oils such as soybean and corn oils are usually extracted by this technique. But this technique comes with some drawbacks such as issues concerning safety, toxicological, environmental and potential health risks associated with using methods like the hexane extraction (Mbaiguinam *et al*., 2007).

In cases where other methods, for example, the mechanical press method is not yielding the desired results, solvent extraction methods are usually employed. This scenario as reported by Abdul-Mumeen *et al*. (2013), is typical 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 (the second Shea nut cake) is directed into the chemical plant for further extraction (Abdul-Mumeen *et al*., 2013). 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. It takes about four hours to have a complete extraction cycle at the chemical plant according to Abdul-Mumeen *et al.* (2013).

After the extraction cycle, a solution of Shea oil in solvent (hexane) is formed. It is then directed into a distillation chamber where the mixture is heated to 68.7 ºC, 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. Hexane is the preferred choice over other solvents as a result of several factors: the physical properties of the solvent, the commercial economics of the product and the edibility of Shea oil from the extraction (Mbaiguinam *et al*., 2007). A combination of the chemical and mechanical methods at the GNL yields 98% extraction efficiency and the company targets only 1.5% of oil left in the Shea cake after extractions (Abdul-Mumeen *et al*., 2013).

The industries producing vegetable oil are in need of suitable and environmentally friendly methods of extraction (Bhattacharjee *et al*., 2006). Johnson and Lusas (1983), revealed that the passion to reduce the use of harmful organic chemicals in large set ups has reignited the interest in souring for alternative removal procedures. Processes are being developed which are more efficient alternative to hexane with the idea of retaining the oil quality, with contingency restoration of essential nutritional value (Chen and Diosady, 2003). Sineiro *et al*. (1998), revealed that the use of water as the most cost-effective is becoming more popular like other bio-renewable chemicals, with the aim of

removing harmful chemicals. Aqueous oil removal has re-surfaced as an appealing exercise for removal of oil from certain oil bearing seeds.

2.4.4 Aqueous Extraction

As reported by Rosenthal *et al*. (1996), in order to substitute for the solvent extraction methods, an aqueous extraction process was developed in the 1950s. This approach was way cheaper and environmentally friendly in terms of oil extraction from oilseeds (Cater *et al*., 1974). Rural areas of many developing countries in Africa use the traditional hot water flotation method of extracting oils from oil bearing seeds (Southwell and Harris, 1992). Different oil seeds have been studied and extracted using the aqueous extraction method and some of them include: peanuts (Bhatia *et al*., 1966; Rhee *et al*., 1972), cottonseed (Sugarman, 1956), sunflower (Hagenmaier, 1974), rapeseed (Embong and Jelen, 1977; Staron and Guillaumin, 1979), coconuts (Hagenmaier *et al*., 1972) and soybeans (Lawhon *et al*., 1981).

2.4.5 Enzyme-Assisted Aqueous Extraction (E.A.A.E)

Enzymatic aqueous oil extraction provide many benefits in comparison with conventional extraction in the fats and oil industry and some of such benefits include lower costs in investments and energy requirements (Rosenthal *et al*., 1996). In circumstances where degumming is a significant issue, enzyme-assisted aqueous extraction can be applied to get rid of this operation as it may allow the elimination of some anti-nutritional substances or toxins from oilseeds (Caragay, 1983). Several

research studies have been carried out on aqueous handling of oil plant seeds (Subrahmanyan *et al*., 1959; Eapen *et al*., 1966; Rhee *et al*., 1972; Hagenmaier *et al*., 1972; Kim, 1989).

The oilseeds are enclosed by a thick cell wall which has to be ruptured to discharge the proteins and oil within the seed. Enzymatic hydrolysis of cell wall is one of the increasing techniques for pre-treatment of oilseeds as it hydrolyses the "complex lipoprotein and lipopolysaccharides elements into simple elements, thus releasing additional oil for extraction" (Deepika and Gagandeeep, 2014). The employment of enzymes can be seen in the extraction of a wide range of substances. An enzyme plays a role by hydrolyzing constituents that makeup the cell wall and thereby releasing intracellular contents.

The lipid body membranes are degraded by proteolytic enzymes which can also generate an effect on the cytoplasmic network thus making the inner structure less tightly bound. This eventually facilitates the removal of lipid and protein from the cell (Rosenthal *et al*., 1996). An aqueous medium can be used to easily separate the released oil from the cotyledon cells. Extraction of oils from soybeans (Rosenthal *et al*., 1998), and oils from coconut (Cintra *et al*., 1986), have also been achieved using enzyme-aided processes. In addition to cellulase and pectinase, α-Amylase can also be used as it reduces the viscosity of an emulsion, which can lead to oil release and improve oil recovery (Huyanh *et al.*, 2013).

Aspergillus niger was used to obtain enzymes and by using 3% of this enzymatic mixture, 50% more oil was obtained for rapeseed and 90% of for soybeans (Fullbrook,

1983). A combination of enzyme preparations including: pectinase, cellulase, and hemicellulase were used to perform aqueous extraction of dehulled rapeseed followed by extraction of the residual oil with petroleum ether (Rosenthal *et al*., 1996). In these studies, the permeability of the seed cell wall was increased by the enzymatic hydrolysis resulting in effective extraction of the oil. More studies show enzymatic processes which had been tried on the seed cell walls to facilitate oil extraction (Cintra *et al*., 1986; Frevert *et al*., 1990; Ho *et al*., 1992; Olson, 1992; Sosulski and Sosulski, 1993; Rosenthal *et al*., 1998).

2.4.6 Hot water floatation (HWF) method

Developing African countries such as Ghana and Nigeria (especially the non-urban locations) still commonly employ the hot water floatation method of oil extraction because its advantages over other small-scale oilseed managing techniques (the use of expellers or ghanis) is its simplicity. The gadgets required (including pestle and mortar, boiling and kneading pans, seeds roaster, calabash) are readily available and less expensive, NRI (1995).

As elaborated by Anebi *et al*. (2014), and Alenyorege *et al*. (2015), traditionally, seeds are crushed with a pestle in a mortar, roasted and pounded in a mortar to a much finner particles for easy milling or grinding. The fine crushing of the seeds is considered the first phase in cell distraction. It facilitates the diffusion of the soluble compounds and the release of the oil. The ground seeds are normally kneaded while adding warm and cold water intermittently to form a paste. Water is important as it hydrolyzes the paste thereby dislodging oil from the paste.

The paste is then suspended into boiling water (100 ^{o}C) and boiled for at least 30 minutes with periodic stirring. A slurry which appears grey in colour starts forming as separation of fat is seen from the oil-water mixture. The oil that forms floats to the surface and sometimes in cases where water is lost as evaporation due to boiling further quantities of water are added to encourage the oil to float to the surface. The oil is then carefully scooped from the surface of the mixture using a calabash or shallow dish and heated to remove residual moisture (NRI, 1995). The oil is then cooled and prepared for packaging or storage. According to Warra (2011), the sesame oil recovery from 0.5 kg seed was 108 ml, equivalent to an oil extraction efficiency of 41% using HWF method. The method (Figure 2.4) has also been used in a number of studies to extract oils from oilseeds (Southwell and Harris, 1992; Tano-Debrah and Ohta, 1995; Anebi *et al*., 2014; Alenyorege *et al*., 2015; Kaviani *et al*., 2015).

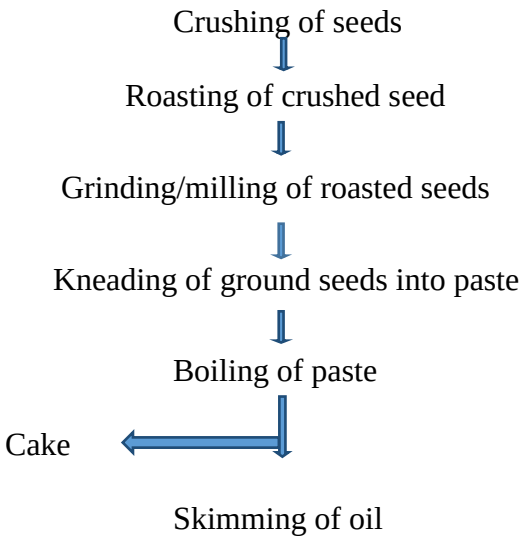


Figure 2.4 Simplified flow chart of the HWF method of oil extraction

2.5 EXTRACTION PARAMETERS

Extraction parameters are important factors to consider in order to achieve desirable oil yield recoveries. When using enzymes to extract, the seeds are usually ground and mixed with water after which it is agitated to result in increased extraction of oils. There are usually seven main parameters that influence enzymatic extraction yields and which are normally taken into consideration, including enzyme mixture, enzyme concentration, pH, oilseed particle size, solid-liquid ratio, temperature and hydrolysis time (Cater *et al*., 1974; Lawhon *et al*., 1981). Studies have been carried out on oil recovery for avocado and reported that better oil was recovered with 1% (w/w) α-amylase, paste-to-water ratio of 1:5 at 65 ^{o}C for 1.5 hours (Buenrostro and Lopez-Munguia, 1986).

The pH is a very important parameter and depending on the composition of the oilseed it varies during extraction processes. According to Rosenthal *et al*. (1996), aqueous extraction at a pH value near the isoelectric point of protein makes it impossible to obtain high oil and protein yields because at this pH, protein can bind the oil in a very stable emulsion. For peanut, a pH of 4 – 5 was found to be suitable for oil extraction with no emulsion phase and protein can be extracted easily at a pH of 8.0 (Rhee, 1972).

Different studies on enzymatic processes were conducted at different temperatures and the optimum temperature range (40 ^{o}C – 60 ^{o}C) was reported for several oilseeds (Kim, 1989; Rustom *et al*., 1991; Dominguez *et al*., 1994). Li *et al*. (2016), reported that when the temperature was higher than 50 ^{o}C, there was a slight decrease in protein yield. Kim (1989), reported no significant increase in the palm oil yield when

extraction temperature was raised above 45 ^{o}C. For the extraction of protein yields from peanuts, an unfavourable effect of temperatures greater than 60 ^{o}C was observed (Rustom *et al*., 1991). This increased temperature may cause denaturation of protein.

The extraction time is dependent on pH and not only on temperature and the type of oilseed used during the extraction process. Rhee *et al*. (1972), reported that 30 minutes was sufficient to obtain maximum oil and protein yields from peanuts, while Rustom *et al*. (1991), reported that increasing extraction times from 15 – 40 minutes had no significant effect on the yields.

2.6 PHYSICAL PROPERTIES OF SHEA BUTTER

2.6.1 Moisture Content

According to Olaniyan and Oje (2007), moisture content decreases as heat temperature rises. Moisture content of oil must be low, this is because oil with great moisture content are inclined to recontamination or rancidity. The reported moisture contents of Shea butter vary from 0.1% (Olaniyan and Oje, 2007) to 66 4.9% (Honfo *et al*., 2013).

2.6.2 Melting Point

This is the point at which Shea butter exist as oil. According to Alander and Andersson (2002), Shea butter melts at approximately 3 ^{o}C – 35 ^{o}C depending on the variety and quality of the nut from which the oil was extracted. Melting point 38.0 ^{o}C – 39.5 ^{o}C was observed as reported by Adomako (1985).

2.6.3 Viscosity

According to Olaniyan and Oje (2007), Shea butter has an optimum viscosity of about 100 centipoise (cP) when the heating temperature is 70 ^{o}C – 90 ^{o}C.

2.6.4 Colour Intensity

Yellow is the dominant colour of Shea butter at all heating temperatures. Heating above 90^{o}C will results in darkening of the oil (Olaniyan and Oje, 2007).

2.6.5 Refractive Index

Refractive index varies with chain length, degree of saturation and to the ratio of cis/trans double bonds. Hence, specific refractive index of fat and oils can be used for identification, checking purity and also provide hints on the oxidative damage. Specifically for Shea butter many authors reported refractive index around 1.46 at 40 °C (Hee *et al*., 2013; Honfo *et al*., 2013).

2.7 CHEMICAL COMPOSITION OF SHEA BUTTER

As a plant fat, Shea butter constitutes approximately about 90% of triglycerides and a minor unsaponifiable fraction (Hee *et al*., 2013). The emollient properties of Shea butter are attributed to the triglycerides it contains, while the unsaponifiable fraction is responsible for Shea butter's medicinal properties as it contains the bioactive substances that include hydrocarbons, tocopherols, sterols, and alcohols (Esuoso *et al.*, 2000).

2.7.1 Triglyceride fraction

Fatty acids usually dominates Shea butter constituency and its composition includes palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acids. Stearic and oleic acids constitutes 40 – 45% of total fatty acids respectively and the range of 5 – 10% constitutes linoleic acid, palmitic acid follows at 4% (Alander, 2004) with lower amounts of arachidic acid and others. Shea butter contains relatively high amounts of saturated fatty acids in comparison with grape seed oil, olive oil and canola oil which have saturated fatty acids less than 20% of total fatty acids (Samman *et al*., 2008). The grape seed oil (total saturated fatty acids: 10.4 – 14.3% of total fatty acids), olive oil (12.7 – 16.2%), and canola oil (5.5 – 7.7%) are generally liquid at room temperature (Baydar *et al*., 2007; Damodaran *et al*., 2008; Samman *et al*., 2008). The high amount of saturated fatty acids in Shea butter is responsible for its solid to semi-solid state at room temperature. Shea butter fatty acids were found to vary across the African countries (Di Vincenzo *et al*., 2005).

2.7.2 Unsaponifiable fraction

Unsaponifiables are identified as substances which dissolves in fat and are insoluble in aqueous solution but soluble in organic solvent after saponification (Hamilton and Rossell, 1986). Unlike the triglyceride fraction which comprises a larger part of the Shea butter, unsaponifiables usually comprises only a small part of the Shea butter. Shea butter contains an unsaponifiable portion consisting of bioactive ingredients that lead to Shea butter's therapeutic properties (Esuoso *et al*., 2000).

The range by weight of unsaponifiables in Shea butter is 4 - 11% (Hamilton and Rossell, 1986; Lipp and Anklam, 1998; Alander, 2004). For many years now, research has continuously been done on the unsaponifiables of plant oils and fats due to their various bioactivities including antioxidant, antimicrobial and anti-inflammatory. The unsaponifiable portion of Shea butter is taken over mostly by triterpene alcohols, followed by hydrocarbons, sterols, and other minimal components such as vitamin E (Itoh *et al*., 1974; Lipp and Anklam, 1998; Alander, 2004). Other chemical compositions of Shea butter includes: acid value, peroxide value and iodine value. Based on dry matter, proximate composition of the kernel and the physico-chemical characteristics of Shea abutter has been documented by Tano-Debrah and Ohta (1994), and shown in the Tables 2.1 and 2.2 respectively.

Table 2.1: Proximate composition of Shea kernel

PARAMETER	PERCENTAGE (%)
Total lipids	59.04
Crude fats	54.85
Proteins	7.81
Total carbohydrates	34.77
Ash	2.57
Starch	7.59
Hemicellulose	10.84
Cellulose	5.95
Pectin Substances	2.93
Total fibre content	20.35

Source: Tano-Debrah and Ohta, 1994.

Table 2.2: Physico-chemical characteristics of Shea kernel

PARAMETER	*PERCENTAGE (%)*
Melting range	34-36
Iodine value	58.53
Saponification value	180.37
Unsaponification matter content	7.48
THE PREDOMINANT FATTY ACIDS	
Palmitic	3.55
Stearic	44.44
Oleic	42.41
Linoleic	5.88
Linolenic	1.66

Source: Tano-Debrah and Ohta, 1994.

