KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI

COLLEGE OF SCIENCE

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY



# PROCESS DEVELOPMENT AND EVALUATION OF TIGER NUT BASED CHOCOLATE PRODUCTS

BY

FRANK ADU ASANTE

SEPTEMBER, 2015

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI

COLLEGE OF SCIENCE

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

# PROCESS DEVELOPMENT AND EVALUATION OF TIGER NUT BASED CHOCOLATE PRODUCTS

THIS DISSERTATION IS PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS OF PhD DEGREE IN FOOD SCIENCE AND TECHNOLOGY

BY

FRANK ADU ASANTE

SEPTEMBER, 2015

Abstract

Dairy milk is a very versatile ingredient in the food industry because of its nutritional, sensory and functional properties. Increasing awareness among consumers about its associated allergies, concerns about its saturated fatty acids, scarcity and cost however have necessitated the development of plant materials as alternative food ingredients. The tiger nut tuber is a plant material that has been identified for the production of novel food ingredients for industry. The development of natural food ingredients of acceptable taste and quantity will however be important considerations in its use in industrial applications. Black and brown varieties of tiger nut tubers from eight different sites and planted in two different periods in Ghana were screened, and the milk extracted after optimizing process variables. The milk and cake were then analyzed and evaluated for their potential use in chocolate products. Proximate analysis showed that the average fat, ash, carbohydrate and fiber contents (g $kg^{-1}$) of the black variety was 192.7, 14.3, 620.5, and 98.2, while that for the brown variety was 219.2, 16.0, 642.9, and 98.3 respectively. Carbohydrates and fiber contents ranged between 497.0 g $kg^{-1}$ - 734.7 g $kg^{-1}$ and 75.3 g $kg^{-1}$ -135.4 g $kg^{-1}$ respectively. Pre-treating equal weights of tubers from different sites by soaking and different combinations of soaking and cooking methods yielded significantly different results for the milk indices determined. Boiling of tubers before soaking showed higher milk yield (4395.5 g $kg^{-1}$ to 6530.4 g $kg^{-1}$) and milk solids (101.0 g $kg^{-1}$ and 139.0 g $kg^{-1}$) for all tubers. The results also showed significant differences in milk solids extracted from black and brown varieties with ranges of 541.4 g $kg^{-1}$

to 619.4 g kg-$^{1}$ and 571.4 g kg-$^{1}$ to 710.2 g kg-$^{1}$ respectively. In establishing the optimum conditions for milk solids extraction, the Response Surface Methodology (RSM) based on three (3) critical factors; milling time ($X_1$), tuber: water ratio ($X_2$) and boiling time ($X_3$) was used. Regression analysis of the data indicated that the optimum extraction conditions for the black variety; was milling time, 17 minutes; meal: water, 1g: 6.4 ml; boiling time, 10 minutes and for the brown variety, milling time, 22 minutes; meal: water, 1 g: 7.0 ml; boiling time, 10 minutes. Under the experimental conditions, the yield of milk solids from the black tubers was 65.07 (0.40) % against the predicted value of 70.66 % while that of the brown cultivars was 74.84 (1.84) % against the predicted value of 80.99 %. The physico-chemical and functional properties of tiger nut milk, powder and cake were evaluated. The milk powder had protein content 76.9 ± 0.8 g kg-$^{1}$, ash 24.4 ± 3.2 g kg-$^{1}$, fat 395.6 ± 3.3 g kg-$^{1}$, anti-oxidant content of 4634 ±17.0 mm frap/100g and total phenol of 187.7 ± 1.80 mg gallic acid equivalent/ml. The results also showed total sugars content of 353.7 ± 1.2 g kg-$^{1}$, sucrose of 316.7 ± 1.5 g kg-$^{1}$, reducing sugars 36.3 ± 1.0 g kg-$^{1}$, bulk density 0.80 ± 0.02 g/cm$^{3}$ and pH of 6.87 ± 0.02. The milk cake recorded water absorption capacity of 15.27 ± 0.16 ml /g, oil absorption capacity of 3.51 ± 0.21ml /g and total dietary fiber content of 559.7 ± 2.4 g kg-$^{1}$. Quantities of sugar, cocoa powder, skimmed milk and tiger nut milk for chocolate beverage production were optimized using a four component constrained mixture design. A consumer panel evaluated appearance, mouthfeel, flavour, after taste and overall acceptability in the 15 formulations from the design. Regression models fitted to the data generated, and the optimum

ingredient formulation for acceptable chocolate beverage was determined. The optimum ingredient formulations for acceptable tiger nut milk chocolate beverage were sugar, 20.56-28.58 %; cocoa powder, 8.73-19.67 %; skimmed milk powder, 5.0 % and tiger nut milk 54.77-60.50 %. The potential of tiger nut milk cake in bar chocolate and spread were also investigated. The ash, fat, protein and fiber contents of developed products increased with increasing substitution of sucrose and maltitol with the tiger nut milk cake. The observed trend from the sensory analysis for chocolate spread revealed that, increasing the proportion of tiger nut in the recipes resulted in a corresponding decrease in the ratings of the sensory attributes. An accelerated shelf life study showed that the beverage pasteurised at 70$^{o}$C for 30 minutes and stored in Polyethylene terephthalate (PET) bottles at 18$^{o}$C has a shelf life of 6 months. The physicochemical, functional and sensory properties of developed chocolate products indicated that processed tiger nut products may be considered as potential ingredient in beverages and high fiber diets.

DECLARATION

I declare that I have wholly undertaken the study reported herein under the supervision of Prof. I. N. Oduro, Prof. W. O. Ellis and Prof. F. K. Saalia and that except portions where references have been duly cited, this dissertation is the outcome of my research.



…….. ……..…….

Date (Signature)

Frank Adu Asante

…….. ……..…….

Date (Signature)

Prof. I. N. Oduro

…….. ……..…….

Date (Signature)

Prof. W. O. Ellis

…….. ……..…….

Date (Signature)

Prof. F. K. Saalia

## CONTRIBUTION TO KNOWLEDGE

The study made meaningful contribution to existing knowledge on tiger nuts tubers, its processing and products. The study established the effect of site, planting period and variety on chemical characteristics of tiger nut tubers. The study also identified the best combination of pre-treatments methods for the extraction of milk and solids. The regression models predicting the various extraction coefficients are important as they would help in predicting the extraction of solids at various levels of the extraction factors as well as the optimum conditions for the extraction of solids. The study produced and provided information on the chemical properties of freeze dried tiger nut milk powder. It has also revealed that processed tiger nut tubers can be used to produce an acceptable chocolate beverage and high fiber chocolate products.

On the whole, this study has increased the knowledge on tiger nut tubers which can be harnessed by food scientists, technologists, nutritionists, industry players as well as policy makers in their efforts to promote its utilization.

### Publications from the study

Asante, F.A., Oduro, I., Ellis, W.O. and Saalia, F.K. (2014). Effect of planting period and site on the chemical composition and milk acceptability of tiger nut (*Cyperus Esculentus* L) tubers in Ghana. *American Journal of Food and Nutrition*, 2 (3): 49-54

Asante, F.A., Ellis, W.O., Oduro, I. and Saalia, F.K. (2014). Effect of soaking and cooking methods on extraction of solids and acceptability of tiger nut (*Cyperus Esculentus* L) milk. *Journal of Agricultural Studies* 2, (2), 76-86

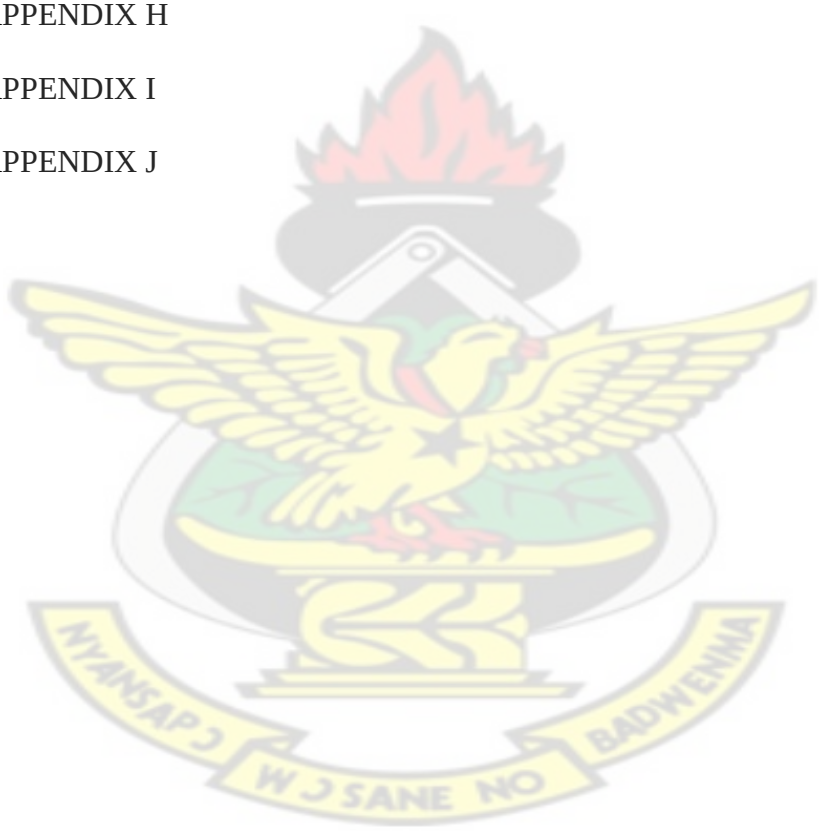
Asante, F.A., Saalia, F.K., Oduro, I. and Ellis, W.O.(2014). Modelling of milk solids extraction from tiger nut (*Cyperus Esculentus* L) tubers using response surface methodology. *International Journal of Food Science, Nutrition and Engineering* 4(3):73-79

*The co-authors of the above listed papers assisted in the design, analysis, interpretation of data and write up of the papers.*

**ACKNOWLEDGEMENTS**

I would like to thank the Lord Jesus Christ without whose grace I would not have come this far. I wish to express my sincere gratitude to my supervisors, Prof. I. N. Oduro, Prof. W. O. Ellis and Prof. F. K. Saalia for their guidance, patience and advice throughout my study. My sincere appreciation goes to staff of the Research and Development Department of Cocoa Processing Company for their immense assistance. I wish to acknowledge Cocoa Processing Company, Tema Ghana for their support. Finally, my gratitude goes to my wife Mrs. Caroline Sarku Asante, my children and relations, Emmanuella Asante, Emmanuel Asante, Elizabeth Asante, Frank Asante, Mary Safoah and Mrs Jessie Okyere for their love and encouragement during my study.

CONTENTS



LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

# APPENDICES

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background

Tiger nut (*Cyperus esculentus* L.) is a root tuber that is a member of the family Cyperaceae. It has other names including "chufa", Zulu nuts and yellow nut sedge (Pascual *et al*., 2000; Rubert *et al*, 2011). In Ghana, it is called, "atadwe" by the Akans, "atangme" by the Gas and "nansaxa" by the Dagombas (Dokosi, 1998). It has been planted extensively in Africa, Asia and European countries for centuries because of its food value and commercial importance (Torrell *et al*., 1993; Arranz *et al*., 2006; Yeboah *et al*., 2011). In Spain, where a well developed industry has known, it has witnessed continuous growth with yields ranging between 15,000 to 20,000 kilograms per hectare recorded (Mosquera *et al*., 1996; Pascual *et al*, 2000; Tiger nuts Traders, S.L., 2010). In 2009 for example, about 5.3 million kilograms of tiger nut tubers valued at approximately 5 million Euros was produced in the Valencia region in Spain (CRDO, 2012).