2.8 QUALITY CONTROL OF SHEA BUTTER: AVAILABLE STANDARDS FOR SHEA BUTTER

ProKarité, a project managed by the World Agroforestry Centre and funded by CFC/FAO (Common Fund for Commodities/Food and Agriculture Organization) has developed a quality standard for unrefined Shea butter. It is also approved by UEMOA (Union Economique Monétaire Ouest Africaine) (Lovett *et al.*, 2005). The ProKarité which is like a regional standard board has proposed sensory, physical and chemical characters that define the quality of Shea butter.

These qualities includes: colour, odour, taste, moisture, free fatty acids, peroxide value, insoluble impurities, volatile matters, relative density, refractive index, saponification value, unsaponifiable matters, iodine value, melting point and soap content

(Hee *et al*., 2013). This organization has developed a grading system using the most important quality characters for Shea butter (Table 2.3).

Table 2.3: Quality characteristics and grades of unrefined Shea butter

	Grade Scale for Crude Shea Butter		
QUALITY PARAMETERS	Grade 1	Grade 2	Grade 3
Moisture content (%)	0 – 0.05	0.05 – 0.2	0.2 – 2.0
Free fatty acid (%)	0 – 1.00	1.0 – 3.0	3.0 – 8.0
Peroxide value (meq/kg)	0 – 10.00	10.0 – 15.0	15.0 – 50.0
Insoluble impurities (%)	0 – 0.09	0.09 – 0.2	0.2 – 2.0

Source: Lovett *et al*., 2005.

Based on these standards, cosmetic and pharmaceutical industries use the best quality unrefined Shea butter of Grade 1, and it can also be used for direct consumption. Food industry for manufacturing confectionary, chocolate, edible oil, and a basis for margarines can use the shea butter of Grade 2. The Shea butter of Grade 3 is recommended to be used in soap-making or further refined for direct consumption (Hee *et al*., 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 INTRODUCTION

This chapter discusses the materials and methods used in the collection and analysis of data to answer the research questions raised in this study.

3.2 THE STUDY AREA

Shea nut kernels were obtained from Tolon (Tolon District) in the Northern Region of Ghana. Tolon is on 156 m elevation at latitude 9.4333 N and longitude 1.0667 W. The Tolon district shares borders with North Gonja to the West, Kumbungu District to the North, Central Gonja to the south and to the East with Sagnerigu District. The Tolon District has a population of seventy-two thousand nine hundred and ninety (72, 990) people with 36,630 being female population representing 50.2% (PHC, 2010).

3.3 MATERIALS

3.3.1 Sources of Materials

The main raw material for this research was Shea nut fruits processed into Shea kernels. The Shea kernel is the product of the general pre-treatment of the Shea nut fruit. Pre-treated Shea kernels were obtained from Northern Region of Ghana (Tolon District). The Shea kernels were prepared at three different levels: **Raw Shea Kernels** (RASK), **Roasted Shea Kernels** (ROSK) and **Shea Kernel Paste** ((SKEP) (finely milled roasted

shea kernels)). They were packaged into separated plastic containers and transported to the laboratory for analyses.

3.3.2 Commercial Enzymes

Commercial Pectinase (E6287) from *Aspergillus aculeatus*, and a commercial Lipase (E0777) from *Thermomyces lanuginosus* obtained from Sigma-Aldrich (3050 Spruce Street, Saint Louis, MO 63103, USA) were used for enzymology of the Shea nut biomass. Cellulase from *Aspergillus niger* obtained from Novozymes (Denmark) was also used in the analysis. Their properties are shown in Table 3.1. All other reagents, chemicals and solvents used were of analytical grade (obtained from Sigma Chemical Co. St. Louis, MO).

Table 3.1 Characteristics of commercial enzymes used in this study

Enzyme	Source	Activity (U/g)	Optimum pH	Optimum Temperature (ºC)	Stability
Pectinase	*Aspergillus aculeatus*	5000	5.0 - 7.0	60	Stable at 2-8 °C
Cellulase	*Aspergillus niger*	1.02	4.5 - 5.0	50	Stable at 8-10 ºC
Lipase	*Thermomyces lanuginosus*	100000	6.5 - 7.5	60	Stable at 2-8 ºC

3.4 PREPARATION OF SAMPLES

The RASK and ROSK samples were finely ground and sieved (0.60-0.71 mm) prior to extraction. The SKEP sample obtained had dried up prior to usage and as a result was re-blended (using a commercial blender) to obtain the slurry paste form.

3.4.1 Extraction of Shea Butter

3.4.1.1 Traditional Hydrothermal Extraction

A preliminary study was carried out according to Tano-Debrah and Ohta (1995). A 4 x 3 factorial design consisting of 4 kneading time levels (0, 10, 20 and 30 minutes) against 3 boiling time levels: 5, 10 and 15 minutes were used to determine the optimized kneading and boiling times. The extraction at each kneading-boiling interaction was done in duplicates. The SKEP sample was used to carry out the preliminary study, and optimized conditions were further used for extraction of Shea butter from the RASK and ROSK samples.

Fifty grams (50 g) of the SKEP sample was weighed using an electronic weighing balance (Model: AS200) and used for extraction of Shea butter using the traditional hydrothermal extraction method in a laboratory setting. The weighed sample was put into a 250 ml beaker as warm water (40 ml, 30 ^{o}C) and cold water (60 ml, 18 ^{o}C) were intermittently added and then kneaded to form an emulsion. The total amount of water added was 100 ml to give a desired solid-to-liquid ratio of 1:2 resulting in workable emulsion phase.

The content of the beaker was treated with 100 ml of boiling water (100 ^{o}C) on a hot plate, boiled for 10 minutes with periodic stirring (total stirring time of about 4 minutes). The beaker with the contents was taken off the hot plate and allowed to cool overnight at room temperature. A whitish brownish mass was formed which was scooped out onto another beaker. It was washed four times with running tap water and heated again on a hot plate (50 ^{o}C) to obtain the crude butter. The oil was further heated for 1 hour at 100

^{o}C using a hot-air oven to expel any residual water. Shea butter extraction yield was then determined and presented as a mean of triplicates. The crude oil was further clarified using vacuum filtration. The clarified oil obtained was transferred into test-tubes with caps and stored in a refrigerator at 4 ^{o}C for further analyses.

3.4.1.2 Solvent Extraction

The RASK, ROSK and the SKEP were subjected to fat extraction using the solvent extraction method recommended by AOAC (1994). Two grams (2 g) each of the samples were placed in cellulose paper cone and extracted using *n*-hexane as solvent in a 5 L Soxlet extractor for 8 hours. The oil extracted was stored in the refrigerator at 4 ^{o}C for further analyses.

3.4.1.3 Enzyme-Assisted Hydrothermal Extraction (E.A.H.E)

Three different commercial enzymes preparations were used: Pectinase (E6287), Lipase (E0777) and Cellulase. A preliminary study was carried out using single enzyme treatment on the three different samples (RASK, ROSK and SKEP). A compromise between optimal conditions were used for enzyme mixes as seen in Table 3.2.

Table 3.2 Different enzyme combinations used on extraction conditions

Enzymatic Treatment	Temperature (°C)	pH
Pectinase	60	6.0
Cellulase	50	5.0
Lipase	70	6.0
Pectinase + Cellulase (1:1 by volume)	50	5.0
Pectinase + Lipase (1:1 by volume)	60	6.0
Cellulase + Lipase (1:1 by volume)	50	5.0
Pectinase + Cellulase + Lipase (1:1:1 by volume)	50	5.5

A set of accurately weighed 50 g meal samples were put into 600 ml conical flasks, combined with water, in a ratio of about 1:4 wt/vol. The content of the flasks were gently boiled (100 °C) for 5 minutes, and then cooled off to about 30 °C (Tano-Debrah and Ohta, 1995). The enzymes were included and thoroughly mixed. Aluminum foil (Everpack products, Ghana) was used to cover the flasks and then placed in a water bath shaker (Thermo Fisher Scientific manufacturer) and incubated at different temperatures (40 °C, 50 °C, 60 °C and 70 °C) and time (1hrs, 2hrs, 3hrs and 4hrs). Another set of samples were prepared similarly but without enzymes and incubated along-side the test samples for the same period of time to serve as control. After the treatment, the digests were transferred into 600 ml beakers and extracted using traditional hydrothermal extraction technique (Tano-Debrah and Ohta, 1995).

The hydrothermal (hot water floatation) extraction method described by Tano-Debrah and Ohta (1995). Hundred milliliters (100 ml) of hot water was added to the mixture and stirred vigorously before 100 ml cold water was added to cool the mixture to

about 30 °C – 40 °C. The mixture was left overnight to settle. Another beaker was used to put in the emulsion which formed the top layer. The emulsion was washed with running tap water and allowed to settle again. The clean emulsion was collected into a beaker and gently boiled (60 ^{o}C) for 5 minutes until clear oil was obtained. Oil obtained was subjected to drying in an air-oven at 100 °C for about 1 hour, the clarified oil which was then decanted into a weighed aluminum dish, cooled and weighed to estimate the extraction yield.

3.5 OPTIMIZATION OF PARAMETERS FOR ENZYME ASSISTED HYDROTHERMAL EXTRACTION

Temperature and pH conditions for the control were set at the optimum conditions each considered enzyme. The optimized conditions for extracting the Shea kernel paste (SKEP) was used for optimization of parameters and the optimized conditions were used to extract Shea butter from the other samples (RASK and ROSK).

3.5.1 Hydrolysis Time

Hydrolysis was done according to the methods of Otu *et al*. (2015). For each run, 50 g of the paste sample was mixed with 200 ml of distilled water to obtain the desired seed/water ratio of 1:4 in a 250 ml beaker and then stirred on a magnetic hot plate at 50 ^{o}C. Four different extraction times were used for the hydrolysis: 1, 2, 3 and 4 hours. Temperature was maintained at 50 ^{o}C for each period**.**

3.5.2 Enzyme concentration

The enzyme concentration was set at: 0.50, 1.00, 2.00, 3.00 and 4.00 % enzyme on seed weight basis and thoroughly mixed (Tano-Debrah and Ohta, 1995; Otu *et al*., 2015). The enzyme-substrate mixture was incubated in a water bath for a period of 1 hour at 50^{o}C. The effect of enzyme concentration was measured in terms of oil extraction yield.

3.5.3 pH

The effect of pH was studied at four different levels: 4, 5, 6 and 7. For each trial, paste sample was mixed with 200 mL of distilled water to get the desired w/vol ratio of 1:4. The temperature was maintained at 50 ^{o}C on a magnetic stirring hot plate and 1 hour hydrolysis time. Variations in pH were achieved by replacing the water used to mix the meal with buffered solutions (Tano-Debrah and Ohta, 1995). The solutions of different pH were prepared using phosphate buffer (British Pharmacopoeia, 2012).

3.5.4 Temperature

The effect of temperature during incubation was evaluated by using four different temperatures: 40 ^{o}C, 50 ^{o}C, 60 ^{o}C and 70 ^{o}C. The pH was adjusted to an optimum level for each enzyme (Table 3.1) and the sample was placed in water bath and extracted after 1 hour hydrolysis time.

3.6 EXTRACTION YIELD

The extraction yield was calculated according to mathematical model formulated by Adeeko and Ajibola (1989) and Olaniyan and Oje (2011). Extraction yield (Ey), is usually expressed as a percentage. It refers to the amount of oil that can be derived from an oil seed. According to Ajala *et al*. (2015), oil yield was determined as the ratio of the weight of oil recovered (Wor) to the weight of the crushed seed sample before extraction (Wcss).

$$Ey = \frac{Wor}{Wcss} \times 100\%$$

Where;

Ey = extraction yield %; ***Wor*** = weight of oil recovered; ***Wcss*** = weight of the crushed seed sample before extraction.

3.7 PHYSICO-CHEMICAL CHARACTERISTICS

3.7.1 Moisture Content %

Moisture content was determined according to Manual of Methods of Analysis of Foods: Oils and Fats (MOHFW) (2005), using a hot air oven. Five grammes (5.0 g) of sample was weighed and placed in a crucible which was then placed in a hot air oven at 105 ^{o}C. Drying was continued (16 hours) until a constant weight was reached and moisture content was estimated as follows:

$$MC\ \% = \frac{\textit{Weight of fresh Shea butter} - \textit{Weight of dried Shea butter}}{\textit{Weight of fresh Shea butter}} \times 100\%$$

3.7.2 Acid value

The acid value was determined according to British Pharmacopoeia (2012). It is the number of milligram (mg) of Potassium hydroxide (KOH) used to react with free acids in a given mass (g) of sample. Five grammes (5.0 g) of the extracted oil was weighed with 25 mL of alcohol added to it. After shaking gently, phenolphthalein solution (two drops) was added and the mixture was shaken vigorously and heated at 65 ^{o}C for 10 minutes to dissolve the oil. The mixture was then titrated against 0.1 M NaOH until a persistent pink colour was observed. The acid value is expressed as:

$$\boldsymbol{Acid\ Value = 5.610n/m}$$

Where; ***n*** = mL of standard sodium hydroxide used, ***m*** = Weight in g of the sample

3.7.3 Peroxide value

The peroxide value was determined according to British Pharmacopoeia (2012). Five grammes (5.0 g) of Shea oil was weighed and reacted with a saturated aqueous solution of potassium iodide. The iodine liberated by the peroxides was titrated with a standard solution of 0.1M sodium thiosulphate. The peroxide value (PV) was expressed as:

$$\boldsymbol{Peroxide\ Value\ (PV) = 10\left(\frac{n_1 - n_2}{W}\right)}$$

Where; $\boldsymbol{n_1}$ = Volume in ml of sodium thiosulphate required for the sample, $\boldsymbol{n_2}$ = Volume in ml of sodium thiosulphate acid required for the blank, $\boldsymbol{W}$ = Weight in grammes of the oil/fat taken for the test.

3.7.4 Saponification value

Saponification value is expressed by potassium hydroxide in mg used to neutralize one gram of sample (Shahidi, 2005). Two grammes (2.0 g) of Shea oil was added into a 200 ml flask, 25.0 mL of the ethanolic solution of potassium hydroxide was added and the mixture was boiled (78 ^{o}C) under a reflux condenser for 1 hour, rotating the contents frequently. Phenolphthalein solution (two drops) was added after the mixture was removed from the heat source and it was titrated with 0.5 M hydrochloric acid until a pink colour was observed. A blank test (without sample) was done 3 times to obtain mean value of titration volume of 0.5 M hydrochloric acid (British Pharmacopoeia, 2012).

$$\boldsymbol{Saponification\ Value = 28.05\ v/w}$$

Where; $\boldsymbol{v}$ = the difference, in ml, between the titrations,

$\boldsymbol{w}$ = the weight, in g, of sample

3.7.5 Iodine value (I_I)

The iodine value I_I is "the number that expresses in grams the quantity of halogen, calculated as iodine that can be fixed in the prescribed conditions by 100 g of the substance" (British Pharmacopoeia, 2012). The iodine value was determined according to British Pharmacopoeia (2012). About 0.25 g of stored shea butter oil was reacted with iodine bromide and the excess iodine liberated, titrated with standard 0.1M sodium thiosulphate. A blank titration was also performed (British Pharmacopoeia, 2012). The iodine value was expressed as milligrams of iodine to completely saturate all the double

bonds in the shea butter using the formula below:

$$\boldsymbol{Iodine\ Value = 1.69\left(\frac{V}{W}\right)}$$

Where; $\boldsymbol{V}$ = difference in volume between the blank and the sample titre, $\boldsymbol{W}$ = weight of the sample used.

3.8 STATISTICAL ANALYSIS

Values are reported as mean ± SD analyzed individually in triplicate (unless otherwise stated). Mean values followed by different superscript letters are significantly different ($P < 0.05$). Analysis of Variance (ANOVA) was used to determine significant differences between groups, considering a level of significance of less than 5% ($P < 0.05$) by using the statistical software IBM SPSS STATISTICS 20 (2016). Graphs are drawn using Microsoft Word 2013.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 INTRODUCTION

The factors which affected Shea oil yield during enzymatic extraction in this study are as follows: pH, enzyme type, enzyme concentration, moisture content, temperature, and incubation time. The effects of these parameters varied according to the differences in the pre-treatment levels of the various substrates. The quantity of the oil produced with enzymes was evaluated, compared with the quantity of oil generated by the traditional and solvent extraction methods.

4.2 MOISTURE CONTENT % OF SAMPLES PRIOR TO HYDROTHERMAL EXTRACTION

Although the three samples showed relatively low moisture contents (Figure 4.1), the raw Shea kernels (ROSK) sample contained the lowest 5.71% (Grade A), roasted Shea kernels (RASK) sample contained 7.22% (Grade B) moisture content and the highest was observed for Shea kernel paste (SKEP) sample, 10.32% (Grade C), according to the grading quality standards of Global Shea Alliance (GSA).

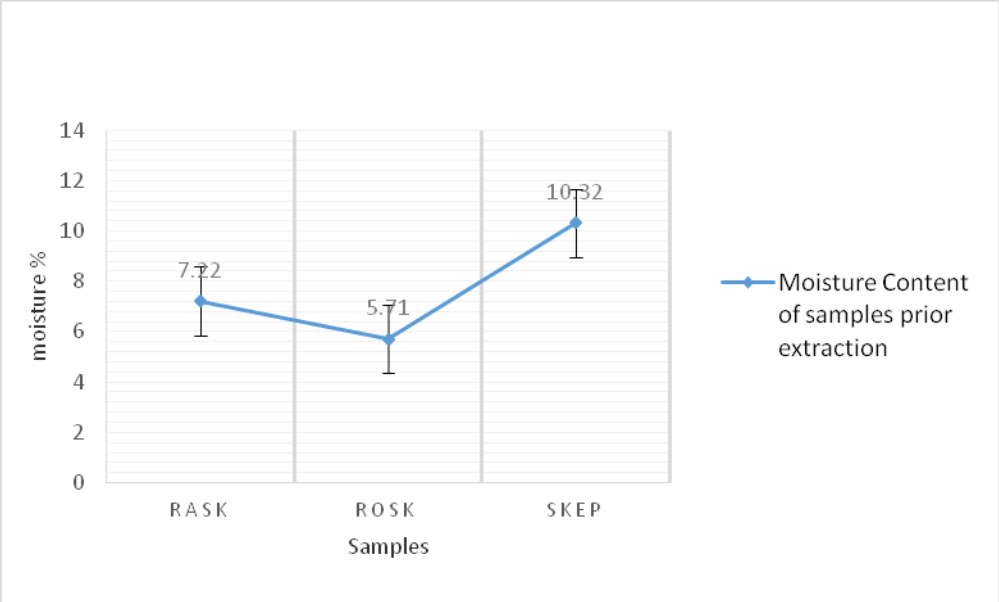


Figure 4.1: Percentage Moisture Content of Pre-treated Shea Samples Prior to Shea butter Extraction

Pre-treatment levels of the SKEP sample involved addition of water to obtain the slurry form of the paste and this attributes to the high amount of moisture content observed from the SKEP sample. According to quality standards by Global Shea Alliance (GSA) (2013), moisture content for Grade A shall be less than 8%, between 8% and 10% for Grade B, and for Grade C, it shall be more than 8%. The traditional floor drying after 14 days of sun-drying produced moisture content of values ranging from 7 TO 7.5% (Aculey et al., 2012) and this is consistent with the moisture content of the raw shea kernels (7.22%) originally processed by the traditional sun-drying method. Further roasting/heating would allow more dehydration of the kernel and so it is not surprising that moisture content of the roasted kernels plunged down to 5.71%. The phenomenon of the increasing moisture content of roasted kernels turned-out into paste (10.32%) could

be attributed to the formation of triacylglycerol (triglycerides) from glycerol and three separate fatty acid chains by condensation during the grinding process. The process of grinding the shea kernel seems to go through three stages chemically: separation of atoms/molecules (bond breaking), rearrangement of atoms/molecules and recombination from which the triglycerides (fat/butter) were formed.

4.3 EFFECT OF KNEADING TIME AND BOILING TIME ON SHEA BUTTER RECOVERY USING SHEA KERNEL PASTE (SKEP)

Results indicated in Table 4.1 showed statistically significantly different at ($P < 0.05$) among kneading, boiling and its interaction. Boiling (100 ^{o}C) time of 10 minutes and kneading time of thirty (30) minutes gave the highest oil recovery yield of 42.96% using SKEP sample. Subsequent extractions were carried out on the RASK and ROSK samples using these standardized times. A study by Apea and Larbi (2013), on Shea butter extraction noted that kneading of Shea nut paste needed 37.4 minutes of time for adequate butter yield (average oil of 493.4 ml/1kg) and thus higher oil recovery.

Table 4.1: Effect of kneading time and boiling time on Shea butter yield using Shea kernel paste (SKEP)

	Kneading time 0 mins	Kneading time 10 mins	Kneading time 20 mins	Kneading time 30 mins
Boiling time 5 mins	10.88±0.61%	25.52±0.56%	37.76±0.10%	38.39±0.84%
Boiling time 10 mins	15.50±0.98%	27.83±0.18%	41.39±0.38%	42.96±0.73%
Boiling time 15 mins	19.07±0.20%	28.89±0.05 %	39.13±0.11%	39.39±0.12%

Values are means ± SD (n = 2)

4.4 EXTRACTION YIELD OF SHEA KERNELS BY TRADITIONAL HYDROTHERMAL EXTRACTION METHOD

The Shea nut kernel contains about 52% oil (Adomako, 1985) although the Netherlands Development Organization (SNV) (2006), gives an estimation of 60% fat. In Table 4.2 below, the highest butter yield using the hydrothermal extraction procedure was 43.65±0.43 % (w/w) from the Shea kernel paste on a dry-weight basis. Oil recovery from RASK by the hydrothermal procedure was lowest (28.47±1.66%) as compared to the ROSK (30.27±0.1%). The SNV (2006), gives the range of extraction rate of about 20% - 31% using the traditional hydrothermal method which is consistent with the rate of extraction (30.27±0.1%) by this research. Thus oil recovery was directly proportional to the particle size of the samples. Apea and Larbi (2013), extracted 0.2513 ml/g from unroasted kernels and 0.3667 ml/g from roasted kernels employing the mechanical press method reporting higher butter yield from roasted kernels. Another study by Ajayi (2013), also showed that roasted Shea kernels gave a yield value of 33.4% while unroasted Shea kernels gave 2.8% yield by the use of electric food processor for kneading.

Table 4.2: **Traditional Hydrothermal Extraction of Pre-treated shea kernels**

	Extraction Yield %
Samples	**Traditional Hydrothermal Extraction**
RASK	28.47 ± 1.66^{a}
ROSK	30.27 ± 0.10^{b}
SKEP	43.65 ± 0.43^{c}

Values are means ± SD (n = 3). Results not connected by same letter in each row are significantly different (P < 0.05). RASK- Raw Shea Kernels, ROSK- Roasted Shea Kernels, SKEP- Shea Kernel Paste.

Following physical examination of the various Shea nut biomasses between the fingers, the SKEP had the highest finely grounded particles relative to the ROSK and RASK respectively. The RASK had the least fine particles and from which the least butter was recovered and thus oil recovery was directly proportional to the particle size of the samples. But Olaniyan and Oje (2007), explained that roasted kennels gave higher butter as a result of the crystalline structures of fats which loosens up for extraction after roasting. Further research by Olaniyan (2010), shows that oil yield of a given sample at any given pressure (at constant pressure) employing the mechanical rig is dependent on the moisture content of that sample. On the contrary a study by Obeng *et al*. (2010), using a low-pressure (45 kg/cm^2) manual screw press employing Intermediate Moisture Content Method (IMC), revealed that roasted kernels (65.9% extraction efficiency) do not give higher butter yield than raw kernels (68.5% extraction efficiency).

Although the crystalline structures of fats loosens up for extraction after roasting (Olaniyan and Oje, 2013), it does still require a larger surface area for effective interaction with the solvent. This possibly explains lower oil yield which was obtained from the ROSK (53.48%) which had lower surface area as compared to a higher yield (57.57%) from the SKEP. The SKEP was finely pulverized and exhibited the highest surface area and therefore could be the reason why it gave the best yield compared to the RASK and ROSK which were hand-ground.

4.5 SHEA BUTTER RECOVERY USING SOLVENT (*N*-HEXANE) EXTRACTION METHOD

Solvent extraction of shea butter oil from the various shea nut meals in general was higher in relation to the traditional extraction. Figure 4.2 shows oil recovery values from RASK, ROSK and SKEP samples, 52.31%, 53.48% and 57.57%, respectively by Solvent Extraction Method. Low oil yield was obtained from both the RASK (52.31%) and ROSK (53.48%) samples as compared to SKEP sample. Statistically significant difference ($P < 0.05$) exists among samples. The high oil recovery value observed for SKEP could be attributed to its high surface area when compared to the other samples.

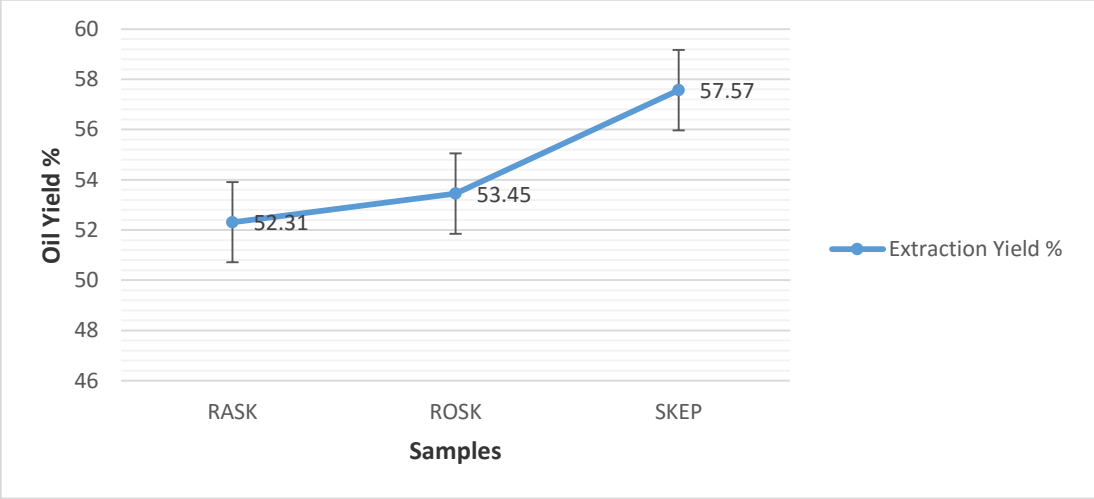


Figure 4.2: Oil recovered using solvent (n-hexane) extraction method.

RASK- Raw Shea Kernels, ROSK- Roasted Shea Kernels, SKEP- Shea Kernel Paste.

Hexane, the solvent in the case of research, is of low polarity. Esters are of low polarity; hexane was able to dissolve all the esters present in the shea nut biomass which gave rise to high oil recovery (Apea and Larbi, 2013). The precision and efficiency with the

solvent extraction method is remarkable but it is overshadowed with the believe that the product resulting is unwholesome for consumption due to presence of traces of solvent that may be retained in oils extracted (Mbaiguinam *et al*., 2007).

4.6 EFFECT OF HYDROLYSIS TIME ON ENZYME-ASSISTED HYDROTHERMAL EXTRACTION USING SHEA KERNEL PASTE (SKEP)

The results on the effects of hydrolysis time on enzyme assisted hydrothermal extraction (E.A.H.E) are shown in Figure 4.3. The maximum oil yield for control, pectinase and lipase was observed at 2.0 hours hydrolysis time and cellulase at 3.0 hours hydrolysis time. Lipase and pectinase had an appreciable effect within two hours but decreased beyond the 2 hour mark except cellulose that appreciated beyond the 2 hour period. However, above the critical value of hydrolysis time, a decrease of reaction rate can result as intermediate products may accumulate (Huyanh *et al*., 2013).

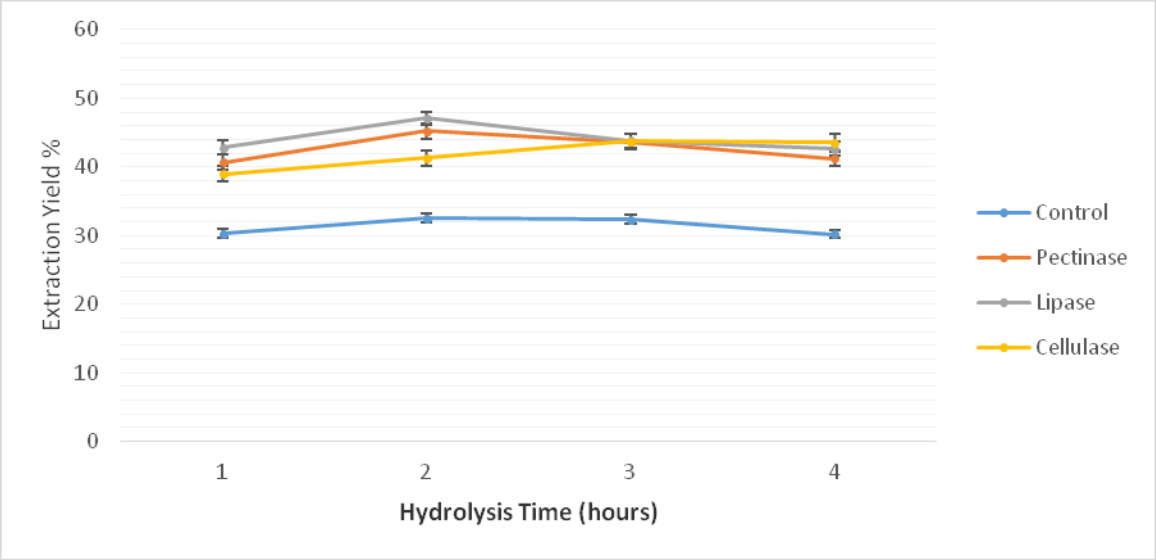


Figure 4.3: Effect of Hydrolysis time on Shea Butter Extraction Yield

It has been investigated that more stable emulsions are formed in a prolonged extraction process (Rosenthal *et al*., 1996). Otu *et al*. (2015), reported maximum oil yield at 60 minutes for control and Viscozyme, and that of crude Pectinase and commercial Pectinase at 90 minutes. Kim (1989), reported 30 minutes period as an optimum time for palm oil extraction, and 40 minutes period for extracting both soybean oil and protein (Lusas *et al*., 1982). A study by Abdulkarim *et al*. (2006), also showed an increase in the oil recovery with an increase in the hydrolysis time from 0 – 2 hours, after which the rate slowed down. Similarly, Dominguez *et al*. (1994), also reported 0.33 – 2.00 hours as being sufficient to result in a significant oil recovery increase.