In West Africa, cultivation, processing, distribution and selling of the crop has been reported in Mali, Niger, Ghana and Nigeria (Pascual *et al*., 2000; Arranz *et al*., 2006). Though it can grow very well in most parts of Ghana, (Dakogre, 2008) it has traditionally been cultivated in large quantities at Kwahu Aduamoa in the Eastern Region as well as Bawjiase and its surrounding villages in the Central Region of Ghana (Tetteh and Ofori, 1998; Nyarko *et al*., 2011). The black and brown varieties are the two main types cultivated in Ghana with yield between 2,210 and 11,300 kilograms per hectare recorded (Irvine, 1969; Tetteh and Ofori, 1998; Ofori-Anti, 2000). Nutritionally, the

tiger nut tuber contains 7.0 % proteins, 58.0 % carbohydrates, and ash 3.0 %. It also contains 10.0 % oil (Kelley and Fredrickson, 1991). The major fatty acid in the tuber is oleic (61 % of total fatty acids), while other fatty acids include linoleic, palmitic and stearic (Kelley and Fredrickson, 1991; Kim *et al*., 2007). Dietary fiber which has been reported to be effective in the treatment and prevention of many diseases has been indicated to be high in the tuber. These diseases include obesity, gastro intestinal disorders, coronary diseases, diabetics and colon cancer (Chevallier, 1996; Djomdi Ejoh and Ndjouenkeu 2006; Anderson *et al.*, 2009; Lattimer and Haub, 2010). Phosphorus and calcium, which are important in the development and maintenance of bones and teeth, are also present in appreciable levels (Holland *et al.*, 1992; Coskuner *et al.*, 2002).

Various studies have reported potential applications for tiger nuts in a number of consumer products made for the gastronomic, pharmaceutical/ medicinal, confectioneries and the bio-fuel industries (Kay, 1987; Barminas *et al.*, 2001; Arafat *et al*., 2009). In the food industry, the tubers are usually baked and processed into flour for baking, as a food additive or chewed as a snack. It has also been used to produce a coffee substitute and vegetable oil (Mulligan and Junkins, 1976). The use of tiger nut tubers and products in developing malt caramel, beverages, fermented foods and flour for baking has also been reported (Kay, 1987; Umeri and Enabeli,1996; Sanful, 2009; Ukwuru *et al*, 2011). A vegetable milk beverage called "*horchata de chufa"* in Spain and "atadwe" milk in Ghana is a popular commercial drink that is made after milling the tubers in water, sieving and sweetening with sugar (Mosquera *et al*, 1996; Dokosi, 1998; Cortes *et al*., 2005; Sanful, 2009). Tiger nut milk has

been reported to be high in starch, reducing sugars and low in fat. It is also rich in minerals such as potassium, phosphorus, as well as vitamins E and C (Tigernuts Traders S.L., 2010) and has been reported to contain myristic, linolenic and large amounts of oleic acid (Belewu and Belewu, 2007).

## 1.2 Statement of Problem

The nutritional composition of tiger nut tubers have been reported by several authors (Fatoki *et al*., 1995; Pascual *et al*., 2000; Anyidoho, 2006) however the effect of site and planting periods on the nutritional quality of tiger nut tubers is yet to be investigated.Traditionally soaking of tiger nut tubers in water is the main pre-treatment method for tiger nut milk extraction after which the tubers are crushed in disc milling machines and mixed with water for extraction (Ofori-Anti, 2000). Though a combination of soaking and cooking methods have been used to pre-treat many plant materials such as sesame seeds to produce milk with improved solids and flavor (Quasem *et al*., 2009) similar studies on extraction of tiger nut milk is yet to be conducted. The effects of many variables such as milling time, tuber: water ratio and cooking time on the extraction of non dairy milk from mant plants including tiger nut tubers have been reported by many researchers (Djomdi Ejoh and Ndjouenkeu 2006, Adekanmi, *et al*., 2009). The determination of the optimum levels of these variables for the maximum yield of tiger nut milk solids is therefore needed to enhance its adoption as an ingredient.

The knowledge of the physico-chemical quality of milk solids is also important for the determination of its potential as an ingredient in food systems (Kelly *et al.,* 2002, Aidoo *et al.,* 2010) thus the need for its

determination and documentation. Beverages like tiger nut milk are important foods for quenching thirst and replenishing essential electrolytes required by the body. Tiger nut milk cake, the main by-product after milk extraction too has been reported to contain very high fiber (Sanchez-Zapata *et al*., 2009) which can be used to develop high fiber foods. The production of ham burgers with improved fiber content using tiger nut milk has been reported (Sanchez-Zapata *et al*., 2009). Considering that cocoa powder has been used to enhance the acceptability of many products (Cacao De Zaan, 1993; Arafat *et al*., 2009) it will be very useful that it's potential in tiger nut based products are studied and documented.



## 1.3 Significance of study

Tiger nuts an annual crop takes 3-4 months to mature and can be cropped in both planting periods and through out the country with increases in its cultivation envisaged because of the perception that it has aprodisiac properties. The development of improved extraction technology will significantly improve yield of milk and bring better economic returns to those involved in its processing. The incorporation of the tiger nut milk and cake into chocolate products also has the potential of increasing the local consumption of cocoa products which remains very low inspite of many initiatives (Awua, 2002). Industrial utilization of tiger nut products will also bring a better focus on the tiger nut industry by policy makers (SRID, 2011).

## 1.4 Research Questions

The main research questions that this study seeks to answer are;

i. Does the nutritional quality of the different varieties of tiger nut tubers cultivated in Ghana vary significantly?

ii. Which of the two main tiger nut varieties would give a higher yield for vegetable milk and solids that would be useful for industrial applications and which one would be more acceptable to consumers?

iii. What is the best cooking method for pretreating tubers before milk extraction?

iv. What are the optimum levels of the factors for the extraction of milk solids?

v. Is it feasible for the tiger nut milk and its cake to be developed into acceptable chocolate products?

vi. What will be the shelf life of these products?

## 1.5 Objectives of the Study

The main objective of this study is to develop a process for producing acceptable tiger nut chocolate products and determine their physico chemical characteristics.

The specific objectives are;

i. To determine the physico-chemical variability among tiger nuts grown in Ghana, based on production locations, varieties, planting periods and acceptability of their milk.

ii. To determine and optimize critical factors for the production of tiger nut milk solids.

iii. To determine the chemical composition and functional properties of tiger nut milk solids and cake.

iv. To formulate, characterize and assess consumer acceptability of tiger nut based milk chocolate beverage, high fiber bar choclate and chocolate spread.

v. To determine the shelf life of developed tiger nut milk chocolate beverage.

# CHAPTER TWO

# 2.0 LITERATURE REVIEW

## 2.1.1 Tiger nut (*Cyperus esculentus* L.)

Tiger nut (*Cyperus esculentus* L.), is a memeber of the family Cyperaceae. Though popularly called "chufa" in many places it has other common names, such as earth nut, tiger nut, yellow nutsedge and "Zulu" nut. It is also called "ayaya" in Hausa and "ofio" in Yoruba (Umerie *et al*., 1997; Pascual *et al*., 2000). Five main varieties have been identified, namely;

- *Cyperus esculentus* var. *esculentus* - very common in the Mediterranean region east to India and Africa;
- *Cyperus esculentus* var. *hermannii* - cultivated in Florida
- *Cyperus esculentus* var. *leptostachyus* - very common in North America
- *Cyperus esculentus* var. *macrostachyus* - cultivated in the United States
- *Cyperus esculentus* var. *sativa* - very common in Asia.

The varieties of interest however are the *esculentus* and the *sativa*, which are commonly studied as a weed and crop in Africa, Asia and Europe (ter Borg and Schippers, 1992; Tiger nuts Traders, S.L., 2010).

## 2.1.2 Physical characteristics and nutritional value of tiger nut tubers

The tubers develop to lengths between 1.0-2.0 cm, showing an obtuse end of irregular form when dry, and round and oval after soaking in water (Coskuner *et al*., 2002). The tubers have a surface color of brown or black depending on the variety and whitish inside. A lightness (L*), redness (a*), and yellowness (b*) for whole and ground tubers has been reported as L*: 31.99; a*: 7.01; and

b*: 10.89 and L*: 58.78; a*: 16.20 and b*: 16.20 respectively by Coşkuner *et al.*, (2002).

The verage nutritional figures for the tubers have been quoted as protein, 3.77 %, 6.72 %; crude fiber, 4.81 %, 8.91 % and sucrose, 19.02 %, 13.4 % by Coskuner *et al*., (2002) and Pascual *et al*., (2000) respectively. Excluding histidine, Bosch *et al*., (2005) has been reported it to contain higher essential amino acids (mg/g protein) than those recommended for satisfying adult needs by the FAO/WHO. Tiger nuts have a fairly good amount of some essential minerals like magnesium, 43µg/g; potassium, 265µg/g; zinc, 158µg/g and 20-28 % of a yellowish non-drying oil (Fatoki *et al*., 1995). Dubois *et al*., (2007) have also reported that the main fatty acids present in tiger nut oil are 14:0 (0.2%), 18:0 (3.2%), 20:0 (0.4%), 16:1 (0.3%), 18:1 (72.6%), 18:2 (8.9%), and 18:3 (0.4%), similar to olive and hazelnut oils.

### 2.1.3 Economic uses of tiger nut tubers

In Spain and Latin American countries, tiger nut is used in making the popular milk drink 'horchata de chufa' (Coskuner *et al*., 2000; Cortes *et al*., 2004). It is a typical product of Spain and of great economic importance. Production is estimated at 40-55million liters per year (Arranz *et al*., 2006). In the United States of America however, tiger nut has mainly used to attract and feed water fowls and cranes as well as ducks (Mosquera *et al*., 1996). In Africa, tiger nuts are an important food crop for many people. They are eaten raw after washing or after it has been soften by soaking in water for sometime. It has also been reported to be roasted and chewed like roasted groundnuts or grated and used for the production of ice creams, biscuits or as a substitute for

coffee (Abbiw, 1990; Dokosi, 1998). They are also used to produce non alcoholic milky looking beverages (Sanful 2009; Ukwuru and Ogbodo, 2011)

### 2.1.4 Tiger nut milk

Tiger nut milk, called *horchata de chufa* in Spain is also very popular in some South American countries (Cortes *et al*., 2004; Corrales *et al*., 2012). The refreshing non-alcoholic beverage of milky appearance is normally produced from dried nuts which are ground and extracted with water (Kay, 1987). The milk beverage has a pH in the range 6.3-6.8 and is rich in starch (Cortes *et al.,* 2005, Belewu and Belewu, 2007). Heating the tiger nut milk beverage above 72°C therefore results in changes in the physical and organoleptic characteristics due to the gellification of the starch (Cortes *et al.,* 2005). The physical, chemical and sensory characteristics of the tiger nut milk beverage have been found to depend on factors, such as the variety of tuber and procedure for beverage production (Belewu and Belewu, 2007; Navarro *et al.*, 1984). The nutritional value of a typical fresh drink with 10 % sugar added has been reported as fat, 3 %; protein, 1 %; starch, 3 % and other carbohydrates, 20 % (De Venanzi, 1991). The composition of Fatty acids of the drink has been found to be very comparable to that of olive oil, with oleic acid constituting about 75 % while palmitic and the other unsaturated fatty acids mainly linoleic and linolenic were 12.5 % and 10 % respectively (Morell and Barber, 1983).

In a study on the composition of tiger nut milk by Cortes *et al*., (2005) arginine, glutamic acid and aspartic acids were the major acids recorded. The following essential amino acids; isoleucine, leucine, lysine, methionine, phenylanine, threonine, tryptophan and valine were also found to be in

appreciable quantities. The flavor of *horchata de chufa* has been described differently by researchers as nutty, pleasant and refreshing, chalky coconut, and vanilla-like (Reid *et al*., 1972; Barber, 1981; Linssen *et al*., 1989, De Venanzi, 1991).