4.7 ENZYME CONCENTRATION EFFECT ON SHEA BUTTER RECOVERY

Figure 4.4 shows the effect on extraction yield from Shea kernel paste (SKEP) sample. Lipase concentration was optimized at 3.0 % while optimized enzyme concentration was 4.0 % for both Pectinase and Cellulase. It has been observed that the oil yield increased with enzyme concentration, but not continuously. According to a study by Huyanh *et al*. (2013), enzyme concentration had a strong influence on the reaction yield. Firstly, as the enzyme concentration increases, oil extraction yield increased. Continual higher enzyme concentration led to a reduction of extraction yield. This could be as a result of the enzyme proteins combining with the oil, thereby producing a stable emulsion that inactivated the enzyme and as a consequence inhibited the liberation of oil (Huyanh *et al*., 2013).

Fullbrook (1983) reported increment in melon seed oil recovery over 100% with respect to control samples during aqueous extraction. It is known that an increase in the enzyme concentration increases the rate at which the oil is separated, but the optimum level must be established (Dominguez *et al*., 1994). Tano-Debrah and Ohta (1995), observed a rapid increase in extracting Shea fat at enzyme concentration (0.0 - 1.0%). Similarly, Abdulkarim *et al*. (2006), used 0.5 - 4% (w/w) enzyme (Celluclast + Pectinex) concentration to extract sunflower oil and found that a 2% (w/w) enzyme concentration was most favourable. Abdulkarim *et al*. (2006), reported high oil recovery with 2.0% (v/w) enzyme using *M. oleifera* seeds and no increase in the oil yields were observed on further increase in enzyme concentration to 2.5% (v/w).

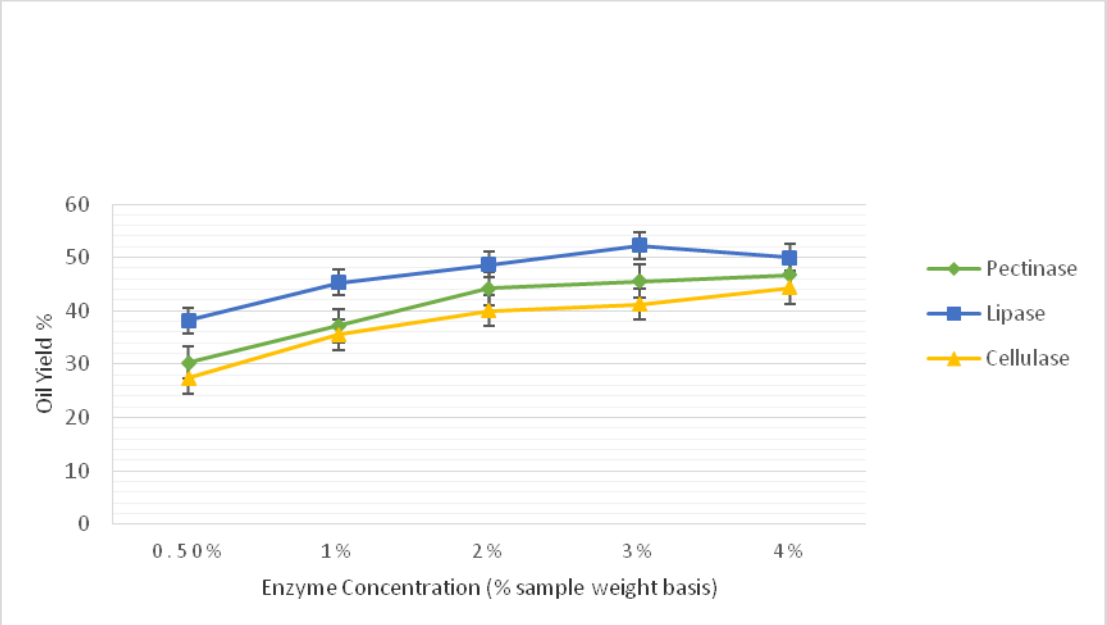


Figure 4.4: Effect of enzyme concentration on oil recovery.

4.8 EFFECT OF pH ON SHEA BUTTER YIELD

Figure 4.5 shows the effect of pH of the extraction medium on oil extraction yield during enzyme assisted hydrothermal extraction. The pH range of 5 - 7 was found to be appropriate in the present study as maximum oil was extracted. Lipase exhibited maximum yield at pH 7.0, cellulase showed maximum yield at pH 5.0, whereas pH of 6.0 was found to be most appropriate for pectinase. According to Rhee *et al*. (1972), deterioration of oil quality through saponification can result from higher pH and cause several changes to the amino acids, such as, the formation of lysinoalanine, lanthionine (De Groot and Slump, 1969).

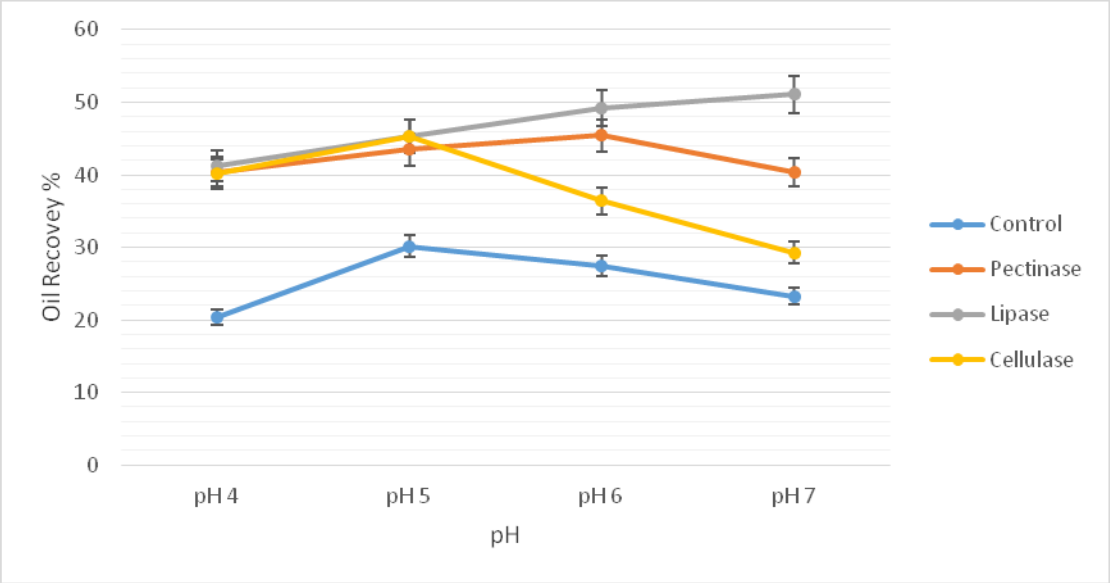


Figure 4.5: Effect of pH on Shea butter yield

4.9 EFFECT OF TEMPERATURE ON EXTRACTION YIELD

The effect of temperature on oil yield during enzyme assisted hydrothermal extraction is shown in Figure 4.6. The oil yield increased significantly as the temperature was raised from 40 ^{o}C – 50 ^{o}C. Similarly, aqueous extraction of palm kernel at extraction temperature of 45 ^{o}C and pH of 7 was reported by Seung (1989). Results also showed a decrease at higher temperature except for the Lipase treated sample.

At temperature of 70 ^{o}C, lipase yielded 54.76% oil. Pectinase offered maximum oil recovery at 60 ^{o}C with a value of 48.21% whereas, cellulase exhibiting low yielding value of 44.55% at 50 ^{o}C. No enhancement in the oil yield was observed above 50 ^{o}C for the cellulase. According to a study by Huyanh *et al*. (2013), the enzymatic reaction rate increased with increase in temperature. However, enzyme activity was inactivated at

higher temperature and as such a temperature range of 50 ^{o}C - 70 ^{o}C was deemed to be satisfactory for Shea butter extraction in this study.

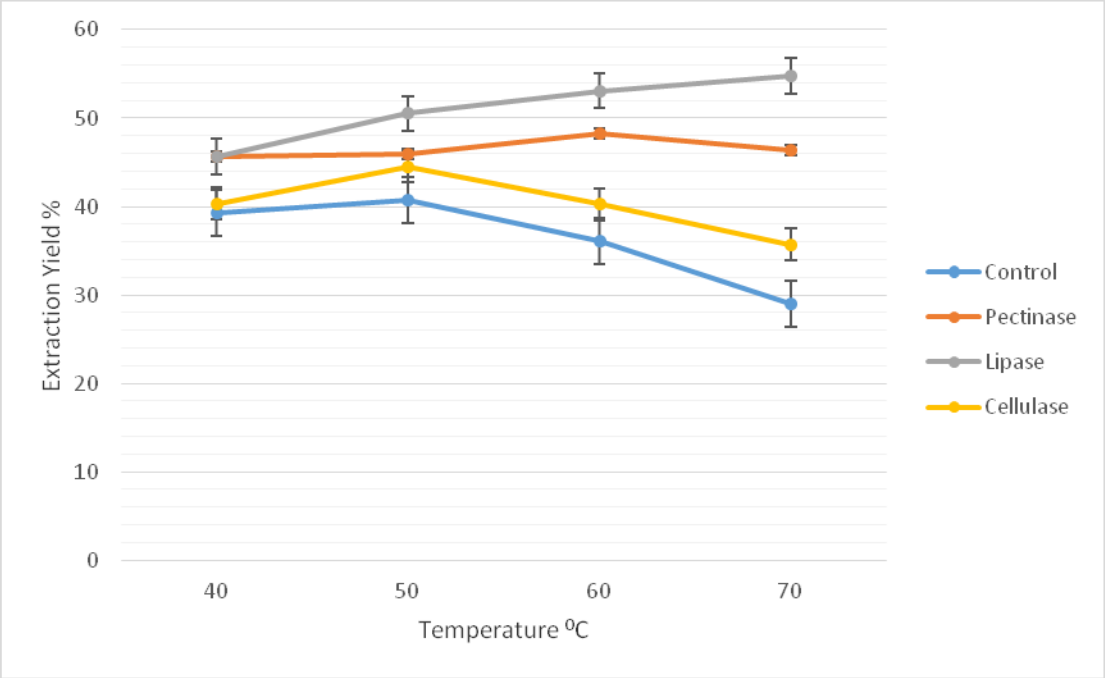


Figure 4.6: Effect of temperature on extraction yield

Lusas *et al*. (1982), reported that the temperature is critical for oil extraction from soybeans during aqueous extraction process and they observed maximum oil recovery between 40 ^{o}C – 60 °C. Hagenmaier (1974), extracted sunflower oil at room temperature. A temperature range of 60 ^{o}C - 65 °C was selected for the extraction of peanut oil (Subrahmanyan *et al*., 1959; Rhea, 1972) and 70 °C for the extraction of rapeseed oil (Embong and Jelen, 1977) whereas 80 °C was maintained during coconut oil extraction (Hagenmaier *et al*., 1972). Aparna *et al*. (2002), used temperature of 37 ^{o}C, 40 ^{o}C, 50 ^{o}C and 60 ^{o}C for enzyme-aided extraction of peanut oil. They observed that a temperature of

40 ^{o}C was the best because of the high oil recovery. Barrios *et al*. (1990), also used temperature of 40 ^{o}C, 50 ^{o}C, 55 ^{o}C and 60 ^{o}C for coconut oil extraction and reported the highest oil recovery at 50 ^{o}C.

4.10 SHEA BUTTER RECOVERY AFTER OPTIMIZATION OF ALL PARAMETERS

The results of the effects of different enzymes on extraction yield are presented in Figure 4.7, showing that a mixture of the three types of enzymes at the ratio of 1:1:1, by volume, has the strongest effect on the Shea butter extraction than individual enzymes or mixtures of two. Increases of about 30 - 50% in enzyme extracted oils with reference to the controls were observed. Enzyme-enhanced aqueous extraction of Shea butter ranged from 22.61% - 69.96% butter extracted. Following the single enzyme treatments, Lipase gave the highest yield followed by Pectinase and then Cellulase. Lipase and pectinase in this study were found to be the best two enzyme mixtures for the raw, roasted and paste samples producing 46.02%, 47.79% and 55.67% respectively and combination of all three enzymes gave highest oil recovery (69.96%). Statistical analysis showed that all the three samples significantly affected extraction yield ($p < 0.05$).

The results from the this study are similar to previous reports showing that a mixture of enzymes in an equal proportion gives the highest yield of oil extraction from rapeseed (Olsen, 1988), sunflower-kernel oil (Dominguez *et al*., 1995), peanut oil (Lanzani *et al*., 1975), palm oil (Cheah *et al*., 1990), Shea fat (Tano-Debrah and Ohta, 1995), rice bran (Hernandez *et al*., 2000), *Moringa oleifera* seeds (Abdulkarim, 2006)

and avocado (Phan, 2008). A mixture of enzymes easily breaks down the cell wall of oilseeds and liberates oil than a single enzyme (Huyanh *et al*., 2013).

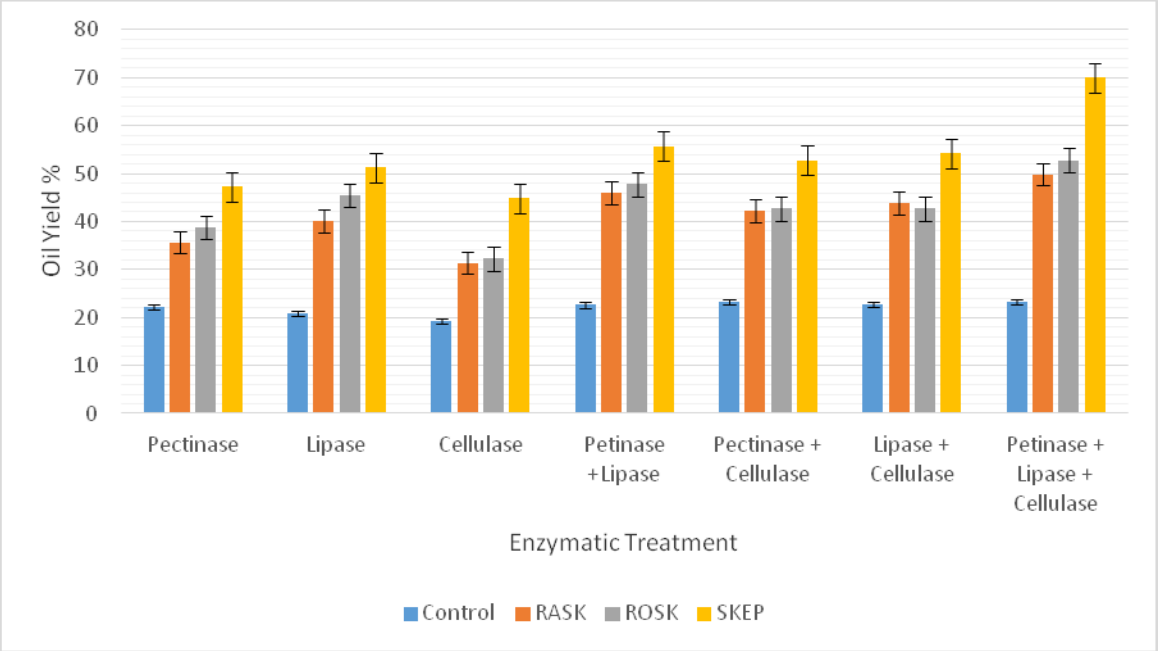


Figure 4.7 Oil recovered after optimization of all parameters.