### 2.1.5 Tiger nut industry in Ghana

In Ghana tiger nuts is called "atadwe" by the "akans", "atangme" by the "Gas", "fie" by the "Ewes" and "nansaxa" by the "Dagombas" (Dokosi, 1998). The main types cultivated in Ghana are the black and brown varieties of which based on shape, colour and size of the nuts, Tetteh and Ofori (1998) identified seven types. These were designated as Kwahu I, II, III and IV and Fanti I, II and Ill. Anyidoho (2006) observed that the brown types were only planted in the forest areas of Kwahu in the Eastern Region while both the brown and the black types were planted in Bawjiase in the Central Region of Ghana.

Commercially, it is grown at very few places though records showed that it could thrive in all the six major agro-ecological zones i.e. rain forest, deciduous forest, forest-savannah transition, coastal savannah and northern (interior) savannah which comprises Guinea and Sudan Savannahs (Irvine, 1969; Tetteh and Ofori, 1986; SRID , 2011). Commercial cultivation is in areas such as Kwahu Aduamoa and Esereso in the Kwahu North District of the Eastern Region. In the Central Region, it is cultivated at Bawjiase and surrounding villages in the Awutu Senya East District, Ampenyi and its surrounding villages in the Komenda Edina Eguafo District, Twifo Praso and surrounding villages in the Twifo Praso Lower Hemang District and New Ebu and surrounding villages in the Abura Aseibu Kwamankese District. They are

also cultivated in commercial quantities in Adansi Danyameso in the Adansi South District in the Ashanti Region, Tanoso and surrounding villages in the Sunyani District of the Brong Ahafo Region and Tampiong in the Savelugu Nantom District in the Northern Region.

There are two main planting periods coinciding with the major and minor cropping seasons. A study conducted in Kwahu Aduamoa by Tetteh and Ofori, (1986) on the cultivation of the tubers showed that women constituted about 70 % of the farmers while the men were about 30 %. Preparation of the land involves clearing of land and making of mounds with the height of the mound ranging from 15-30 cm depending on soil conditions (Tetteh and Acheampong, 1999). The mounds are for conservation of water and provision of large volume of loose soil for nut growth and development (Tetteh and Acheampong, 1999). Tubers are normally propagated either by hand or with a cutlass after being soaked in water for 24-36 hours. It is also normally intercropped with maize and takes between 3-4 months to maturity (Wills, 1962; Dakogre, 2008). Unlike Valencia in Spain where the industry is well developed, harvesting and primary processing of the tubers in Ghana are very laborious. The tubers are manually harvested and the plant dried for a few days before the tubers are separated for cleaning, sorting and further drying in the sun on mats. The tubers are mainly cleaned of soil particles by rubbing the nuts together in a basket in many growing areas. In a few places like Kwahu Aduamoa however, tubers are washed in water bodies to remove the adhering soil before drying and storage. While Wills (1962) reported a yield of about 1500lbs per acre (1680kg/ha), Ofori (1994) in a study at Kwahu Aduamoa quoted a yield of 2210 -11300kg/ha. The nuts deteriorate very fast when

stored in containers such as polythene bags due to the heat generated. Thus they are normally stored in jute sacks and baskets and placed on raised platforms in airy places for long storge (Tetteh and Ofori, 1998).

Harvested tubers are distributed through intermediaries who buy bulk and retail from almost all the major markets, in the regions and districts. According to Opare (2005), the unit of measurement and sale of tiger nut by wholesalers is the use of jute sacks and "olonka" (1000 g) while the retail unit is the tomato purée tin (70 g) and the milk tin (170 g). Locally, direct sales of the nuts are done by vendors who repackage the nuts purchased into plain polythene bags and sell in head pans or on table tops in communities. The popular places one can find tiger nuts include areas with heavy vehicular traffic and heavily populated places such as lorry stations, areas around motor tollbooths and road intersections. Presently, the bulk of harvested tubers grown in Ghana are eaten raw as a snack or used as pudding. Normally, this pudding is prepared and sold at lorry stations, market places and construction sites. It is eaten by varied number of people including babies and adults. Drivers who travel for long hours are known to carry the nuts along their journey to chew to prevent them from dosing off. Furthermore, it is also chewed by some men based on the perception that it improves sexual performance (NVTI, 1985). Records however indicate that since the early 70's some quantities of tubers were exported to Spain, Japan, USA and England (Harris, 1973; Ofori, 1994; Pascual *et al.,* 2000). In 2004 for example, about 3 million kilograms were exported to Spain from Ghana, Nigeria and Togo (Arranz *et al.*, 2006).

## 2.2 Dairy milk

Dairy milk has been defined as the secretion, excluding colostrums, from a lactating mammal (Egan *et al.,* 1981). It is remarkable among natural foods because of the great variety of processed foods prepared from it. From liquid milk; whole milk powder, full cream unsweetened evaporated milk, full cream sweetened evaporated milk, full cream sweetened condensed milk and a host of others are produced. It can be considered as containing three basic components, namely water, fat and non fatty solids. The organic matter in the non-fatty portion consists mainly of the protein casein, albumin and globulin, lactose, lactic and citric acids (Egan *et al.*, 1981). Table 2.1 is an example of the average composition of milk from different mammals.

**Table 2.1 Composition of milk from different mammals**

| | Constituents (%) | | | |
|---|---|---|---|---|
| **Mammal** | **Water** | **Protein** | **Fat** | **Lactose** |
| Cow | 87.3 | 3.3 | 3.3 | 4.5 |
| Human | 87.1 | 1.0 | 4.5 | 6.8 |
| Buffalo | 83.4 | 3.8 | 6.9 | 5.2 |
| Goat | 86.9 | 3.6 | 4.1 | 4.5 |
| Sheep | 80.7 | 6.0 | 7.0 | 5.4 |

Source: (Pennington and Church, 1985)

Though milk from different ruminants like goats and buffalo are used for food by humans, the most consumed is cow milk (Minifie, 1989, Ramli *et al*., 2006, Krupa *et al*., 2011). Nutritionally, milk is known to be an important source of proteins, calcium, phosphorous and magnesium. It is also a good source of

vitamins A and D, thus contributing significantly to the nutritional quality of foods containg milk and its products (Lees and Jackson, 1985; Minifie, 1989; Krupa *et al*., 2011). Because of the balance of essential amino acids and a good amount of riboflavin ($B_2$) and niacin, the protein is classified as high quality (Harper and Hall, 1976; Park *et al*., 2007). Lactose, a disaccharide, also called milk sugar is the main carbohydrate in milk. The pH of fresh milk is between 6.4-6.7, with the value kept within this range by proteins, phosphate and citrate, acting as buffers (Fox and Cameron, 1989). During fermrntation the taste of the milk turns sour as the pH drops till it reaches 5.2 when the milk curdles and the casein is precipitated in the form of flocculent curds (Fox and Cameron, 1989).

Milk products have for a very long time constituted a very important ingredient in the food industry because of the many properties it imparts to products. It increases significantly the nutritional value of products due to its minerals and proteins. It is also known to improve the shelf life of chocolate products by inhibiting fat bloom and plays an important role in taste and color (Shukla, 1994). Milk fat is also commonly used in chocolate because of its lower price compared to cocoa butter. Adding milk fat to chocolate however influences the physical properties of chocolate masses such as softening the texture of the final product (Attaie *et al*., 2003). Though processing of milk has resulted in many products which have become very useful ingredients in the chocolate confectionery industry (Lees and Jackson, 1985; Minifie,1989), the bulk of the milk products used in chocolate confectionery in Ghana are full cream milk powder, skimmed milk powder and whey powder (Awua, 2002).

### 2.2.1 Deficiencies and challenges of dairy milk

In spite of the versatility of milk and its products, its composition has not made it the first choice for some consumers. Some proteins in cow milk such as α- lactoglobulin and ß- lacto globulin are known to be the most frequent causes of food allergy in infants (Ah-Leung *et al.*, 2007). It is also well known that allergy to these proteins may continue in childhood through to adulthood and can be severe (El-Algamy, 2007). Lactose intolerance, a condition due to the poor digestion of lactose is also another condition limiting the consumption of milk and its products. It is very common in Asians, South Americans and Africans. Of the world's population, 75 % is estimated to be lactose-intolerant with the most common form primarily affecting adults (Ouhrabkova *et al.,* 2010). Another factor is the growing awareness of the risk in consuming foods with high saturated fatty acids and cholesterol such as milk products (Kouane *et al.*, 2005). The relative high cost of dairy milk compared to some of the alternatives like soymilk and its products has also become a major hindrance to its mass utilization as it has puts it out of the reach of the vulnerable groups of society.

## 2.3 Non dairy milk

The use of plant materials in the production of dairy like milk is one possibility that has been identified as a means of meeting the ever increasing demand for proteins, vitamins, minerals, carbohydrates and health beneficial ingredients that would have been derived from consuming dairy milk. Non-dairy or vegetable milk are the general names of plant based milk. It is normally obtained by water extraction of plant materials such as oil seeds and

legumes with a color unique to every plant material (Edwards, 1998). Vegetable milks are colloidal emulsions and suspensions which have particles or droplets with at least one linear dimension in the size range of 1 nm to about 1 mm (Dickinson, 1992). The composition of the emulsion is usually the small fat or oil droplets, stabilized with surface-active agents such as proteins and lipids. The suspensions however are the undissolved solid particles, such as protein, starch, fiber and other cellular material, which are present in the aqueous solution. While the insoluble particles in the solution can aggregate and form sediments, the less stable droplets in emulsion can also merge and increase in size over time (Dickinson & Stainsby, 1988).

Non dairy milk has attracted a lot of interest because of its potential as a low cost and healthy alternative to cow milk (Wang, 1980; Diarra *et al*., 2005). In comparison to cow milk, they have no significant quantities of cholesterol or lactose, which makes them an attractive alternative to bovine milk for people conscious of their health and/or diet, vegetarians, and those who are lactose intolerant. The intensification in the demand for non dairy milk products by consumers has lead to development of alternatives to animal milk products such infant formulas (Benward and Benward, 2000); low sugar and low calorie milks for dietetic and diabetic purposes (Demirag *et al.*, 1999); and non dairy fermented products (Martensson *et al.*, 2000; Shirai *et al.*, 1992). Table 2.2 is the proximate compositions of dairy milk as compared to some selected vegetable milk.

**Table 2.2: Proximate composition of dairy milk and some selected vegetable milks**

| Milk Samples | Constituents (%) | | | | |
|---|---|---|---|---|---|
| | **Moisture** | **Protein** | **Fat** | **Carbohydrate** | **Ash** |
| Dairy milk [1] | 87.20 | 3.50 | 3.70 | 4.90 | 0.70 |
| Soy milk [1] | 92.50 | 3.40 | 1.50 | 2.10 | 0.50 |
| Peanut milk [2] | 90.00 | 2.10 | 4.40 | 3.47 | 0.02 |
| Cowpea milk [3] | 91.22 | 3.23 | 2.05 | 3.29 | 0.21 |

Sources: Banzon and Velasco (1982) [1]; Nadutey (1999) [2]; Sanni *et al.*, (1999) [3]



### 2.3.1 Pre-treatment of plant materials and Production of vegetable milk

The preparation of plant materials before milk extraction is a very important activity for ensuring high quality milk and solids (Kuntz *et al*., 1978; Sopade and Obekpa, 1990; Ndjouenkeu and Djomdi Ejoh, 2007). It is commonly done after sorting out of extraneous matter such as unwanted leaves, under developed materials, sand particles etc which can impart negatively on the taste and quality of the final product. It includes winnowing, soaking, cooking and application of chemicals (Nelson *et al*., 1987; Chan and Beuchat, 1992; Tunde-Akintunde and Souley, 2009). According to Pan and Tangratanavalee, (2003), water absorption during soaking is directly related to the changes in textural characteristics and grinding properties of plant materials. It is beneficial in reducing its size and separating the fiber from other components in the grinding process besides reducing the grinding time and energy.