4.11 PHYSICO-CHEMICAL CHARACTERISTICS OF EXTRACTED OILS

Physico-chemical properties such as moisture content, acid value, free fatty acids, peroxide value, saponification value and iodine value of shea butter oils were carried out according to British Pharmacopoeia (2012). Physico-chemical properties of the traditional hydrothermal extraction, solvent-extraction, and E.A.H.E oils are presented in Tables 4. Where significant differences were observed as a result of ANOVA at $P < 0.05$, further comparisons was conducted using Post-Hoc Tukeys Multiple Comparison Test at $P < 0.05$.

Table 4.3: Physico-chemical properties of extracted Shea butter from the traditional hydrothermal extraction method

Parameters	RASK	ROSK	SKEP
Moisture content %	0.20 ± 0.01^{a}	$\mathbf{0.}17\pm0.01^{b}$	0.15 ± 0.01^{c}
Acid value (mg KOH/g)	3.66 ± 0.50^{a}	4.19 ± 0.42^{b}	4.17 ± 0.35^{c}
Free Fatty Acids (mg KOH/)	1.85 ± 0.21^{a}	2.09 ± 0.21^{b}	2.09 ± 0.14^{c}
Peroxide value (mEq/g)	4.86 ± 0.64^{a}	5.12 ± 0.21^{a}	5.15 ± 0.14^{a}
Saponification value (mg KOH/g)	182.85 ± 0.12^{b}	183.25 ± 0.13^{b}	183.22 ± 0.57^{b}
Iodine value (mg I_2/g)	49.53 ± 0.16^{c}	47.62 ± 0.70^{c}	47.85 ± 0.86^{c}

Values are means ± SD (n = 2). Results not connected by same letter in each row are significantly different (P < 0.05). RASK- Raw Shea Kernels, ROSK- Roasted Shea Kernels, SKEP- Shea Kernel Paste.

Table 4.4: Chemical properties of extracted Shea butter from solvent extraction method

Parameters	RASK	ROSK	SKEP
Acid value (mg KOH/g)	7.70 ± 0.40^{a}	8.26 ± 0.23^{a}	8.12 ± 0.50^{a}
Free Fatty Acid (mg KOH/g)	3.85 ± 0.20^{b}	4.13 ± 0.11^{b}	4.07 ± 0.21^{b}
Peroxide value (mEq/g)	9.95 ± 0.14^{a}	11.39 ± 0.92^{b}	11.46 ± 0.64^{c}
Saponification value (mg KOH/g)	184.59 ± 0.69^{c}	184.42 ± 0.50^{c}	184.28 ± 0.85^{c}
Iodine value (mgI_2/g)	58.16 ± 0.13^{d}	58.48 ± 0.85^{d}	55.59 ± 0.21^{d}

Values are means ± SD (n = 2). Results not connected by same letter in each row are significantly different (P < 0.05). RASK- Raw Shea Kernels, ROSK- Roasted Shea Kernels, SKEP- Shea Kernel Paste.

Table 4.5: Physico-chemical properties of extracted Shea butter from the Enzyme Assisted Hydrothermal Extraction (Pectinase + Lipase + Cellulase).

Parameters	RASK	ROSK	SKEP
Moisture content %	0.18 ± 0.01^{a}	0.17 ± 0.01^{b}	0.17 ± 0.01^{b}
Acid value (mg KOH/g)	3.21 ± 0.99^{a}	4.20 ± 0.78^{b}	5.41 ± 0.27^{c}
Free Fatty Acid (mg KOH/g)	1.60 ± 0.50^{a}	2.10 ± 0.42^{b}	2.71 ± 0.13^{c}
Peroxide value (mEq/g)	3.67 ± 0.57^{a}	5.34 ± 0.15^{b}	5.39 ± 0.20^{c}
Saponification value (mg KOH/g)	183.16 ± 0.40^{b}	181.06 ± 0.52^{b}	180.41 ± 0.54^{b}
Iodine value (mgI_2/g)	49.53 ± 0.16^{c}	47.64 ± 0.68^{c}	48.18 ± 0.65^{c}

Values are means ± SD (n = 2). Results not connected by same letter in each row are significantly different ($P < 0.05$). RASK- Raw Shea Kernels, ROSK- Roasted Shea Kernels, SKEP- Shea Kernel Paste.

4.11.1 Moisture content

Moisture content of Shea oils obtained from enzyme-assisted hydrothermal extraction was higher than values obtained from traditional hydrothermal extraction (Table 4.3 and Table 4.3). There was statistically significant difference ($P < 0.05$) among RASK, ROSK and SKEP samples for both extraction methods (See Appendix D2 and D4). Shelf life property of a food sample is indicated by its moisture content. By the application of enzyme assisted hydrothermal extraction method, the raw Shea kernels (RASK) with the highest percentage moisture content value (0.18±0.01) indicates shorter shelf life while samples roasted Shea kernels (ROSK) (0.17±0.01) and Shea kernel paste (SKEP) (0.17±0.01) indicates longer shelf life.

Comparing these values to the Ghana standards for unrefined Shea butter (Ghana Standard, 2006) and those of the Union Economique Monetaire Quest Africaine (UEMOA) (Lovett *et al*., 2005), it was found that all the butter samples fell within the grades for useful Shea butter. According to both standards, the samples are classified with average percentage moisture content since the observed moisture contents were either within narrow limits (0.177 ± 0.01%) to or above the lower limit of 0.2 for average moisture as indicated by the standards. None of the samples had moisture level in excess of the maximum values of both standards (Ghana Standard, 2006; Lovett *et al*., 2005). The standards therefore indicated that all the samples were of the third grade and thus met the requirement of soap making industries and also be refined for direct consumption. It can be concluded that oil recovery increases with decreasing moisture content as was recorded in studies by Olaniyan (2010) and Otu *et al.* (2015).

4.11.2 Acid Value and Free Fatty Acids Value

The sustainability for industrial use and edibility of oil is greatly indicated by the acid value. A low acid value (AV) is preferred because the exposure of an oil to rancidification is reduced as it contains less free fatty acids (Roger *et al.,* 2010). The lowest acid value of Shea butter reported was 0 mg KOH/g (Honfo *et al*., 2013) and 21.2 mg KOH/g was the highest (Nkouam *et al*., 2007). From the present study, acid value and free fatty acids value obtained from the traditional hydrothermal extraction method among samples: RASK, ROSK and SKEP were significantly different at $p < 0.05$ ($p = 0.02$). The results of solvent extraction method were 7.70 mg KOH/g , 8.26 mg KOH/g

and 8.12 mg KOH/g for RASK, ROSK and SKEP respectively and are considered high because they are above the 1% threshold reported by Kyari (2008).

Values from enzyme assisted hydrothermal extraction method were 3.21 mg KOH/g , 4.20 mg KOH/g and 5.14 mg KOH/g for RASK, ROSK and SKEP respectively using a combination of the three enzymes in equal volumes. The quality of Shea butter samples analysed from traditional hydrothermal and enzyme assisted hydrothermal extraction methods were of second grade (1.1 - 3.0), while that of solvent extracted Shea butters were third grade quality (3.1 - 8) with respect to free fatty acids (Ghana Standard, 2006; Lovett *et al*., 2005).

4.11.3 Peroxide Value

In fats and oil, high peroxide value (>10 meq kg^{-1}) is generally associated with the development of rancidity, and it is the most typical determinant of lipid oxidation which gradually limits their use in the food market. It should, therefore, be supervised or managed (Shahidi, 2005). The peroxide values obtained by enzyme assisted hydrothermal extraction method were most acceptable among sample groups (RASK, ROSK and SKEP) even though the other two methods (traditional hydrothermal method and solvent extraction method) possess acceptable peroxide values. Solvent extracted butter using *n*-hexane showed high value (9.95 - 11.46 mEq/g) than reported by Francis (2009) and Okullo *et al*., (2010), where both used *n*-hexane and found 2.2 mEq/g and 2.5 mEq/g respectively. This might be due to long extraction time (8 hours) used for extraction.

Comparing results obtained from the present study to the available standards, all samples from using traditional hydrothermal extraction method and enzyme assisted hydrothermal extraction method were found to be high quality as the level of peroxides obtained were below 10.0, categorizing the samples as first grade (Ghana Standard, 2006; Lovett *et al*., 2005). This indicates suitability extracted Shea butter for the pharmaceutical and cosmetics industries and also for direct consumption. The findings from the enzyme assisted hydrothermal extraction, RASK (3.67 mEq/g) was much similar with the value (3.7 mEq/g) report by Yonas (2014), using screw expeller and by Francis (2009), using mechanical cold pressing (3.55 mEq/g). Likewise, extracted butter from RASK (enzyme-assisted method) showed the lowest peroxide value at 3.67 mEq/g indicating the better quality of extracted Shea butter compared to the roasted Shea kernels (ROSK) and Shea nut paste (SKEP). According to Divine *et al*. (2011), pre-treatments performed on Shea nut products had an important influence on the quality of oil produced, proteins material, triglycerides, and some minimal elements of Shea butter.

4.11.4 Saponification value

Various studies reported saponification values in the range between 132 mg KOH/g (Ezema and Ogujiofor, 1992) and 261.3 mg KOH/g (Olaniyan and Oje, 2007), the latter was extracted through dry extraction process using mechanical expression rig. The average saponification value of Shea butter is reported as 180.9 mg KOH/g (Honfo *et al*., 2013). The butters obtained from enzyme assisted hydrothermal extraction and traditional hydrothermal method in this study showed low saponification values

compared to Shea butter extracted using solvent (*n*-hexane) extraction method. According to William and Odom (2015), a low saponification value could suggest non-suitability of oil sample for industrial use as it indicates adulteration.

Using Analysis of Variance (One-Way ANOVA), findings among the three sample groups showed no statistically significant difference ($p > 0.05$) for both the traditional hydrothermal method ($p = 0.059$) and solvent extraction method ($p = 0.753$). The Enzyme assisted hydrothermal extraction method showed statistically significant difference ($p < 0.05$) indicating that different saponification values would be obtained by the three different pre-treatment stages (RASK = 181.16 mg KOH/g, ROSK = 181.06 mg KOH/g, and SKEP = 180.41 mg KOH/g). Results obtained from the three different extraction methods especially the enzyme-assisted hydrothermal method shows comparable advantage (ability to saponify) with other oil bearing seeds.

4.11.5 Iodine Value

The low iodine indices (<100 I_2/100g) from this study categorizes the extracted Shea butter oil as a non-drying oil according to Joseph *et al*., 2014. The higher the amount of unsaturation, the more iodine is absorbed and the higher the iodine value (Gunstone, 2004; Nielsen, 2010). So far, the lowest reported value was 21.7 g I_2/100g, that was found in butter extracted by supercritical CO_2 (Nkouam *et al*., 2007), and the highest value was 89.5 g I_2/100 g (Honfo *et al*., 2013). In this study, butters obtained by traditional method (47.67 - 49.53 I_2/100 g) and enzyme-assisted hydrothermal extraction (47.64 – 49.53 I_2/100g) were higher in value than 55.59 – 58.48 I_2/100 g, which was

obtained by solvent extraction. The findings from solvent extraction was higher than reported by Okullo *et al*., (2010) (39.21 – 41.37 g I_2/100 g) and Francis (2009) (36.60 g I_2/100 g), where both authors used n-hexane for solvent extraction. Values from all three pre-treated levels of samples and methods of extraction used in this study were higher than 38.73 g I_2/100 g, which was obtained by mechanical cold pressing (Francis, 2009).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSIONS

Based on the findings of this study, it can be concluded that grounded raw Shea kernels yielded an appreciable amount of Shea butter regardless of extraction methods used contrary to popular beliefs that suggest unroasted Shea kernels yielded no or very little butter yield. In an evaluation of the different levels of pre-treated samples, significant differences were not necessarily seen in all the cases. The roasted Shea kernels and Shea kernel paste produced high butter yield compared to the raw Shea kernel samples, thereby implying that a roasting step in the extraction of Shea butter may be considered necessary for higher butter yield. However, to obtain butter of better quality, roasting step can be eliminated which results in Shea butter with low peroxide values.

The process for enzyme mixtures was optimized at pH of 6, 3% enzyme-substrate concentration, 2 hour hydrolysis time at a temperature of 60 ºC. The highest extraction yield of 69.96% was recovered from 1:1:1 combination of all three industrial enzymes. Thus enzyme assisted hydrothermal hydrolysis of Shea nut biomass for Shea butter production is a promising technology capable of eliminating the drudgery with the mechanical extraction, the safety concerns associated with the chemical methods and the arduous and cumbersome nature of the traditional village extraction methods. This makes the technology an environment-friendly alternative to conventional hexane oil extraction.

5.2 RECOMMENDATIONS

1. One major drawback in the enzyme-assisted hydrothermal extraction process is the cost of enzymes. To make this process financially practical, further work is suggested to minimize cost with applications of modified mixes of crude enzymes.
2. Further study should also be done using raw (unroasted) Shea kernels to have a detailed evaluation of its nutritional composition and physicochemical properties.

REFERENCES

Abdulkarim, S. M., Lali, O. M., Muhammed, S. K., Long, K. and Ghazali, H. M. (2005). Some physico-chemical properties of *Moringa oleifera* seed oil extracted using solvent and aqueous enzymatic methods. *Food Chemistry*, **93***:* 253-263

Abdulkarim, S. M., Lali, O. M., Muhammed, S. K., Long, K. and Ghazali, H. M. (2006). Use of enzymes to enhance oil recovery during aqueous extraction of *Moringa oleifera* seed oil. *Journal of Food Lipids*, **13***:* 113-130.

Abdul-Mumeen, I., Zakpaa, H. D., Mills-Robertson, F. C. (2013). Biochemical and microbiological analysis of shea nut cake: A waste product from shea butter processing. *Journal of Agricultural Biotechnology and Sustainable Development*, **5**(4): 61-68.

Aboyella, C. (2002). "An economic analysis of shea nuts and cashew production in the Bawku East District". A Bsc. Dissertation submitted to the Department of Economics & Agribusiness, University of Ghana.

Addaquaye, J. (2004). Shea butter Value Chain: Refining in West Africa, WATH Technical Report No. 3

Adeeko, K. A and Ajibola, O. O (1989). Processing factors affecting yield and quality of mechanically expressed groundnut oil. *Journal of Agricultural Engineering Research*, **5**: 31-43.

Ademola, A. O., Oyesola, O. B. and Osewa, S. O. (2012). Assessment of Shea butter processing among rural dwellers in Atisbo Local Government Area of Oyo, Nigeria. *European Journal of Business and social Sciences*, **1** (7): 1-08.

Adomako, D. (1985). Prospects for the development of the shea nut industry in Ghana. Cocoa Research Institute., Ghana, *Technical Bulletin*, **11:** 8 - 10.

Agyente-Badu, K. C. (2010). The effect of *Cochlosperum planchonii* roo*t* dye on the shelf-life of shea butter during storage. MPhil thesis submitted to the Department of Biochemistry and Biotechnology. Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Ajala, E. O., Aberuagba, F., Olaniyan, A. M. and Onifade, R. K. (2015). Optimization of solvent extraction of shea butter (*Vitellaria paradoxa*) using response surface

methodology and its characterization. *Journal of Food Science and Technology,* **53(1)***:*730.

Ajayi, A. B**.,** (2013). Upgrade of Indigenous Methods of Production of Shea Butter. B.Sc dissertation submitted to the Department of Chemical Engineering. Covenant University, Ogun State-Nigeria.

Akihisa, T., Kojima, N., Katoh, N., Ichimura, Y., Suzuki, H., Fukatsu, *et al.* (2010). Triterpene alcohol and fatty acid composition of Shea nut from seven African countries. *Journal of Oleo Science,* **59(**7): 351-360.

Aculey, P. C., Lowor, S.T., Kumi, W. O. and Assuah, M. K. (2012). The Effect of Traditional Primary Processing of the Shea Fruit on the Yield and Quality. *American Journal of Food Technology*, **7**(2): 73-81.

Alander, J. and Andersson, A. C. (2002). The Shea Butter Family – the Complete Emollient Range for Skin Care Formulations. Cosmetics and Toiletries Manufacture Worldwide, pp 28–32.

Alander, J. (2004). Shea Butter: A Multi-Functional Ingredient for Food and Cosmetics. *Lipid Technology,* **16**: 202-205.

Alenyorege, E. A., Hussein, Y. A. and Adongo, T. A. (2015). Extraction Yield, Efficiency And Loss Of The Traditional Hot Water Floatation (HWF) Method Of Oil Extraction From The Seeds Of *Allanblackia Floribunda. International Journal of Scientific and Technology Research,* **4**(02): 92–95.

Al-hassan, S. (2012). "Market Access Capacity of Women Shea Processors in Ghana", *European Journal of Business and Management*, **4**(6):2222-2839.

Anebi, G. J., Umogbai, V. I. and Obetta, S. E. (2014). Development of a Soya Bean Oil Extracting Machine. *Asian Journal of Science and Technology*, **5**(12): 782-788.

Association of Official Analytical Chemist (AOAC). (1994). *Methods of Analysis of the Association of Official Analytical Chemists*, 14th Ed., AOAC, Washington, DC.

Aparna, S., Khare, S. K. and Gupta, M. N. (2002). Enzyme assisted aqueous extraction of peanut oil. *Journal of American Oil Chemists' Society,* **79**: 215-218.

Apea, O. B and Larbi, E. (2013). Indigenous Technology and Scientific Research as Ingredients for Economic Development: A Case of Shea Butter Industry. *Journal of Contemporary Integrative Ideas*, **1**(1): 16-26.

Badifu, G. I. O. (1989). Lipid Composition of Nigerian *Butyrospermum paradoxum* Kernel. *Journal of Food Composition and Analysis,* **2**: 238-244.

Bail, S., Krist, S., Masters, E., Unterweger, H. and Buchbauer, G. (2009). Volatile Compounds of Shea Butter Samples Made under Different Production Conditions in Western, Central and Eastern Africa. *Journal of Food Composition and Analysis,* **22**: 738-744.

Barrios, V. A., Olmos, D. A., Noyola, R. A., and Lopez-Munguia, C. A. (1990). Optimization of an enzymatic process for coconut oil extraction. *Oleagineux.* **45:** 35–42.

Baydar, N. G., Ozkan, G., Cetin, E. S. (2007). Characterization of Grape Seed and Pomace Oil Extracts. *Grasas Aceites,* **58**: 29-33.

Bhatia, D. S., Darpra, H. A. B and Balga, B. P. (1966). Peanut protein isolate-production and properties. *Journal of Food Science and Technology,* **3**: 2-5.

Bhattacharjee, P., Singhal, R. S., and Tiwari, S. R. (2006). Supercritical carbon dioxideextraction of cottonseed oil. *Journal of Food Engineering*, **79**: 892-989.

British Pharmacopoeia (2012). His /Her Majesty's Stationery Office (**HMSO)**, Volume II, London, Appendix X.

Buenrostro, M. and Lopez-Munguia, C. A. (1986). Enzymatic extraction of avocado oil. *Biotechnology Letters*, **8**: 505-506.

Caragay, A. B. (1983). Pacing technologies in the fats and oils industry. *Journal of American Oil Chemists' Society,* **60:** 1614-1644.

Carette, C., Malotaux, M., Leeuwen, M. and Tolkamp, M. (2009). *Shea nut and butter in Ghana opportunities and constraints for local processing*. Hans Eenhoorn and Resilience Foundation. pp: 6-8. PDF document available at http://www.resilience-foundation.nl/docs/Shea.pdf (Accessed on 5th September, 2015).

Caroline, C., Mayumi, M., Mirjam, V. L. and Mirjam, T., (2009). Shea nut and butter in Ghana Opportunities and constraints for local processing *Wageningen University, Wageningen, Holand.*

Cater, C. M., Rhee, K. C., Hagenmaier, R., and Mattil. K F. (1974). Aqueous extraction and alternative oilseed milling process. *Journal of American Oil Chemists' Society,* **51:** 137-141.

Chaffin, B. (2004). Shea Butter Republic. Routledge. *New York, NY. Intro, Chapter 1.*

Cheah, S. C., Augustin, M. A and Ooi, L. C. L. (1990). Enzymatic extraction of palm oil. *Palm Oil Research Bulletin*, **20**: 30-36.

Chen, B-K., and Diosady, L. L. (2003). Enzymatic aqueous processing of coconuts. *International Journal of Applied Science and Engineering*, **1**: 55-61.

Cintra, M. O., Lopez-Munguia, A. and Vernon, J. (1986). Coconut oil extraction by a new enzymatic process. *Journal of Food Science*, **51**: 695-697.

CRIG. (2002). A case study: Research and development of shea trees and shea products; the cultivation and processing of shea nuts. *Cocoa Research Institute of Ghana (CRIG)* in collaboration with the University of Ghana**.**

Damodaran, S., Parkin, K. L., Fennema, O. R (2008). Fennema's Food Chemistry. *4th edn. CRC Press. Boca Raton, FL. Chapter. 4.*

De Groot, A. P., and Slump, P. (1969). Effects of severe alkali treatment of proteins on amino acid composition and nutritive value. *Journal of Nutrition*, **98**: 45-48.

Deepika, S. and Gagandeep, K. S. (2014). Methods used for extraction of maize (*Zee Mays, l.)* germ oil – A review. *Ind.J.Sci.Res. and Tech,* 2(4):48-54.

Di Vincenzo, D., Maranz, S., Serraiocco, A., Vito, R. and Wiesman, Z. (2005). Regional Variation in Shea Butter Lipid and Triterpene Composition in Four African Countries. *Journal of Food Composition and Analysis*, **53**: 7473-7479.

Divine, N. B., Ce´sar, K., Louis, M., Bernard, M. and Ze´phirin, M. (2011). Influence of physical pretreatments of sheanuts (*Vitellaria paradoxa*) on butter quality. *European Journal of Lipid Science and Technology*, **113**: 1152–1160.

Dodiomon, S., Koffi, N., and Kafana, S. (2011). "Variability of production out of fruits of Shea trees", *Agriculture & Biology Journal of North America*, **2(**2): 239-243.

Dominguez, H., Nunez, M. J and Lema, J. M. (1994). Enzymatic pretreatment to enhance oil extraction from fruits and oilseeds: A review. *Food Chemistry,* **49**: 271-286.

Dominguez, H., Nunez, M. J and Lema, J. M. (1995). Aqueous processing of sunflower kernels with enzymatic technology. *Food Chemistry,* **53**: 427-434.

Eapen, K. E., Kalbag, S. S and Subrahmanyan, V. (1966). Operations in the wet-rendering of peanut for the separation of protein, oil, and starch. *Journal of American Oil Chemists' Society*, **43**: 585-589.

Embong, M. B. and Jelen, P. (1977). Technical feasibility of aqueous extraction of rapeseed oil: A laboratory study. *Canadian Institute of Food Science and Technology Journal Aliment*, **10**: 239-243.

Esuoso, K. O., Lutz, H., Bayer, E., and Kutubuddin, M. (2000). Unsaponifiable Lipid Constituents of Some Underutilized Tropical Seed Oils. *Journal of Food Composition and Analysis*, **48**: 231-234.

Ezema, D. O., and Ogujiofor, K. O. (1992). The evaluation of *Butyrospermum paradoxum* as a suppositroy base. *International Journal of Pharmaceutics*, **30:** 117-123.

Francis, O. (2009). Post-harvest handling practices and physic- chemical characteristics of Shea (*Vitellaria paradoxa*) fruit in Uganda. Makerere University, Uganda.

Ferris, R. S. B., Collinson, C., Wanda, K., Jagwe, J. and Wright, P. (2001). Evaluating the Market Opportunities for Shea nut and Shea nut processed products in Uganda. Natural Resourses Institute, Submitted by USAID in October 2001, p. 4.

Fluery, J. M (1981). "The butter tree". *IDRC-reports*, **10**(2): 6-9.

Fold, N. (2000). "A matter of good taste? Quality and the construction of standards for chocolate in the European Union. *Cahiersd' Economieet Sociologie Rurales*, **55/56**: 92–110.

Frevert, J., Frische, R., Hart, J. and Wittkind, J. (1990). Enzymatic solventless recovery of oils from plant materials. Ger. Offen. DE 38432027.

Fullbrook, P. D. (1983). The use of enzymes in the processing of oilseeds. *Journal of American Oil Chemists' Society*, **60**: 428-430.

Gaertner, Karl Friedrich von. (1807). Supplementum Carpologiae. Leipzig. Cited in Tropicos.org. Missouri Botanical Garden. (http://www.tropicos.org, accessed on 12th August, 2015).

Garrett, H. R and Grisham, M. C. (1995). Protein. In: Biochemistry. Saunders College Publishers. Pp 94.

George, Y. O., Michjaloo, A. and Peter, O. (2010). Effect of Temperature, Moisture Content, Particle Size and Roasting on Shea Butter Extraction Efficiency. *International Journal of Food Engineering*, **6**(2).

Ghana standard, GS (Unrefined Shea Butter), 2006, pp. 1-6.

Global Shea Alliance Quality Standards GSA 001. (2013). Specifications for Shea nuts establishes the quality requirements for trading of the whole nuts and kernels from the fruit *Vitellaria paradox*.

Goreja, W. G. (2004). Shea Butter: The Nourishing Properties of Africa's Best-Kept Natural Beauty. Amazing Herbs Press. New York, NY.

Gunstone, F. D. (2004). The chemistry of oils and fats sources, composition, properties and uses.

Hagenmaier, R. D. (1974). Aqueous processing of full-fat sunflower seeds, yields of oil and protein. *Journal of American Oil Chemists' Society,* **54**: 470-471.

Hagenmaier, R. D., Cater, C. M. and Mattik, K. F. (1972). Critical unit operations of the aqueous processing of fresh coconuts. *Journal of American Oil Chemists' Society,* **49**: 178-181.

Hamilton, R. J. and Rossell, J. B. (1986). Analysis of Oils and Fats. Elsevier Applied Science. *New York. NY. Chapter 1*.

Hee, S. N., Rodolfo, H. J. and James, E. S. (2013). Quality characteristics of Shea butter Vitellaria paradoxa. *African Natural Plant Products: Discoveries and Challenges in Chemistry, Health, and Nutrition*, **11**: 164-168.

Hernandez, N., Rodiquez-Alegia, M. E., Gonzalez, F. and Lopez-Munguia, C. A. (2000). Enzymatic treatment of rice bran to improve processing. *Journal of American Oil Chemist's Society,* **77**: 177-180.

Ho, C. C., Chow, M. C., and Ong, S. H. (1992). Recovery of residual oil from centrifuge sludge palm oil mill: effect of enzyme digestion and surfactant treatment. *Journal of American Oil Chemist's Society*, **69**: 276-282.

Honfo, F. G., Anita R. L., Noel A., Mohamed, M. S., and Martinus A. J. S. (2013). Characteristics of traditionally processed shea kernels and butter. *International Journal of Food Science & Technology,* **48**(2): 1714-1721.

Huyanh, C. M., Vinh, T. and Frederic, D. (2013). Optimization of Enzyme-Assisted Extraction of Oil Rich in Carotenoids from Gac Fruit (*Momordica cochinchinensis Spreng*.). *Food Technology & Biotechnology*, **58**(4): 488-499.

Itoh, T., Tamura, T., and Matsumoto, T. (1974). Sterols, Methylsterols, and Triterpene Alcohols in Three Theaceae and Some Other Vegetable Oils. *Lipids*, **9**:173-184.

Johnson, L. A., and Lusas, E. W. (1983). Comparison of alternative solvents for oil extraction. *Journal of American Oil Chemist's Society*, **60**: 229-242.

Joseph, C. O., Victor, A. C., Njoku, C. C., Okonkwo, C, and Anaduaka, G. E. (2014). Potentials of non-edible *Abrus precatorius* seed oil towards biodiesel production. *African Journal of Biotechnology*, **13**(44): 4226-4235.

Kaviani, M., Darjani, Z., Tomovska, J., Mazandarani, Z. and Shariati, M. A. (2015). Comparing Different Extraction Methods of Sesame Oil. *International Journal of Pharmaceutical Research & Allied Science*, **4** (2): 22-25.

Kim, H. K. (1989). Aqueous extraction of oil from palm kernel. *Journal of Food Science*, **54**: 491-492.

Kyari, M. Z. (2008). Extraction and characterization of seed oils. *International Agrophysics*, **22**: 139-142.

Lanzani, A., Petrini, M. C., Cozzoli, O., Gallavresi, P., Carola, C. and Jacini, G. (1975). On the use of enzymes for vegetable-oil extraction. A preliminary report. *La Rivista Italiana Della Sostanze Grasse*, **52**: 226-229.

Lawhon, J. T., Manak, L. J., Rhee, K. C, and Lucas, E. W. (1981). Production of oil and protein food products from raw peanuts by aqueous extraction and ultrafication. *Journal of Food Science*, **46**: 3911-395.

Li, P., Mohammed, A. A., Gasmalla, W. Z.., Junjun, L., Rui, B., and Ruijin, Y. (2016). Effects of roasting temperatures and grinding type on the yields of oil and protein obtained by aqueous extraction processing. *Journal of Food Engineering*, **173**: 15-24.

Lipp, M., and Anklam, E. (1998). Review of Cocoa Butter and Alternative Fats for Use in Chocolate-Part A. *Compositional Data. Food. Chem*, **62**: 73-97.

Lovett, P. N. (2004). The Shea Butter Value Chain. WATH Technical Report No. 2. Publication produced for review by the United States Agency for International Development (USAID) Available at http://felmart.com/valuechain.pdf (Accessed on 3rd July, 2015).

Lovett, P. N. (2005). Shea Butter industry expanding in West Africa. *Inform.* **16**: 273–275.

Lovett, P., Miller, M., Mensah, P., Adams, V., and Kannenberg, C. (2005). Shea butter export Guide, pp. 7-8.

Lusas, E.W., Lawhon, J.T. and Rhee, K.C. (1982). Producing edible oil and protein from oilseeds by aqueous processing. *Oil Mill Gazet*, **4**: 28–34.