Heating conditions are one of the important variables in the processing of materials for non dairy milk extraction (Quasem *et al*., 2009). It is reported to destroy moulds and bacteria, which can affect the quality of the milk (Nout,

1993) as well as destroy anti-nutritional factors like trypsin inhibitors (Bressani and Elias, 1983). It has also been reported to modify the functional properties of seeds for the enhancement of emulsions and dispersions, and improvement in yields of total solids and flavor (Shi and Ren, 1983; Quasem *et al*., 2009). The heat treatments given also affect the colour and the flavor of the milk. The heat applied should however not be excessive since that can reduce the quality of protein in the milk through denaturation, maillard reactions as well as the destruction of thermolabile nutrients like vitamins A, B, C and E. It may also lead to the gelling of starch resulting in the reduction of extraction of milk (Han *et al*., 1988).

The widely differing characteristics of plant materials i.e. leafs, stems, roots and fruits, the composition of useful nutrients as well as development in technology and equipment however have given rise to such diverse protocols for the production of non dairy milk (Boloorforshan and Markakis, 1979; Priepke *et al.*, 1980; Zaruwa *et al*., 2005; Belewu and Belewu, 2007; Aidoo *et al.*, 2009). The major process factors involved in extraction of ingredients from plant materials are particle size of material, ratio of solvent to raw material and extraction temperature (Mani *et al.*, 2007; Djomdi Ejoh and Ndjouenkou, 2006). In non dairy milk extraction, plant materials are either dry milled with attrition or hammer mills or wet milled with industrial blenders (Harjai and Singh, 2007; Gesinde *et al.*, 2008; Hajirostamloo, 2009). Water is the main solvent for the production of plant milk. The production is basically based on the penetration of water into cells of pulverized meal. The pulverized nature of the meal permits its better dispersion in the aqueous medium, while the availability of more liquid increases the driving force of solids out of the

plant material (Milani *et al*, 2011). In most cases heat and agitation of the medium are applied to speed up the extraction process. The heating of water increases the temperature of the medium and the tenderization of the tissues while the agitation influences the rate of water absorption (Kuntz *et al*; 1978; Sopade and Obekpa, 1990) and a transfer of materials from the cells into the water resulting in better quality milk. The extracted milk is normally heat treated to ensure food safety and extended shelf life through the inactivation of undesirable biologically active compounds such as trypsin inhibitors and lipoxygenase (Liener, 1994).



### 2.3.2. Nutritional and sensory quality of non dairy milk

Non dairy milk is mainly made up of insoluble solid particles, such as undissolved protein, starch, fiber and other cellular material, which are suspended in the aqueous solution (Nadutey, 1999; Sanni *et al*., 1999; Belewu and Azeez, 2008). Compared to animal milk, non dairy milks have no significant cholesterol or lactose. Many vegatable milk are however lower in protein, calcium, vitamins such as D and B12, as well as riboflavin than cow's milk (Banzon and Velasco, 1982; Nadutey, 1999; Belewu and Azeez, 2008). This shortfall should not be a bother for those whose diets contain other adequate sources of these nutrients. However for consumers who rely on non dairy milk for a significant portion of their daily protein needs, or for those who need additional protein such as young children and lactating mothers, they can go for beverages such as soy milk that contains as much as 3.50 % protein (about the same as cow milk), 2.00 % fat, 0.50 % ash and 2.90 % carbohydrate (Riaz, 1999). To address the concerns of many consumers, many producers of non dairy beverage are now improving upon the quality of their

products by fortifing them with vitamins and minerals (Vegetarian Nutrition, 2008).

More systematic efforts to overcome some of the nutritional deficiencies in phyto milk have led to studies into composite milk products. These are milk products obtained from more than one plant source. Typical examples are the combination of cowpea, which is known to have low energy and beany flavor with peanut to improve upon energy and reduce the unacceptable flavor (Nadutey, 1999; Asiamah, 2005; Aidoo *et al.*, 2010). Creating synergy by blending vegetable milk with dairy milk has also been recognized as an option (Berry, 2002). "kishk" is a fermented cereal milk with cow milk mixture available in Lebanon (Tamime and O'Conner, 1995). Rasogolla (an Indian delicacy made out of milk) has also been successfully prepared from a blend of cow milk and safflower milk (50:50). The product made from such milk blend was 13.0 % cheaper than the one made exclusively from cow milk (Lokhande *et al.*, 2010)

The broad spectrum of sensory characteristics, including appearance, after-taste, aroma and flavor are the important factors for judging the acceptability of non dairy milk. Generally the beverages assume the characteristic flavors, colour and mouth feel of the material from which it is produced. Grassy-beany flavors or green off-flavors, suspension instability and chalky mouth feel associated with soymilk have been found to be associated with compounds that pre-exit in the maturing of soybeans and those generated during processing (Nelson *et al.*, 1976; Kuntz *et al.*, 1978; Rackis *et al.*, 1979; Rubico *et al.*, 1987; Galvez *et al.*, 1990).

Colour, a very important indicator for determinig the quality of many non dairy milk products is derived from the natural pigments in the plant materials (Wang and Murphy, 1994). Generally White or cream coloured products are more readily accepted by consumers since such products resamble cow milk (Vegetarian Nutrition, 2008). Enzymatic and non-enzymatic browning reactions if not properly controlled could however lead to the formation of water soluble brown, gray, and black colored pigments which are unacceptable to many consumers (Vegetarian Nutrition, 2008). The need for improved sensory attributes (flavor and mouth feel) of non dairy milk beverages have led to production of milk from different pre-treated materials (Hinds *et al.*, 1997; Sanful, 2009) and blending of non dairy milk with cocoa products (Iserliyaska *et al.*, 2012). De-fatting materials before the production of milk; removal of flavor compounds by evaporation (deodorization using steam under pressure) after milk production; masking the bitterness and off-flavor by sweetening and flavoring (e.g. with chocolate and coffee flavor) have also been used to improve upon the quality of milk produced at the small scale level (Wang *et al.*, 1997).

### 2.3.3 Anti-nutritional factors in non dairy milk

Anti-nutritional factors reduce the nutritional value of foods The major sources of vegetable milk are from legumes which are known to contain several anti-nutritional factors such as trypsin inhibitors, lectins, phytates and poly-phenols (Sathe & Salunkhe, 1984). Several studies however show that pre-treatment for milk extraction such as soaking and cooking eliminated or reduce significantly these anti-nutrients (Desphande *et al.*, 1984; Salunkhe and Kadam, 1989; Liener, 1994). The use of genetically engineered plant materials

devoid of anti-nutritional factors e.g. lipoxygenase in the production of soymilk have also been explored to improve upon the quality of vegetable milk (Wang *et al*., 1997).

### 2.3.4 Vegetable milk powder

Dehydration of milk gives the product some advantages. These are longer shelf life, less storage space requirement and lower cost of bulk packing. To date several dehydration processes have been used for the production of non dairy milk powder, including roller, spray and freeze drying (Schwartz *et al*., 1984; Aidoo *et al*., 2010). A typical dehydration process is the patented process by BUHLER called "The BUHLER Process for Soy Micro-powder". Unlike the conventional soymilk powder production, this process results in de-hulled, micro-milled, full-fat soy flour. It involves a hot de-hulling process and micro milling of the cotyledon into powder (Gavin and Wettstein, 1990).

## 2.4 New Product and Process Development

New product and process development are central to many firms' future success. Smith and Reinertsein (1991) have stated that while every company's motivation will vary, the following are some of the reasons: more sales, better return on investments, staying ahead of competitors, reaction to changing trends and opportunities on the market and maintaining of its leadership position. The task of developing products that meet customer's expectation however is a complex one. The initial creative step is to examine the various formulations and processing technologies to select those that seem to have the potential to deliver the desired product characteristics. The second step is to determine the optimum formulation and processing conditions while the third

step is to generate the appropriate formulation and processing tolerance (Joglekar and May, 1990).

There are many analytical tools that increase the effectiveness of the product or process development process. The usual approach is to conduct one-variable-at-a-time experiments. Though it is easy and simple to set up, its weakness as a method include inability to determine the most acceptable combination of factors involved as well as the effect of their interactions on the desired results (Joglekar and May, 1990; Montgomery, 1997). Response surface methodology (RSM) is a collection of statistical and multiple regression tools that are used to evaluate a response of interest from experimental designs. The result is graphically represented as response surfaces which can be used to describe how the test variables affect the response, determine the interrelationships among the test variables and establish the optimum response region (Giovanni 1983, Montgomery, 2005).

Basically RSM is a four-step process. First, two or three factors that are the most important to the product or process under study are identified. Second, the ranges of factor levels, which will determine the samples to be tested, are defined. Third, the specific test samples are determined by the experimental design and then tested. Fourth, the data from these experiments are analyzed by RSM and then interpreted (Giovanni, 1983).

RSM has been used in food research in optimizing ingredients (Espinola et al., 2011; Kuila et al., 2011; Ahmed et al., 2011) and process variables (Floros and Chinnan 1988; Mudahar et al., 1990; Galvez et al., 1990; Vainionpaa, 1991) or both (Bastos et al., 1991).

### 2.4.1 Crude fiber

Dietary fiber is an essential component of food materials. It has been defined as the component of food that is not broken down by secretions in the digestive tract (Fox and Cameron, 1989). It is almost entirely composed of polysaccharides, especially cellulose, hemicelluloses, pectin, protopectin and lignin. Accumulating evidence from recent studies now favours the view that increase intake of dietary fiber can have beneficial effects in humans (Anderson et al., 2009). Intake of dietary fiber is believed to reduce the risk for developing cardiovascular diseases, certain gastrointestinal disorders as well as improving serum lipid concentrations and improving immune function (Anderson et al., 2009).

Dietery fiber can be classified into soluble and insoluble fractions based on their water solubility. Insoluble dietary fibre (IDF) includes celluloses, some hemicelluloses and lignin. Foods that are rich in insoluble fiber are reported to decrease transit time of food in bowels as well as increase fecal weight and volume. A good example is wheat bran. Soluble dietary fibre (SDF) includes β-glucans, pectins, gums, mucilages and some hemicelluloses. Foods rich in soluble fiber such as dried beans, oat products and vegetables are known to delay gastric emptying and enhance satiety (Anderson, 1986; Jenkins et al., 1987).

### 2.4.2 Fiber fortification in foods

Dietary fiber may be used in recipes formulations for its physiological and functional properties (Lario *et al.*, 2004). Physiologically it can be incorporated into foods to manage health conditions that have been associated with the reduced intake of fiber such as diabetics, and cardio vascular diseases

(Anderson *et al.*, 2009). Functionally it can be used as a fat and sugar replacer, texture, mouth feel and volume enhancer, binder, bulking agent and stabilizer (Alesson-Carbonell *et al*., 2005a, 2005b; Borderías *et al*., 2005; Fernández-López *et al*., 2008; Sendra *et al*., 2008; Hu *et al*., 2009). The source of fiber however affects the nutritional and functional properties due to the differences in the structure and constitution of plant cells (García *et al*., 2007; Fernández- López *et al*., 2007; Besbes *et al*., 2008).