Maranz, S., Wiesman, Z. and Garti, N. (2003). Phenolic constituents of Shea (*Viellaria paradoxa)* Kernels, *Journal of Agriculture and Food Chemistry*, **51**: 6268-6273.

Maranz. S., Wiesman, Z., Bisgaard, J., Bianchi, G. (2004). Germplasm resources of *Vitellaria paradoxa* based of variations in fat composition across the species distribution range. *Agroforestry systems (in cooperation with ICRAF).* **60**:71.

Masters, E. T., Yidana, J. A. and Lovett, P. N. (2004). Reinforcing Sound Management through Trade: Shea Tree Products in Africa. *Unasylva*, **210**: 46-52.

Mbaiguinam, M., Mbayhoudel, K. and Djekota, C. (2007). Physical and Chemical Characteristics of Fruits, Pulps, Kernels and Butter of Shea *Butyrospermum parkii* (Sapotaceae) from Mandoul, Southern Chad. *Asian Journal of Biochemistry*, **2**: 101-110.

Moharram, H., Ray, J., Ozbas, S., Juliani, H. and Simon, J. (2006). Shea Butter: Chemistry, Quality, and new Market Potentials (Wang M.; Shengmin S.; Hwang L.S.; Ho C.T. 2006. Challenges in Chemistry and Biology of Herbal Research. American Chemical Society. Washington DC. USA. Ch. 25. Pp. 326-340).

MOHFW. (2005). Manual of Methods of Analysis of Foods: Oils and Fats (Lab. Manual 2). Directorate General of Health Services. Ministry of Health and family Welfare Government of India, New Dehli.

Ndukwe, I. G., Amupitan, J. O., Isah, Y. and Adegoke, K. S. (2007). Phytochemical screening of the crude extract of the roots, stem bark and leaves of *Vitallaria paradoxa* (GAERTN. F). *African journal of Biotechnology,* **6(**16): 1905-1909.

Nielsen, S. S. (2010). Food Analysis (4th Edition), Food Science Texts Series, DOI 10.1007/978-1-4419-1478-1_27, Springer Science+Business Media, LLC 2010.

Nkouam, G. B., Barth, D., Dirand, M., and Tchatcheng, J. B. (2007). Oil Extraction from Shea Kernel (*Vitellaria paradoxa*, Gaertn.) and Canarium Pulp (*Canarium schweinfurthii*, Engl) using Supercritical CO2 and Hexane: A Comparative Study. *Research Journal of Applied Sciences*, 2: 646-652.

NRI Natural Resource Institute (1995). **S**mall Scale Vegetable Oil Extraction 5, 105p. Available at http://www.appropedia.org/original:Small_Scale_Vegtable_Oil_Extraction_6 (Accessed March 6th, 2016).

Obeng, G. Y; Adjaloo, M. K, Donkor, P. (2010). Effect of Temperature, Moisture Content, Particle Size and Roasting on Shea Butter Extraction Efficiency. *International Journal of Food Engineering*, **6**(2): 1-12.

Ogbonnaya, C. and Adgidizi, P. P. (2008). Evaluation of some Physico-chemical properties of Shea butter (*Butyrospermum paradoxum*) related to its value for food and industrial utilization. *International Journal of Post-Harvest Technology and Innovation*, **1(**3**):** 320-326.

OJEC-Official Journal of the European Communities. (2000). Directive 2000/36/EC of the European Parliament and of the Council of 23rd June 2000 relating to cocoa and chocolate products intended for human consumption. L 197.

Okullo, J. B. L., Omujal, F., Agea, J. G., Vuzi, P. C., Namutebi, A., Okello, J. B. A., and Nyanzi, S. A. (2010). Physico-Chemical characteristics of Shea butter (*Vitellaria paradoxa* CF Gaertn.) oil from the Shea district of Uganda. *African Journal of Food Agriculture Nutrition and Development*, **10:** 2070-2084.

Olaniyan, A. M. (2010). Effect of Extraction Conditions on the Yield and Quantity of Oil from Castor Bean. *Journal of Cereals and Oilseeds*, **1**(2): 24-33.

Olaniyan, A. M., and Oje, K. (2007) Quality Characteristics of Shea Butter Recovered from Shea Kernel through Dry Extraction Process. *Journal of Food Science and Technology*, **44**(4): 404-407.

Olaniyan, A. M., and Oje, K. (2011). Development of model equations for selecting optimum parameters for dry process of shea butter extraction. *Journal of Cereals and Oilseeds*, **2**(4): 47-56.

Olsen, H. S. (1988). Aqueous enzymatic extraction of oil from seeds. In: Asian food conference proceedings Bankgok, Thailand. Reprinted by: Novo Industry A/S, pp A-0604la.

Otu, S. A., Dzogbefia, V. P., Kpikpi, E. N. and Essuman, E. K. (2015). Comparative Effect of Crude and Commercial Enzyme in Shea Fat Extraction. IOSR *Journal of Biotechnology and Biochemistry (IOSR-JBB)*, **1**(3): 18-27.

Paulsen, G. (1981). Important forest products in Africa other than wood – A preliminary study (Project Report RAF/78/025). FAO, Rome.

Phan, T. D. (2008). Study of oil extraction process from avocado (*Persea americana* Mill.) for food and cosmetic application, *University Research Report*, Nong Lam University, Ho Chi Minh City, Vietnam (in Vietnamese).

PHC (2010). Population and Housing Census. Ghana Statistical Service. (Accessed May 14th, 2016).

Rhee, K. C., Cater, C. M. and Mattil, K. F. (1972). Simultaneous recovery of protein and oil from raw peanuts in an aqueous system. *Journal of Food Science,* **37**: 90-93.

Roger, A. B., Rebecca, R. A., George, A., and Mathias, I. O. (2010). Chemical characterization of oil form Germinated nuts of several coconut cultivars *(Cocos nuciferh L.). European Journal of Scientific Research,* **391**: 514-522.

.

Rosenthal, A., Pyle, D. L. and Niranjan, K. (1996). Aqueous and enzymatic processes for edible oil extraction. *Enzyme Microbial Technology*, **19**: 402–420.

Rosenthal, A., Pyle. D. L. and Niranjan, K. (1998). Simultaneous aqueous enzymatic extraction of oil and protein from soybean: mechanisms for process design. *Trans Institute of Chemical Engineers,* **76**: 224-230.

Russo, L. and Etherington, T. (2001). Non wood news. *An information bulletin on non-wood forest products*, **8**: 38-39.

Rustom, I. Y. S., Lopez-Levia, M. H. and Nair, B. M. (1991). A Study of factors affecting extraction of peanut (*Arachis hypogaea* L.) solids with water. *Food Chemistry,* **42:** 153-165.

Salunkhe, D. K.; Chavan, J. K.; Adsule, R. N. and Kadam, S. S. (1992). World Oilseeds: Chemistry, Technology and Utilization, Van Nostrand Reinhold, New York. p 554.

Samman, S., Chow, J. W. Y., Foster, M. J., Ahmad, Z. I., Phuyal, J. L., *et al.* (2008). Fatty Acid Composition of Edible Oils Derived from Certified Organic and Conventional Agricultural Methods. *Food Chemistry,* **109**: 670-674.

Seung, H. K. (1989). "Aqueous Extraction of Oil from Palm Kernel". *Journal of Food Science,* **54**(2): 491-492.

Shahidi, F. (2005). Quality Assurance of Fats and Oils. In: Bailey's Industrial Oil and Fat Products, 6th Ed., John Wiley and Sons Inc., USA.

Sineiro, J., Dominguez, H., Nunez, M. J., and Lema, J. M. (1998). Optimization of the enzymatic treatment during aqueous oil extraction from sunflower seeds. *Food Chem*istry, **61**:467–474.

Soladoye, M. O., Orhiere, S. S. and Ibimode, B. M. (1989). Ethanobotanical Study of two Indigenous Multipurpose Plants in the Guinea Savanna of Kwara State - *Vitellaria paradoxa* and *Parkia biglobosa. Biennial Conference of Ecological Society of Nigeria. Forestry Research Institute, Ibadan*. p.13.

Sosulski, K. and Sosulski, F. W. (1993). Enzyme-aided vs two-stage processing of canola: technology, product quality and cost evaluation. *Journal of American Oil Chemist's Society,* **70**: 825-829.

Southwell, K. H., & Harris, R. V. (1992). Extraction of oil from oilseeds using the hot water flotation method. *Tropical science,* **32**(3): 251-262.

Staron, T., and Guillaumin, A. (1979). A method for extracting rapeseed protein and oil by water. *Revenue Francais des Corps Gras,* **26**: 441-446.

Netherlands Development Organization SVN (2006). Improving Market Access for Small Farmers: Concept note on developing shea market chains, Shea Subsector Study.

Subrahmanyan, J., Bhatia, D. S., Kalbag, S. S. and Subramanian, N. (1959). Integrated processing of peanut for the separation of major constituents. *Journal of American Oil Chemists' Society,* **36**: 66-70.

Sugarman, N. (1956). Process for simultaneously extracting oil and protein from oleaginous material. *U. S. Patent No.* 2 762 820.

Tano-Debrah, K. and Ohta, Y. (1995). Enzyme-assisted aqueous extraction of shea fat: A rural approach. *Journal of American Oil Chemists' Society,* **72**: 251-256.

Tano-Debrah, K., and Ohta, Y. (1994). Enzyme-assisted aqueous extraction of fat from kernels of the shea tree, *Butyrospermum parkii*. *Journal of the American Oil Chemists' Society*, **71**(9): 979-983.

Tella, A. (1979). Preliminary Studies on Nasal Decongestant Activity from the Seed of Shea Butter Tree, *Butyrospermum parkii*. *British Journal of Clinical Pharmacology,* **7:** 495-497.

Walter, S., Cole, D., Kathe, W., Lovett, P., & Paz Soldan, M. (2003)**.** Impact of Certification on the Sustainable use of NWFP: Lessons-learnt from Three Case Studies. (Paper submitted for presentation at the International Conference on Rural Livelihoods, Forests and Biodiversity 19-23 May 2003, Bonn, Germany)

Warra, A. A. (2011). Sesame (*sesamumindicum l.*) Seed oil methods of extraction and its prospects in cosmetic industry: a review. *Bayero Journal of Pure and Applied Sciences,* **4**(2): 164 – 168.

William, O., and Odom, V. E. (2015). Evaluation of Saponification value, Iodine value and Insoluble properties in Coconut Oil from Jomoro District in the Western Region of Ghana. *Asian Journal of Agriculture and Food Science,* **3**(5): 2321.

Yonas, A. (2014). Evaluation of processing factors in screw expeller and comparison with other extraction methods of Ethiopian Shea (*Vitlleria paradoxa*) butter, MSc thesis, Addis Ababa University Institute Of Technology School of Chemical And Bio-Engineering.

APPENDICES

Appendix A1:

4 x 3 Factorial design using Two-Way ANOVA to determine if there is any effect of kneading, boiling or its interaction on oil recovery.

Descriptive Statistics

Dependent Variable: yield

Kneading	boiling	Mean	Std. Deviation	N
0 mins	5mins	10.8850	.61518	2
	10mins	15.5000	.09899	2
	15mins	19.0750	.20506	2
	Total	15.1533	3.68421	6
10mins	5mins	25.5200	.05657	2
	10mins	27.8300	.18385	2
	15mins	28.8900	.05657	2
	Total	27.4133	1.54389	6
20 mins	5mins	37.7550	.10607	2
	10mins	41.3900	.38184	2
	15mins	39.1300	.11314	2
	Total	39.4250	1.65192	6
30 mins	5mins	38.3900	.08485	2
	10mins	42.9600	.73539	2
	15mins	39.3850	.12021	2
	Total	40.2450	2.17560	6
Total	5mins	28.1375	11.97987	8
	10mins	31.9200	11.93162	8
	15mins	31.6200	8.96918	8
	Total	30.5592	10.70356	24

Appendix A2:

Tests of Between-Subjects Effects: 4 x 3 Factorial design

Dependent Variable: yield

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2413.778[a]	11	219.434	8917.077	.000
Intercept	22627.129	1	22627.129	919490.495	.000
Kneading	**2348.458**	**3**	**782.819**	**31811.149**	**.000**
Boiling	**37.738**	**2**	**18.869**	**766.775**	**.000**
kneading * boiling	**27.582**	**6**	**4.597**	**186.807**	**.000**
Error	.295	12	.025		
Total	25041.202	24			
Corrected Total	2414.074	23			

Appendix B1

Descriptives: Extraction Yield Using Hot Water Extraction Method

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
raw	3	**28.4733**	**1.66569**	.96169	24.3355	32.6111	26.56	29.60
roasted	3	**30.2867**	**.10066**	.05812	30.0366	30.5367	30.18	30.38
paste	3	**43.6533**	**.43097**	.24882	42.5827	44.7239	43.32	44.14
Total	9	**34.1378**	**7.23126**	2.41042	28.5793	39.6962	26.56	44.14

Test of Homogeneity of Variances

Extraction Yield

Levene Statistic	df1	df2	Sig.
9.961	2	6	**.012**

Appendix B2

One Way ANOVA: Extraction Yield Using Hot Water Extraction Method

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	412.388	2	206.194	208.249	.000
Within Groups	5.941	6	.990		
Total	418.329	8			

Appendix C1

Descriptives: Extraction Yield Using Solvent Extraction Method

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
raw	3	**52.3067**	**2.65055**	1.53030	45.7223	58.8910	49.43	54.65
roasted	3	**53.4833**	**.64532**	.37257	51.8803	55.0864	52.74	53.90
paste	3	**57.5733**	**.75725**	.43720	55.6922	59.4545	56.75	58.24
Total	9	**54.4544**	**2.78121**	.92707	52.3166	56.5923	49.43	58.24

Test of Homogeneity of Variances

Yield

Levene Statistic	df1	df2	Sig.
3.485	2	6	**.099**

Appendix C2

One Way ANOVA: Extraction Yield Using Solvent Extraction Method: Descriptives

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	45.850	2	22.925	8.581	.017
Within Groups	16.031	6	2.672		
Total	61.881	8			

Appendix D1: Descriptives for moisture content (physical quality of oil) from traditional hydrothermal extraction

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
RASK	3	.2033	.01155	.00667	.1746	.2320
ROSK	3	.1733	.01155	.00667	.1446	.2020
SKEP	3	.1533	.00577	.00333	.1390	.1677
Total	9	.1767	.02345	.00782	.1586	.1947

Appendix D2: ANOVA result for moisture content (physical quality of oil) from traditional hydrothermal extraction

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.004	2	.002	19.000	.003
Within Groups	.001	6	.000		
Total	.004	8			

Appendix D3: Descriptives for moisture content (physical quality of oil) from enzyme-assisted hydrothermal extraction

	N	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
RASK	**3**	**.1833**	**.00577**	**.00333**	**.1690**	**.1977**
ROSK	**3**	**.1667**	**.00577**	**.00333**	**.1523**	**.1810**
SKEP	**3**	**.1600**	**.01000**	**.00577**	**.1352**	**.1848**
Total	**9**	**.1700**	**.01225**	**.00408**	**.1606**	**.1794**

Appendix D4: ANOVA result for moisture content (physical quality of oil) from enzyme-assisted hydrothermal extraction