## 2.5 Chocolate products

### 2.5.1 Chocolate manufacture

Chocolate is made from the basic ingredients cocoa butter and cocoa liquor with milk solids, emulsifiers and sugars. The composition of chocolate varies according to the type of chocolate. Traditionally the mixture is kneaded and refined in two stages by roll refiners. The refined mass is then transferred to a hollow vessel called a conche within which the mixture is continuously mixed with more butter, lecithin and vanillin for 18 hours or more after which the masse is pumped into tanks for use (Lees and Jackson, 1985; Minifie, 1989).

### 2.5.2 Chocolate milk beverage

Chocolate milk is defined as whole, low-fat or skim milk which has had cocoa (chocolate) and a sweetener added to it. It is a very effective way of imparting the cocoa taste particularly when consumed hot as the liquid character means almost instant exposure to the flavor components (Cacao De Zaan, 1993, Awua, 2002). Chocolate milk beverage has great amount of carbohydrates, proteins and electrolytes more than that of plain milk (Abd El-Khair, 2009). The challenges of chocolate milk drink however lie in the stabilization of what

is inherently an unstable system (Cacao De Zaan, 1993). Only a part of the cocoa powder normally dissolves in the milk with the particles that are heavier than the milk sedimenting over a period of time. In order to hold the cocoa particles in suspension a relatively high viscosity is required. Kappa carageenan is a stabilizer that is used to achieve this effect (Cacao De Zaan, 1993).

### 2.5.3 Chocolate spread



It is a high fat chocolate confectionery produced using cocoa powder. It comprises of vegetable oil, sweeteeners, milk, nuts and cocoa powder for colour and flavor. They are normally applied on pastries and biscuits for snack or for breakfast. Its manufacture normally consists of the following processes; mixing of ingredients, milling to desired particle size, tempering and finally packing.

### 2.5.4 Quality characteristics of chocolate products

Quality explains the specifications of a product or service and the satisfaction the consumer receives (Hoyer *et al.,* 2001). Chocolate products, like many foods have many features and characteristics that are of interest to consumers and legislative bodies.

#### 2.5.4.1 Nutritional and health benefits of cocoa/chocolate products

Cocoa/Chocolate like all foods have nutritional value that are directly related to the amount and kind of proteins, carbohydrates, fats, minerals and vitamins they contain and the presence and absence of minor ingredients such as alkaloids, tannins and similar compounds.

### 2.5.4.2 Fats

Chocolate products nornally contain appreciable quantity of Cocoa butter which contains useful amounts of vitamin D and E (Cacao De Zaan, 1993). Considering the fact that fats have a high calorific value than other food ingredients (FAO, 2006), cocoa butter in food products increases its energy value. Again the fact that digestion of fat is the slowest of food ingredients also makes cocoa/chocolate food supply energy to the body slowly; giving the body a longer feeling of satisfaction (Sørenson and Astrup, 2011). Though well documented that saturated fats present a high risk of atherosclerosis, and thus heart diseases (Mitchell *et al*., 1989; Oh *et al*., 2005), studies show that the main lipid in many chocolate products, a stearate does not seem to augment the risk of heart diseases (Kris-Etherton, 1997). The underlying reason appears to be one of limited absorption from the intestinal tract, in contrast to most other lipids that are readily absorbed (Deaker, 1996; Kris-Etherton, 1997; Kris-Etherton *et al.*, 2000) thus exerting a neutral effect on the total and the LDL cholesterol levels, which are known to make one prone to cardiovascular diseases.

### 2.5.4.3 Carbohydrates and fibers

Carbohydrates constitute a major portion of many chocolate recipes with sucrose dominating (Chocosuisse, 2001). Though the energy levels of the carbohydrates are not as high as that of the fat (FAO, 2006) it is readily available, making chocolate especially useful for those in physical pursuits. This is the reason why chocolates, candy, cookies, chocolate milk and similar foods are so commonly used by workers, athletes and children between meal breaks (ICCO, 2005). Fiber however is mostly contributed by cocoa liquor,

cocoa powder, nuts or cereals which are included in some recipes or in bar chocolates. In recent times the health benefits from consuming large amounts of fiber including that of cocoa have continued to receive attention (Anderson *et al.,* 2009). Some of the health benefits include the prevention of obesity, diabetes, arteriosclerosis, colon/rectal cancer and constipation (Cummings *et al.,* 1976; Dukehart *et al.,* 1989).

**2.5.4.4 Proteins**

One important value of proteins is for the building of body tissues. More important however are the levels and the variety of amino acids present in the proteins. Though cocoa contains some protein (Cacao De Zaan, 1993) its content and quality is not adequate to meet the nutritional and physiological requirements of an active person. The addition of other ingredients like milk powder to chocolate products however is known to improve the profile of amino acids in developed products (Awua, 2002).

**2.5.4.5 Vitamins**

Cocoa and chocolate products are good sources of fat soluble vitamins because of cocoa butter which contains appreciable quantities of fat soluble vitamins A, D, E, and K. The addition of other ingredients like milk, nuts and fruits however improves the water soluble vitamins such as B1, B2, B6, C and folic acid contents of the products (Cacao De Zaan, 1993).

**2.5.4.6 Minerals**

The cocoa component of chocolate products is a rich supply of minerals including manganese, copper, sodium, iron, phosphorus and zinc, which perform important roles in the physiology of humans (Cacao De Zaan, 1993). Natural cocoa powder however is the second highest natural food source of

magnesium (Wester, 1987) which is a very important micronutrient in human nutrition. It is found in the intracellular fluids of the body. The required daily intake is around 300 mg. In the body it is essential for the production and activation of many enzymes that regulate the metabolism of proteins, carbohydrates, lipids, nucleic acids and nucleotides. Manganese also helps with the formation of bones and teeth and assists in the adsorption of calcium and bones and thus helps to prevent hypertension, heart disease and joint problems by keeping muscles and joints supple (Wester, 1987).



**2.5.4.7 Cocoa polyphenols**

Diseases such as cancer, heart diseases and some cerebrovascular deseases have been found to be caused by free racicals (Lee *et al.,* 2003). In recent times however some natural foods have also been found to contain antioxidants such as vitamins and phenoloic compounds which prevent the damage caused by these free radicals. Several scientific sudies now shows that cocoa and chocolate products have polyphenols which have proven natural antioxidant composition (Adam *et al.,* 1931; Knapp and Hearne, 1939; Forsyth, 1955; Lee *et al.,* 2003). Ariefdjohan and Savaiano, (2005) and Engler and Engler, (2006) have reported that there was a 50 % lower rate of death due to cardiovascular disease and stroke in older men who consumed the highest amount of chocolate and or cocoa compared to those who did not due to the fact that the flavanols found in cocoa chocolate products reduced oxidation of LDL cholesterol, reduced platelets aggregation, increased arterial blood flow and decreased blood pressure.

### 2.5.5 Analyzing chocolate products

#### 2.5.5.1 Sensory evaluation

Sensory analysis is a scientific discipline that is concerned with providing answers to questions about product quality, questions concerning new and existing in-house and competitor products and provides input for decision making in new product development. Product tasting in the chocolate industry is as important as chemical and microbial evaluation of products for quality. However, though specific minimum and maximum levels have been developed for nutritional and microorganism levels, there are serious disagreements on what a good quality chocolate product should taste like (Awua, 2002). This is because chocolate products are consumed not only because it creates good feelings, for its antioxidants or for its nutrition but also because it tastes good (Awua, 2002).

Sensory analysis has to do with measurement. However, since it is largely a subjective process it is statistically transformed to an objective assessment to be of use to a food manufacturer in the areas of new product creation or improvement, and quality control (Gacula and Singh, 1984; Cacao De Zaan, 1993). Sensory analysis can be grouped into three main categories; discrimination- which evaluates whether or not a difference exists between two or more products, description- which describes and measures any differences that are found to exist between products and preference or Hedonics-which identifies liking or acceptability amongst producdts (Carpenter *et al.*, 2000).

### 2.5.5.2 Important sensory attributes of chocolate products

Appearance and color are important visual attributes for deciding the acceptability of new product including chocolate (Carpenter *et al.*, 2000; Aidoo *et al.*, 2010). These properties mainly depend on composition, added coloring agents, stabilizing agents and processing parameters, especially heat treatment (Cacao De Zaan, 1993). With chocolate flavored drinking beverages, the amount of powder is one of the main factors that controls color and appearance of the product (Cacao De Zaan, 1993). Flavor is also an important sensory attribute of chocolate products because it is a characteristic that consumers can readily determine (Lund, 1982). The flavor of the product however depends on other ingredients present in the chocolate recipe which can also contribute to the products flavor. Flavor is a very important factor in products formulated using non dairy milk because of flavors extracted from the raw materials. Raw legumes and oilseeds for example are rich in lipoxygenases and other metallo-proteins, which are precursors for unacceptable aldehydes and ketones flavor compounds (Rackis *et al.,* 1979).

Mouth feel of food is an attribute that is associated with consumer acceptability of many foods (Folkenberg *et al.,* 1999). In chocolate, the type of ingredients, the milling technology and the particle size of ingredients in the final product are the major factors that affect the mouth feel of the products (Awua, 2002). In many chocolate products, mouth feel has been found to have a relationship with the physical and flow properties of the product (Folkenberg *et al.,* 1999). The presence of ingredients like nuts and flour products that leave grainy particles in the mouth and throat have been found to negatively affect the mouth feel of products imparting a chalky feel Kuntz *et al.,* (1978).

After-taste is the unacceptable lingering sensation such as bitterness or astringency that persists in the mouth after eating food (Wang *et al*., 2001). In chocolate products, a lingering chocolaty flavor is the expected after-taste. Its absence or masking suggests poor quality of cocoa products or other ingredients in the recipe. Overall acceptability is a sensory attribute, which defines combination of all the different attributes together. Higher overall acceptability scores indicate that the product has good chances of being purchased and tried by consumers if launched in the market. Most of the times, the consumer response is judged on the overall acceptability in optimization studies along with other characteristics such as flavor, after-taste, colour and mouth feel.

To date sensory evaluation has been applied in the development of several products including non dairy milk beverages and chocolate products (Aidoo *et al*., 2010; Quansem *et al*., 2009; Sanful, 2009).

## 2.6 Shelf life studies

Shelf life of a product is an indication of the length of time that a product is acceptable to consumers. The shelf life of a food product is vital to its success in the marketplace and is an extremely important attribute for any new product. Product shelf life is affected by many factors including the post harvest handling of the raw materials, ingredients in the recipe formulation, the processing technology, packaging and distribution (Robertson, 1993).

The principal mechanisms involved in the deterioration of processed foods have been noted by Robertson (1993), as follows:

1. Microbiological spoilage; sometimes accompanied by pathogen development.
2. Chemical and enzymatic activity causing lipid breakdown, color, odor, flavor, and texture changes.
3. Moisture and/or other vapor migration producing changes in texture, water activity and flavor.

In the design of shelf life studies important consideration is given to the likely causes of deterioration that will limit the shelf life of the product. Some of the sources of this information may studies conducted in the organization data on competitive products, scientific literature and information on ingredient made available by suppliers (Labuza and Schmidl, 1985; Saguy and Karel, 1987).