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	**.001**	**2**	**.000**	**7.800**	**.021**
Within Groups	**.000**	**6**	**.000**		
Total	**.001**	**8**			

Appendix E1
Chemical Properties on HWF Extraction: Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Acid	RASK	2	3.6850	.04950	.03500	3.2403	4.1297	3.65	3.72
	ROSK	2	4.1900	.04243	.03000	3.8088	4.5712	4.16	4.22
	SKEP	2	4.1750	.03536	.02500	3.8573	4.4927	4.15	4.20
	Total	6	4.0167	.25913	.10579	3.7447	4.2886	3.65	4.22
FFA	RASK	2	1.8450	.02121	.01500	1.6544	2.0356	1.83	1.86
	ROSK	2	2.0950	.02121	.01500	1.9044	2.2856	2.08	2.11
	SKEP	2	2.0900	.01414	.01000	1.9629	2.2171	2.08	2.10
	Total	6	2.0100	.12869	.05254	1.8750	2.1450	1.83	2.11
Peroxide	RASK	2	4.8550	.06364	.04500	4.2832	5.4268	4.81	4.90
	ROSK	2	5.1150	.02121	.01500	4.9244	5.3056	5.10	5.13
	SKEP	2	5.1500	.01414	.01000	5.0229	5.2771	5.14	5.16
	Total	6	5.0400	.14738	.06017	4.8853	5.1947	4.81	5.16
Saponification	RASK	2	182.8450	.12021	.08500	181.7650	183.9250	182.76	182.93
	ROSK	2	183.2450	.13435	.09500	182.0379	184.4521	183.15	183.34
	SKEP	2	183.2200	.05657	.04000	182.7118	183.7282	183.18	183.26
	Total	6	183.1033	.21750	.08879	182.8751	183.3316	182.76	183.34

Iodine	RASK	2	49.5300	.15556	.11000	48.1323	50.9277	49.42	49.64
	ROSK	2	47.6200	.70711	.50000	41.2669	53.9731	47.12	48.12
	SKEP	2	47.8450	.85560	.60500	40.1577	55.5323	47.24	48.45
	Total	6	48.3317	1.05971	.43262	47.2196	49.4438	47.12	49.64

Appendix E2
Chemical Properties HWF Extraction: ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Acid	Between Groups	.330	2	.165	90.064	**.002**
	Within Groups	.005	3	.002		
	Total	.336	5			
FFA	Between Groups	.082	2	.041	111.409	**.002**
	Within Groups	.001	3	.000		
	Total	.083	5			
Peroxide	Between Groups	.104	2	.052	33.160	.009
	Within Groups	.005	3	.002		
	Total	.109	5			
Saponification	Between Groups	.201	2	.100	8.438	.059
	Within Groups	.036	3	.012		
	Total	.237	5			
Iodine	Between Groups	4.359	2	2.179	5.204	.106
	Within Groups	1.256	3	.419		
	Total	5.615	5			

Appendix E3
Chemical Properties HWF Extraction: Post Hoc Tests

Dependent Variable	(I) Samples	(J) Samples	Mean Difference (I-J)	Std. Error	Sig.
Acid	**RASK**	**ROSK**	**-.50500***	.04282	.003
		SKEP	**-.49000***	.04282	.003
	ROSK	**RASK**	**.50500***	.04282	.003
		SKEP	.01500	.04282	.936
	SKEP	**RASK**	**.49000***	.04282	.003
		ROSK	-.01500	.04282	.936
FFA	**RASK**	**ROSK**	**-.25000***	.01915	.002
		SKEP	**-.24500***	.01915	.002
	ROSK	**RASK**	**.25000***	.01915	.002
		SKEP	.00500	.01915	.964
	SKEP	**RASK**	**.24500***	.01915	.002
		ROSK	-.00500	.01915	.964
Peroxide	**RASK**	**ROSK**	**-.26000***	.03958	.015
		SKEP	**-.29500***	.03958	.010
	ROSK	**RASK**	**.26000***	.03958	.015
		SKEP	-.03500	.03958	.685
	SKEP	**RASK**	**.29500***	.03958	.010
		ROSK	.03500	.03958	.685
Saponification	RASK	ROSK	-.40000	.10909	.070
		SKEP	-.37500	.10909	.082
	ROSK	RASK	.40000	.10909	.070
		SKEP	.02500	.10909	.972
	SKEP	RASK	.37500	.10909	.082
		ROSK	-.02500	.10909	.972
Iodine	RASK	ROSK	1.91000	.64711	.117
		SKEP	1.68500	.64711	.154
	ROSK	RASK	-1.91000	.64711	.117
		SKEP	-.22500	.64711	.937
	SKEP	RASK	-1.68500	.64711	.154
		ROSK	.22500	.64711	.937

*The mean difference is significant at the 0.05 level.

Appendix F1
Descriptives: Chemical properties for Solvent Extraction

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
Acid	RASK	2	7.7000	.39598	.28000	4.1423	11.2577
	ROSK	2	8.2600	.22627	.16000	6.2270	10.2930
	SKEP	2	8.1150	.04950	.03500	7.6703	8.5597
	Total	6	8.0250	.33116	.13520	7.6775	8.3725
FFA	RASK	2	3.8500	.19799	.14000	2.0711	5.6289
	ROSK	2	4.1300	.11314	.08000	3.1135	5.1465
	SKEP	2	4.0650	.02121	.01500	3.8744	4.2556
	Total	6	4.0150	.16634	.06791	3.8404	4.1896
Peroxide	RASK	2	9.9500	.14142	.10000	8.6794	11.2206
	ROSK	2	11.3850	.09192	.06500	10.5591	12.2109
	SKEP	2	11.4550	.06364	.04500	10.8832	12.0268
	Total	6	10.9300	.76402	.31191	10.1282	11.7318
Saponification	RASK	2	184.5950	.68589	.48500	178.4325	190.7575
	ROSK	2	184.4150	.04950	.03500	183.9703	184.8597
	SKEP	2	184.2800	.08485	.06000	183.5176	185.0424
	Total	6	184.4300	.34059	.13904	184.0726	184.7874
Iodine	RASK	2	58.1600	.12728	.09000	57.0164	59.3036
	ROSK	2	58.4800	.08485	.06000	57.7176	59.2424
	SKEP	2	58.5950	.02121	.01500	58.4044	58.7856
	Total	6	58.4117	.21311	.08700	58.1880	58.6353

Appendix F2

One Way ANOVA: Chemical properties for Solvent Extraction

		Sum of Squares	df	Mean Square	F	Sig.
Acid	Between Groups	.338	2	.169	2.408	.238
	Within Groups	.210	3	.070		
	Total	.548	5			
FFA	Between Groups	.086	2	.043	2.457	.233
	Within Groups	.052	3	.017		
	Total	.138	5			
Peroxide	Between Groups	2.886	2	1.443	133.205	.001
	Within Groups	.033	3	.011		
	Total	2.919	5			
Saponification	Between Groups	.100	2	.050	.312	.753
	Within Groups	.480	3	.160		
	Total	.580	5			
Iodine	Between Groups	.203	2	.102	12.782	.034
	Within Groups	.024	3	.008		
	Total	.227	5			

Appendix F3

Chemical Properties for Solvent Extraction: Post Hoc Tests

Multiple Comparisons (Tukeys HSD)

Dependent Variable	(I) Samples	(J) Samples	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Acid	RASK	ROSK	-.56000	.26486	.234	-1.6668	.5468
		SKEP	-.41500	.26486	.382	-1.5218	.6918
	ROSK	RASK	.56000	.26486	.234	-.5468	1.6668
		SKEP	.14500	.26486	.855	-.9618	1.2518
	SKEP	RASK	.41500	.26486	.382	-.6918	1.5218
		ROSK	-.14500	.26486	.855	-1.2518	.9618
FFA	RASK	ROSK	-.28000	.13222	.233	-.8325	.2725
		SKEP	-.21500	.13222	.362	-.7675	.3375
	ROSK	RASK	.28000	.13222	.233	-.2725	.8325
		SKEP	.06500	.13222	.880	-.4875	.6175
	SKEP	RASK	.21500	.13222	.362	-.3375	.7675
		ROSK	-.06500	.13222	.880	-.6175	.4875
Peroxide	RASK	ROSK	-1.43500*	.10408	.002	-1.8699	-1.0001
		SKEP	-1.50500*	.10408	.001	-1.9399	-1.0701
	ROSK	RASK	1.43500*	.10408	.002	1.0001	1.8699
		SKEP	-.07000	.10408	.794	-.5049	.3649
	SKEP	RASK	1.50500*	.10408	.001	1.0701	1.9399
		ROSK	.07000	.10408	.794	-.3649	.5049
Saponification	RASK	ROSK	.18000	.40004	.898	-1.4917	1.8517
		SKEP	.31500	.40004	.735	-1.3567	1.9867
	ROSK	RASK	-.18000	.40004	.898	-1.8517	1.4917

Iodine	SKEP	SKEP	.13500	.40004	.940	-1.5367	1.8067
		RASK	-.31500	.40004	.735	-1.9867	1.3567
		ROSK	-.13500	.40004	.940	-1.8067	1.5367
	RASK	ROSK	-.32000	.08916	.074	-.6926	.0526
		SKEP	-.43500*	.08916	.033	-.8076	-.0624
	ROSK	RASK	.32000	.08916	.074	-.0526	.6926
		SKEP	-.11500	.08916	.490	-.4876	.2576
	SKEP	RASK	.43500*	.08916	.033	.0624	.8076
		ROSK	.11500	.08916	.490	-.2576	.4876

*. The mean difference is significant at the 0.05 level.

Appendix G1

Chemical Properties for Enzyme Extraction: Descriptives

		N	Mean	Std. Deviation	Std. Error
acid	RASK	2	3.2100	.09899	.07000
	ROSK	2	4.1950	.07778	.05500
	SKEP	2	5.4100	.26870	.19000
	Total	6	4.2717	.99455	.40602
FFA	RASK	2	1.6050	.04950	.03500
	ROSK	2	2.1000	.04243	.03000
	SKEP	2	2.7050	.13435	.09500
	Total	6	2.1367	.49726	.20301
peroxide	RASK	2	3.6850	.57276	.40500
	ROSK	2	5.3350	.14849	.10500
	SKEP	2	5.3900	.19799	.14000
	Total	6	4.8033	.91042	.37168

saponification	RASK	2	183.1600	.39598	.28000
	ROSK	2	181.0600	.52326	.37000
	SKEP	2	180.4150	.54447	.38500
	Total	6	181.5450	1.33923	.54674
iodine	RASK	2	49.5300	.15556	.11000
	ROSK	2	47.6400	.67882	.48000
	SKEP	2	48.1750	.09192	.06500
	Total	6	48.4483	.92625	.37814

Appendix G2

Chemical Properties for Enzyme Extraction: ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Acid	Between Groups	4.858	2	2.429	82.754	.002
	Within Groups	.088	3	.029		
	Total	4.946	5			
FFA	Between Groups	1.214	2	.607	81.661	.002
	Within Groups	.022	3	.007		
	Total	1.236	5			
Peroxide	Between Groups	3.755	2	1.878	14.468	.029
	Within Groups	.389	3	.130		
	Total	4.144	5			
Saponification	Between Groups	8.241	2	4.120	17.002	.023
	Within Groups	.727	3	.242		
	Total	8.968	5			
Iodine	Between Groups	3.796	2	1.898	11.540	.039
	Within Groups	.493	3	.164		

Total	4.290	5			

Appendix G3

Chemical Properties for Enzyme Extraction: Post Hoc Tests

Multiple Comparisons: Tukey HSD

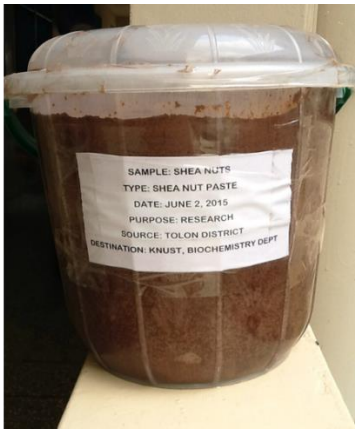
Dependent Variable	(I) Samples	(J) Samples	Mean Difference (I-J)	Std. Error	Sig.
Acid	RASK	ROSK	-.98500*	.17132	.021
		SKEP	-2.20000*	.17132	.002
	ROSK	RASK	.98500*	.17132	.021
		SKEP	-1.21500*	.17132	.012
	SKEP	RASK	2.20000*	.17132	.002
		ROSK	1.21500*	.17132	.012
FFA	RASK	ROSK	-.49500*	.08622	.021
		SKEP	-1.10000*	.08622	.002
	ROSK	RASK	.49500*	.08622	.021
		SKEP	-.60500*	.08622	.012
	SKEP	RASK	1.10000*	.08622	.002
		ROSK	.60500*	.08622	.012
Peroxide	RASK	ROSK	-1.65000*	.36023	.039
		SKEP	-1.70500*	.36023	.036
	ROSK	RASK	1.65000*	.36023	.039
		SKEP	-.05500	.36023	.987
	SKEP	RASK	1.70500*	.36023	.036
		ROSK	.05500	.36023	.987
Saponification	RASK	ROSK	2.10000*	.49229	.047
		SKEP	2.74500*	.49229	.023

Iodine	ROSK	RASK	-2.10000*	.49229	.047
		SKEP	.64500	.49229	.481
	SKEP	RASK	-2.74500*	.49229	.023
		ROSK	-.64500	.49229	.481
	RASK	ROSK	1.89000*	.40557	.038
		SKEP	1.35500	.40557	.088
	ROSK	RASK	-1.89000*	.40557	.038
		SKEP	-.53500	.40557	.477
	SKEP	RASK	-1.35500	.40557	.088
		ROSK	.53500	.40557	.477

***. The mean difference is significant at the 0.05 level.**

RASK- Raw Shea Kernels

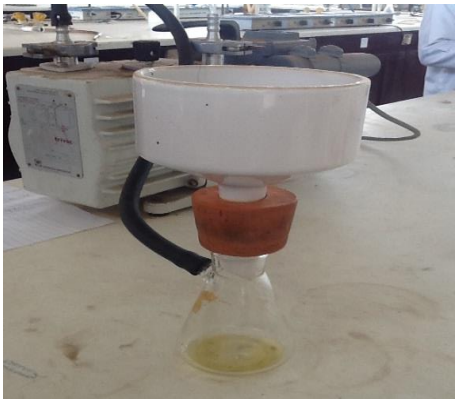
ROSK- Roasted Shea Kernels



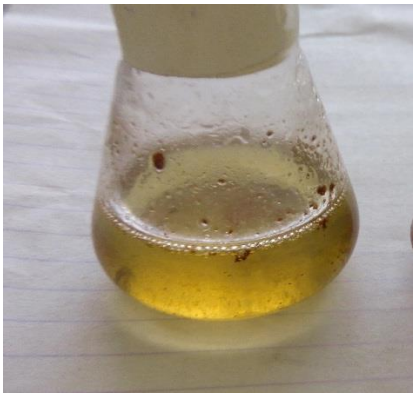
SKEP- Shea Kernel Paste

Traditional hydrothermal extraction

Samples of Enzyme Extraction



Vacuum filtration of shea oil



Sample of Shea oil obtained

Appearance as white mass starts forming during kneading in traditional hydrothermal extraction

Dried Paste sample

Ground raw shea kernels

Ground roasted shea kernels

Crude Shea butter