# CHAPTER THREE

## 3.0 EFFECT OF PLANTING PERIOD AND SITE ON THE CHEMICAL COMPOSITION AND MILK ACCEPTABILITY OF TIGER NUT (*CYPERUS ESCULENTUS* L) TUBERS IN GHANA

### 3.1 Introduction

Tiger nut (*Cyperus esculentus* L.) is a perennial crop that is cultivated extensively in Spain, almost exclusively in the Valencia region, Turkey, Southern India, Thailand, Ghana, Nigeria, Sierra Leone and Cameroon (Rosengarten, 1984; Fatoki *et al*., 1995; Er *et al*., 2009; CRDO, 2012). Nutritionally, the tubers contain between 200-280 g kg-$^{1}$ of yellow non-drying pleasantly flavored oil, similar to olive or sweet almond oil (Kim *et al*., 2007; CRDO, 2012). The oil consists of 170-180 g kg-$^{1}$ saturated fatty acids, much of which (120-140 g kg-$^{1}$) is palmitic acid and the rest mainly stearic acid. Of the unsaturated fatty acids present, about 75 g kg-$^{1}$ is oleic acid and between 90-155 g kg-$^{1}$ linoleic (Kim *et al*., 2007). The oil extract has been used in cooking and for the manufacture of body and hair creams (Eyeson and Ankrah, 1975). Of the tuber weight, about 500.0 g kg-$^{1}$ is digestible carbohydrates, 40.0 g kg-$^{1}$ protein and 90.0 g kg-$^{1}$ crude fiber with a high fraction of it being indigestible carbohydrates mainly cellulose and lignin (Linssen *et al*., 1989). Tiger nuts also have a good profile of some essential minerals like zinc, 158 mg kg-$^{1}$; magnesium 430 mg kg-$^{1}$ and potassium, 265 mg kg-$^{1}$(Fatoki *et al*., 1995).

The tubers are used as food around the Mediterranean, especially in Egypt and Spain as well as countries in West Africa, particularly in Cameroon, Nigeria and Ghana. Dried nuts are ground into flour and incorporated into various

foods. It is also baked and milled into powder for use as food additives, spices or made into a refreshing beverage called "Horchata De Chufas" or tiger nut milk (Mosquera *et al*, 1996; Umeri and Enabeli, 1996; Belewu and Belewu, 2007).

In Ghana, the black and brown varieties are mainly cultivated. Though it can be cropped throughout the country, it has traditionally been cultivated at only a few places in the Eastern, Central and the Brong-Ahafo Regions (Tetteh and Ofori, 1998). Harvested tubers are first sorted, rubbed in baskets or washed in water and dried before distribution through intermediaries. The nuts are chewed raw, dried or roasted like sweets, or made into a highly cherished milk-like beverage referred to as "Atadwe" milk. Currently, nearly all nuts grown in Ghana are consumed locally. Due to increasing awareness of the nutritional potential, nutraceutical benefits of the tubers and the perception that it has aphrodisiac properties there is increased cultivation of tiger nuts and explorations of novel uses for its milk (Ablordeppey,2004; Sanful, 2009).

The variety of a plant, location and period of planting has been shown to have significant effects on physico-chemical properties of plant materials (Adjei-Nsiah, 2010; Makeri, *et al*, 2011). However though considerable studies have been done on the proximate composition and mineral content of tiger nuts tubers (Fatoki *et al*., 1995; Pascual *et al*., 2000; Coskuner *et al*., 2002; Oladele and Aina, 2007; Adejuyitan *et al*., 2009) there is very little information on the effect of site and planting period on the chemical composition of its tubers and acceptability of its milk. The objectives of this study therefore were to determine the chemical composition and acceptability of milk of two varieties of tubers from different sites and planting periods in Ghana.

## 3.2. Materials and methods

### 3.2.1. Material samples

Sixteen samples of two varieties of tiger nut tubers (*Cyperus esculentus* L.) grown at Ampenyi, Bawjiase, Danyameso, New Ebu, Tampiong, Tanoso, Kwahu Aduamoa and Twifo Praso in the two planting periods (major; April to July and minor; September to November) were studied using a complete block design. The tubers were sorted, washed and dried in a Sanyo oven (Model MOV-212, Japan) at 55$^{o}$ C till constant moisture content was between 7 to 10 %.The oven-dried tiger nut tubers were milled in a Waring blender (Model 38BL41, USA) to pass through a sieve of pore size 0.5 mm and then stored in airtight containers in an Ocean freezer (Model, NJ55 TB ECO, Italy) at -18$^{o}$C until used for laboratory analysis.

### 3.2.2. Physical and Chemical Analysis

Moisture and crude protein (N x 6.25) contents were determined in triplicate using the air oven drying method at 105$^{0}$C (AOAC 925.10, 2000) and Kjeldahl method (AOAC 960.52, 2000) respectively. Total ash and crude fat, were also determined in triplicate according to the AOAC official methods (AOAC 923.03, 2000; AOAC 920.39, 2000). Crude fibre was determined according to methods described in Pearson's Composition and Analysis of Foods (Egan *et al.*, 1981). Crude protein (N x 6.25) was determined by the Macro-Kjeldahl method using 1.0 g samples. Ash was determined by the incineration of a 1.0 g sample placed in a muffle furnace maintained at 550°C for 6 hours (until ash was obtained). Crude fat (ether extract) was determined by exhaustively extracting 5.0 g of the milled sample with petroleum ether by use of a Soxhlet apparatus. The level of carbohydrate was obtained by the difference method,

that is, by subtracting the sum of the protein, fat (lipid), fiber and ash from the total dry matter. The calorific value was calculated by multiplying the mean values of the crude protein, fat and carbohydrates by the Atwater factors of 4, 9 and 4 respectively, (FAO, 2006). Free fatty acids (FFA) were determined by mixing 10.0 g of Soxhlet extracted oil with 25.0 ml diethyl ether, 25 ml ethanol and 1 ml phenolphthalein and titrating with 0.1M NaOH until a pink color persisted for 15 seconds (IOCCC, 1996). The FFA content was calculated as oleic acid (1.0 ml NaOH is equivalent to 0.0282g oleic acid). Determinations were carried out in triplicate.

3.2.3. Mineral analysis

A wet digestion method using nitric acid (AOAC, 999.11, 2000) was used to eliminate all organic matter from the samples before samples were analyzed for the individual minerals. One gram (1.0 g) of the sample was weighed into a 250 ml beaker. Twenty five millilitres (25 ml) of conc. $HNO_3$ was added and the beaker was covered with a watch glass. The sample was digested with care on a hot plate in a fume chamber until all the organic matter had been oxidized (20-30 min.). The pale yellow solution was cooled and 1 mL of 70 % $HClO_4$ was added with care. Digestion was continued until the solution was almost colorless (until all the $HNO_3$ was removed). The solution was then cooled slightly after the digestion process, and about 30 ml distilled water were added and allowed to boil for about 10 minutes using a Jenway hotplate and stirrer (Model, 1103.2, Essex UK) then filtered when hot through Whatman filter paper number 1 into a 100 ml volumetric flask. The beaker was washed well with distilled water and filtered. The flask was then cooled and made up to the 100 ml mark. This solution was used for all the mineral analysis. The

following minerals; Sodium (Na), Potassium (K), Magnesium (Mg), Calcium (Ca), Zinc (Zn), Iron (Fe), Phosphorus (P), Manganese (Mn) and Copper (Cu) were all determined in triplicate with the use of the Perkin Elmer Atomic Absorption Spectrophotometer (Model AA 220FS, Massachussetts, USA).

3.2.4. Milk extraction methods

The extraction of milk (Figure 3.1) was carried out by the modification of the traditional method of milk extraction as described by Ofori-Anti (2000). One hundred grams (100g) of oven-dried nuts were soaked in covered vessels with 600 mL of water for 12 hours. The water was discarded and the nuts milled in a Waring blender (Model 38BL41, USA) at high speed for 5 minutes with fresh water equivalent to twice the new weight of the soaked tubers. The milk slurry was pressed through a cheese cloth, and the milk obtained was used for analysis.

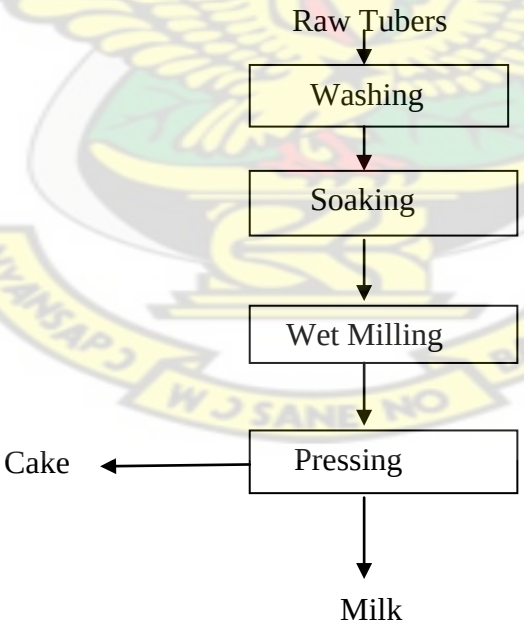



**Figure: 3.1: Flow Diagram for milk production from tiger nut tubers (Ofori Anti, 2000).**

3.2.5. Sensory evaluation of milk

Consumer preference of milk extracted from the tubers was determined using simple ranking test in a randomized complete block design (Montgomery, 2001). The tiger nut milk from each site was sweetened with 20 g $kg^{-1}$ of sugar before ranking by the panelists. Ranking of milk was done by a panel of 10 trained persons on a scale of 1-6. The most acceptable was ranked 1 and the least acceptable was ranked 6 (Appendix B1). Water was available for panelists to rinse their mouth and they were allowed to re-taste samples.

3.2.6. Statistical analysis

The data obtained were evaluated on dry weight basis and subjected to ANOVA using MINITAB 14 statistical package (MINITAB Inc., U.S.A.). Tukey's test ($p < 0.05$) was used to identify significant differences among means and the Friedman's Rank Test was used to determine the rank sums of the treatments used for sensory analysis.

## 3.3. Results and Discussion

The Proximate composition, an indication of the nutritional potential of the tubers and the free fatty acid content, an indication of the effect of post harvest handling and storage on the quality of tiger nuts obtained from different locations in Ghana are presented in Table 3.1.

**Table 3.1: Chemical composition of two varieties of tubers from different sites and periods**

| Variety/ Planting period/ Site | CHEMICAL COMPOSITION | | | | | | |
|---|---|---|---|---|---|---|---|
| | FAT (g kg-$^{1}$) | PROTEIN (g kg-$^{1}$) | ASH (g kg-$^{1}$) | CHO (g kg-$^{1}$) | FIBER (g kg-$^{1}$) | ENERGY (kcal kg-$^{1}$) | FREE FATTY ACIDS (%) |
| ***Black (major)*** | | | | | | | |
| Ampenyi | 262.8(5.6)$^{a}$ | 40.4(1.9)$^{a}$ | 12.3(1.2)$^{f}$ | 596.3 | 84.9(1.4)$^{a}$ | 5016.2 | 0.43 (0.01)$^{a}$ |
| Bawjiase | 174.7(2.8)$^{q}$ | 22.7(1.3)$^{j}$ | 15.4(0.1)$^{a}$ | 678.0 | 109.2(2.5)$^{bc}$ | 4452.9 | 0.81 (0.01)$^{b}$ |
| Danyameso | 155.4(1.0)$^{b}$ | 44.7(1.4)$^{b}$ | 19.4(0.4)$^{g}$ | 677.1 | 103.4(2.2)$^{b}$ | 4370.4 | 1.18 (0.01)$^{c}$ |
| Ebu | 166.0(6.6)$^{q}$ | 33.9(2.8)$^{k}$ | 16.7(0.3)$^{b}$ | 709.2 | 74.2(2.9)$^{i}$ | 4555.1 | 1.11 (0.01)$^{d}$ |
| Tampiong | 242.3(1.8)$^{f}$ | 38.2(1.8)$^{a}$ | 13.6(0.4)$^{h}$ | 617.1 | 88.8(0.6)$^{a}$ | 4879.0 | 0.44 (0.01)$^{a}$ |
| Tanoso | 203.1(5.0)$^{g}$ | 43.4(1.2)$^{b}$ | 17.7(0.6)$^{b}$ | 636.3 | 103.8(8.5)$^{b}$ | 4652.7 | 0.37 (0.01)$^{e}$ |
| ***Black (minor)*** | | | | | | | |
| Bawjiase | 254.0(2.4)$^{s}$ | 73.4(0.2)$^{c}$ | 15.2(0.2)$^{e}$ | 553.8 | 104.1(9.4)$^{b}$ | 4795.7 | 0.47 (0.01)$^{f}$ |
| Danyameso | 295.4(3.2)$^{h}$ | 72.9(1.7)$^{c}$ | 17.6(0.1)$^{p}$ | 497.4 | 118.4(3.8)$^{c}$ | 4939.8 | 0.42 (0.01)$^{g}$ |
| ***Brown (major)*** | | | | | | | |
| Ampenyi | 222.6(2.0)$^{i}$ | 29.8(4.3)$^{e}$ | 14.6(0.3)$^{c}$ | 643.8 | 85.9(3.1)$^{d}$ | 4791.5 | 0.58 (0.01)$^{h}$ |
| Bawjiase | 128.7(3.5)$^{c}$ | 39.1(3.0)$^{d}$ | 15.6(0.3)$^{c}$ | 734.7 | 85.3(2.7)$^{d}$ | 4315.6 | 0.60 (0.01)$^{h}$ |
| Danyameso | 165.5(2.6)$^{j}$ | 56.9(1.7)$^{n}$ | 15.6(0.5)$^{cd}$ | 672.2 | 89.9(1.1)$^{d}$ | 4489.0 | 0.52 (0.01)$^{i}$ |
| Ebu | 128.7(3.5)$^{c}$ | 38.7(3.1)$^{d}$ | 16.4(1.2)$^{d}$ | 717.2 | 102.9(2.2)$^{j}$ | 4241.9 | 1.12 (0.01)$^{j}$ |
| Kwahu Aduamoa | 191.4(4.2)$^{k}$ | 67.6(2.3)$^{f}$ | 15.5(0.4)$^{cd}$ | 646.6 | 75.3(2.2)$^{e}$ | 4691.1 | 0.33 (0.01)$^{k}$ |
| Twifo Praso | 176.6(1.5)$^{l}$ | 56.6(4.8)$^{c}$ | 12.3(0.7)$^{i}$ | 641.6 | 116.2(7.3)$^{k}$ | 4462.4 | 0.43 (0.01)$^{l}$ |
| ***Brown (minor)*** | | | | | | | |
| Bawjiase | 252.8(2.8)$^{m}$ | 75.1(0.3)$^{g}$ | 14.4(0.3)$^{q}$ | 580.0 | 78.6(0.4)$^{de}$ | 4895.6 | 0.92 (0.01)$^{m}$ |
| Danyameso | 275.3(3.5)$^{n}$ | 73.1(1.2)$^{g}$ | 10.0(0.2)$^{j}$ | 506.9 | 135.4(3.7)$^{l}$ | 4795.7 | 0.84 (0.01)$^{n}$ |
| ***Major*** | | | | | | | |
| All Black Tubers | 200.7(41.2)$^{o}$ | 37.2(7.7)$^{l}$ | 15.9(2.5)$^{e}$ | 662.2 | 94.0(13.20)$^{h}$ | 4654.0 | 0.72 (0.01)$^{o}$ |
| All Brown Tubers | 168.9(34.4)$^{p}$ | 48.1(13.8)$^{m}$ | 15.0(1.8)$^{e}$ | 676.0 | 92.6(14.1)$^{h}$ | 4499.0 | 0.62 (0.01)$^{p}$ |
| ***Minor*** | | | | | | | |
| All Black Tubers | 264.0(12.6)$^{r}$ | 74.1(1.4)$^{i}$ | 12.2(2.4)$^{l}$ | 543.5 | 107.0(31.3)$^{g}$ | 4845.7 | 0.45 (0.01)$^{q}$ |
| All Brown Tubers | 274.7(12.6)$^{r}$ | 73.2(1.1)$^{i}$ | 16.4(1.3)$^{k}$ | 525.6 | 111.2(10.1)$^{g}$ | 4868.0 | 0.88 (0.01)$^{r}$ |
| ***Variety/period*** | | | | | | | |
| All Black Tubers | 219.2(44.9)$^{d}$ | 46.2(17.2)$^{h}$ | 16.0(2.8)$^{m}$ | 620.5 | 98.3(14.4)$^{f}$ | 4707.7 | 0.59 (0.01)$^{s}$ |
| All Brown Tubers | 192.7(51.8)$^{d}$ | 54.6(16.5)$^{h}$ | 14.3(2.1)$^{n}$ | 642.9 | 98.2(20.0)$^{f}$ | 4585.4 | 0.75 (0.01)$^{t}$ |

*Means in the same column but with different superscripts are significantly different (P<0.05)*

There were significant differences (p<0.05) in the indices measured between locations and varieties. Ampenyi black tubers planted in the major period

recorded the highest energy value of 5016.2 k cal kg-$^{1}$, while Bawjiase brown tubers from the major planting period recorded the highest carbohydrate content of 734.7 g kg-$^{1}$. There were also significant differences in the parameters analysed ($p<0.05$) between planting periods. For example, Bawjiase and Danyameso which were the two sites that planted both varieties in major and minor periods recorded significant differences in their protein and fat content. The black tiger nut tubers obtained from Danyameso in the minor planting period recorded the highest fat content of 295.4 g kg-$^{1}$, while the major planting crop recorded 155.4 g kg-$^{1}$. Whereas Bawjiase brown tubers planted in the minor period recorded the highest protein content of 75.1 g kg-$^{1}$ the major period crop recorded 39.1 g kg-$^{1}$. These differences in indices of tubers from different planting periods, sites and varieties are similar to observations from other studies on plant materials (Djomdi Ejoh and Ndjouenkeu, 2006; Xiang *et al.*, 2008; Addo-Quaye *et al.,* 2011) which has been attributed to differences in rainfall, climate, soil and crop variety (Howeler, 2002). Comparatively, the protein content of brown tubers planted in the minor period at Bawjiase (75.1 g kg-$^{1}$) was lower than the 80.7 g kg-$^{1}$ obtained for raw tubers by Ekyeanyanwu and Ononogbo, (2010) but higher than the 50.0 g kg-$^{1}$ recorded in another study by Arafat *et al.*, (2009). The mean fat content recorded for all black and brown tubers for both seasons (219.2 g kg-$^{1}$, 192.7 g kg-$^{1}$) were also relatively lower than the 296.7 g kg-$^{1}$ recorded in an earlier study (Umeri *et al.*, 1997) and the 510.0 g kg-$^{1}$ recorded for cocoa beans which is a major economic crop in Ghana (Takrama *et al.*, 2007). However, considering that the fatty acids composition of tiger nut oil is

comparable to that of olive oil which is of relatively higher value (Dubois *et al*., 2007) it will make nutritional and commercial sense to exploit it.

Free fatty acid (FFA) was determined as an index for the effect of primary processing and handling on the tubers. The Free fatty acids (FFA) values obtained ranged from 0.37-1.18 % for Tanoso black tubers of the major planting period and Danyameso black tubers of the major planting period respectively. The significant differences ($p<0.05$) in the FFA values among sites could be attributed to the impact of different post harvest handling and storage practices on the break down of the oil into fatty acids and glycerol. Currently, these practices are not uniform. While harvested tubers in Kwahu are normally washed in water to remove residual soil, at other sites like New Ebu and Twifo Praso the soil is rubbed off the tubers in big baskets. There was also no obvious trend in the free fatty acid content with respect to period of harvest; however it appeared that the black variety of tiger nuts showed higher free fatty acids in the major harvesting period than in the minor period (Table 3.1). The reverse was observed for the brown variety. If free fatty acids content is also taken as an index of stability of fatty food, then Black tiger nuts harvested in the minor period would keep longer (i.e. be more stable) than those harvested in the major period. The average free fatty acid of 0.66 % for the tubers however is less than the industry limit of 1.75 % for cocoa beans (Chaiseri and Dimick 1989). This suggests that the incorporation of tiger nut into cocoa products will not increase the leves of free fatty acids. There was no significant differences in mean fiber values for black and brown tubers for both major and minor periods (94.0 g $kg^{-1}$, 92.6 g $kg^{-1}$; and 107.0 g $kg^{-1}$,111.2 g $kg^{-1}$) respectively. Comparatively, these figures were higher than the 8.8 g

kg-$^{1}$ recorded for sweetpotatoes and 11.8 g kg-$^{1}$ recorded for cassava (Olayiwola *et al.,* 2009; Maieves *et al.,* 2012). The reported relationship between consumption of high fiber diets and reduction of coronary heart diseases, diabetes mellitus and obesity suggests that the consumption of tiger nut tubers may reduce the risk of developing these diseases (Anderson *et al.,* 2009). However, considering the traditional uses of tiger nut tubers in Ghana (tubers are mainly chewed for the juice and milk extraction) it can be said that till recipes are developed to make use of the whole tuber the many benefits of high tiger nut fiber diet cannot be realized.

The mineral content of the two tiger nut varieties obtained from different locations of the two planting period are shown in Table 3.2.

**Table 3.2: Elemental composition of two varieties of tiger nut tubers from different sites and periods**

| Variety/Planting period/Site | Na (mg kg-$^{1}$) | Ca (mg kg-$^{1}$) | K (mg kg-$^{1}$) | Mg (mg kg-$^{1}$) | Ph (mg kg-$^{1}$) | Fe (mg kg-$^{1}$) | Zn (mg kg-$^{1}$) | Cu (mg kg-$^{1}$) | Mn (mg kg-$^{1}$) |
|---|---|---|---|---|---|---|---|---|---|
| ***Black (major*)** | | | | | | | | | |
| Ampenyi | 550.30(1.47)$^{e}$ | 7.67(0.42)$^{a}$ | 6750.0(219.0)$^{h}$ | 535.0(36.0)$^{a}$ | 283.09(2.27)$^{a}$ | 3.21(0.02)$^{e}$ | 36.77(0.73)$^{i}$ | 3.32(0.28)$^{k}$ | 0.18(0.03)$^{a}$ |
| Bawjiase | 778.25(1.68)$^{f}$ | 11.27(0.31)$^{b}$ | 10891.0(227.0)$^{i}$ | 662.0(26.0)$^{b}$ | 375.37(2.93)$^{f}$ | 2.72(0.03)$^{f}$ | 47.52(0.65)$^{j}$ | 1.83(0.17)$^{a}$ | 1.00(0.06)$^{b}$ |
| Danyameso | 924.07(6.27)$^{a}$ | 15.80(0.60)$^{c}$ | 11659.0(430.0)$^{i}$ | 689.0(19.0)$^{b}$ | 279.33(1.91)$^{a}$ | 4.65(0.08)$^{g}$ | 45.10(0.39)$^{a}$ | 4.62(0.59)$^{b}$ | 1.42(0.09)$^{c}$ |
| Edu | 920.62(1.58)$^{a}$ | 8.27(0.50)$^{a}$ | 8877.0(321.0)$^{a}$ | 581.0(15.0)$^{a}$ | 477.41(0.89)$^{g}$ | 3.41(0.02)$^{h}$ | 32.88(0.41)$^{k}$ | 1.36(0.28)$^{a}$ | 8.14(0.07)$^{c}$ |
| Tampiong | 523.44(0.94)$^{b}$ | 12.27(0.30)$^{b}$ | 8879.0(187.0)$^{a}$ | 653.0(23.0)$^{b}$ | 294.29(0.60)$^{h}$ | 5.47(0.03)$^{i}$ | 23.08(0.16)$^{l}$ | 1.45(0.20)$^{a}$ | 0.12(0.02)$^{a}$ |
| Tanoso | 521.19(0.55)$^{b}$ | 11.13(0.50)$^{b}$ | 9023.0(360.0)$^{a}$ | 660.0(9.0)$^{b}$ | 354.20(0.60)$^{b}$ | 5.17(0.01)$^{j}$ | 60.58(0.72)$^{m}$ | 4.83(0.20)$^{b}$ | 9.22(0.48)$^{h}$ |
| ***Black (minor)*** | | | | | | | | | |
| Bawjiase | 682.15(1.36)$^{g}$ | 12.80(1.40)$^{b}$ | 10035.0(44.0)$^{k}$ | 747.0(23.0)$^{b}$ | 362.55(9.33)$^{b}$ | 4.05(0.07)$^{k}$ | 46.38(0.65)$^{a}$ | 1.99(0.02)$^{a}$ | 1.35(0.43)$^{bc}$ |
| Danyameso | 700.63(1.32)$^{h}$ | 15.27(1.92)$^{c}$ | 12780.0(449.0)$^{l}$ | 694.0(103.0)$^{b}$ | 283.95(9.31)$^{a}$ | 4.47(0.02)$^{l}$ | 46.35(1.42)$^{a}$ | 4.71(0.75)$^{b}$ | 1.90(0.15)$^{c}$ |
| ***Brown (major*)** | | | | | | | | | |
| Ampenyi | 763.16(1.41)$^{i}$ | 7.93(0.42)$^{h}$ | 8316.0(299.0)$^{b}$ | 551.0(30.0)$^{c}$ | 366.00(1.63)$^{c}$ | 2.93(0.01)$^{m}$ | 34.89(0.83)$^{b}$ | 1.24(0.09)$^{c}$ | 1.98(0.05)$^{i}$ |
| Bawjiase | 1075.80(4.70)$^{j}$ | 12.67(0.76)$^{l}$ | 12523.0(221.0)$^{m}$ | 695.0(29.0)$^{ef}$ | 478.37(1.95)$^{d}$ | 3.11(0.04)$^{n}$ | 41.05(1.02)$^{c}$ | 2.85(0.20)$^{d}$ | 7.44(0.15)$^{d}$ |
| Danyameso | 937.98(2.23)$^{k}$ | 15.60(0.40)$^{c}$ | 9758.0(220.0)$^{cd}$ | 678.0(45.0)$^{e}$ | 253.53(0.90)$^{e}$ | 5.37(0.08)$^{o}$ | 55.34(0.59)$^{d}$ | 4.90(0.37)$^{h}$ | 1.47(0.12)$^{e}$ |
| Ebu | 642.12(0.17)$^{l}$ | 10.13(0.61)$^{d}$ | 8593.0(213.0)$^{b}$ | 587.0(16.0)$^{cd}$ | 476.85(1.63)$^{d}$ | 2.77(0.08)$^{p}$ | 38.45(0.56)$^{e}$ | 1.09(0.11)$^{c}$ | 0.68(0.04)$^{j}$ |
| Kwahu Aduamoa | 538.56(1.05)$^{m}$ | 11.33(0.42)$^{d}$ | 9364.0(260.0)$^{c}$ | 587.0(10.0)$^{cd}$ | 371.35(0.91)$^{c}$ | 4.96(0.13)$^{a}$ | 33.57(0.76)$^{b}$ | 1.13(0.08)$^{c}$ | 0.39(0.02)$^{k}$ |
| Twifo Praso | 484.51(1.19)$^{n}$ | 16.80(0.35)$^{c}$ | 8052.0(264.0)$^{b}$ | 640.0(14.0)$^{e}$ | 398.31(0.61)$^{i}$ | 3.86(0.01)$^{q}$ | 38.43(0.70)$^{e}$ | 0.35(0.04)$^{i}$ | 1.01(0.06)$^{l}$ |
| ***Brown (minor)*** | | | | | | | | | |
| Bawjiase | 870.24(2.22)$^{o}$ | 16.13(0.42)$^{c}$ | 10176.0(348.0)$^{d}$ | 655.0(29.0)$^{e}$ | 258.65(2.25)$^{e}$ | 4.80(0.02)$^{a}$ | 55.84(1.66)$^{d}$ | 5.54(0.19)$^{j}$ | 1.65(0.18)$^{e}$ |
| Danyameso | 595.75(4.51)$^{p}$ | 14.40(0.92)$^{i}$ | 14241.0(427.0)$^{n}$ | 740.0(12.0)$^{f}$ | 469.88)(11.34)$^{d}$ | 3.64(0.08)$^{r}$ | 42.40(0.76)$^{c}$ | 3.03(0.09)$^{d}$ | 7.64(0.68)$^{d}$ |
| All Black Tubers (major) | 702.98(183.37)$^{d}$ | 11.07(2.79)$^{g}$ | 9330.0(1650.0)$^{e}$ | 630.0(58.4)$^{g}$ | 343.95(71.86)$^{j}$ | 4.11(1.07)$^{b}$ | 40.99(12.24)$^{f}$ | 2.90(1.51)$^{e}$ | 2.30(3.25)$^{g}$ |
| All Brown Tubers (major) | 740.36(217.45)$^{d}$ | 12.41(3.17)$^{g}$ | 9430.0(1560.0)$^{e}$ | 623.0(58.2)$^{g}$ | 390.74(78.56)$^{j}$ | 3.83(1.04)$^{b}$ | 40.29(7.40)$^{f}$ | 1.93(1.58)$^{e}$ | 2.16(2.49)$^{g}$ |
| All Black Tubers (minor) | 691.39(10.19)$^{c}$ | 14.03(2.02)$^{f}$ | 11410.0(1530.0)$^{f}$ | 720.0(72.4)$^{h}$ | 323.25(43.85)$^{k}$ | 4.26(0.24)$^{c}$ | 46.36(0.99)$^{g}$ | 3.36(1.56)$^{f}$ | 1.62(0.42)$^{f}$ |
| All Brown Tubers (minor) | 732.99(150.38)$^{c}$ | 15.27(1.14)$^{f}$ | 12210.0(2250.0)$^{f}$ | 699.0(50.5)$^{h}$ | 364.27(115.29)$^{k}$ | 4.22(0.64)$^{c}$ | 49.12(7.45)$^{g}$ | 4.29(1.38)$^{f}$ | 4.65(3.31)$^{f}$ |
| All Black Tubers | 700.08(157.80)$^{q}$ | 11.81(2.89)$^{k}$ | 9849.0(1837.0)$^{g}$ | 653.0(73.0)$^{i}$ | 338.78(65.72)$^{l}$ | 4.14(0.93)$^{d}$ | 42.33(10.80)$^{h}$ | 3.02(1.50)$^{g}$ | 2.13(2.82)$^{m}$ |
| All Brown Tubers | 738.51(199.69)$^{q}$ | 13.13(3.03)$^{k}$ | 10218.0(2099.0)$^{g}$ | 642.0(64.0)$^{i}$ | 384.12(87.29)$^{l}$ | 3.93(0.96)$^{d}$ | 42.50(8.24)$^{h}$ | 2.52(1.83)$^{g}$ | 2.79(2.86)$^{m}$ |

Tukey's test was used to locate the differences in means.

*Means in the same column but with different superscripts are significantly different (P<0.05)*

Sodium, potassium, magnesium and phosphorus which are very useful for very important reactions and functions of organs in the body (Igoe and Hui, 2001) were the major macro elements in the tubers while iron, copper, manganese were the least abundant. This was similar to studies conducted by other researchers on tubers from other countries (Glew *et al*., 2006; Oladele and Aina, 2007). Sodium ranged from 484.51 mg kg-$^{1}$ to 1075.80 mg kg-$^{1}$ with Bawjiase brown of major planting period recording the highest. The minor period black variety in Bawjiase recorded the highest magnesium of 747.0 mg kg-$^{1}$, while the brown variety planted in the major period recorded the highest phosphorus of 478.37 mg kg-$^{1}$. Brown tubers planted in the minor period at Danyameso however recorded the highest potassium of 14241.0 mg kg-$^{1}$ with the black tubers planted in the major period at the same site recording the highest sodium 924.07 mg kg-$^{1}$ (Table 3.2). The trace amounts of calcium and iron which are essential in the development of strong bones and blood synthesis suggest that there is the need for the fortification of the vegetable milk produced from the tubers to prevent micro-nutrient deficiencies (Igoe and Hui, 2001). If used in cocoa products however there will be very little need for additional magnesium because of the high quantities in cocoa (Wester, 1987). Zinc was the highest recorded minor element with the highest amount of 60.58 mg kg-$^{1}$ recorded for Tanoso black. This was however only 26 % of the required daily intake for adults and children above 4 years (FDA, 2009). Over all, Black tubers harvested in the major period had mineral profile that was not significantly different from those of the brown variety.

The consumer preference for milk extracted from the tiger nut tubers was obtained through a simple ranking test. The rank sums are indicated in Table 3.3.

**Table 3.3: Consumer preference of tiger nut milk extracted from tubers from different sites**

| VARIETY | SITE | ACCEPTABILITY | RANKING |
|---|---|---|---|
| BLACK | Ampenyi | 4.5(1.3)[a] | 5TH |
| | Bawjiase | 2.6(1.4)[a] | 3RD |
| | Danyameso | 2.2(1.4)[a] | 2ND |
| | Ebu | 2.2(0.9)[a] | 1ST |
| | Tampiong | 3.9(1.2)[a] | 4TH |
| | Tanoso | 5.7(0.7)[b] | 6TH |
| BROWN | Ampenyi | 3.8(2.2)[a] | 6TH |
| | Bawjiase | 3.3(2.0)[a] | 1ST |
| | Danyameso | 3.5(1.8)[a] | 5TH |
| | Ebu | 3.5(1.1)[a] | 3RD |
| | Kwahu Aduamoa | 3.4(2.0)[a] | 2ND |
| | Twifo praso | 3.5(1.4)[a] | 4TH |

*Means in the same column but with different superscripts differ significantly ($p<0.05$)*

Friedman's ranking showed that inspite of the different rankings for brown tubers, there were no significant differences ($p < 0.05$) in the acceptability of their milk unlike the black tubers where acceptability of milk from the black tubers in Tanoso fell below expectation due to unacceptable flavor. The unacceptable flavor may be due to the post harvest handling of the tubers, soil structure and the climatic conditions in the Tanoso area. These factors have been determined to affect the flavor of *horchata de chufas* (Heath and Reineccius, 1986; Pattee and Singleton 1980).

### 3.4. Conclusion

Tiger nut tubers have high fat, carbohydrate, fiber and energy value which could be exploited for industrial applications. The site and planting period of tiger nut tubers have significant effects on some of its chemical properties such as protein, fat and energy content, minerals as well as acceptability of its milk. Minor season produces tubers with high protein, fat, fiber and energy content while major season produces tubers with high carhydrates content. With the exception of milk from tanoso, milk from tubers harvested from other sites can be used to formulate chocolate recipes without any adverse effects on its acceptability.

*The next chapter reports on the study to determine the best method for pre-treating tubers before milk extraction.*

# CHAPTER 4

## 4.0 EFFECTS OF SOAKING AND COOKING METHODS ON EXTRACTION OF SOLIDS AND ACCEPTABILITY OF TIGER NUT (*CYPERUS ESCULENTUS* L) MILK

### 4.1. Introduction

Non dairy milk has for some time now attracted a lot of interest because of the scarce supply of cow milk in developing countries and its relatively lower cost. Dietary constraints, allergens, religious convictions, ethical reasons and the growing awareness of its nutritional value are other reasons that have heightened its interest (Wang, 1980; Edwards, 1998; Diarra *et al*., 2005, El-Algamy, 2007). Non dairy products that have attracted a lot of interest are Soy milk, safflower milk, rice milk, peanut milk and tiger nut milk (Edwards, 1998; Russell and Delahunty, 2004; Kashid *et al*., 2007; Isanga and Zhang, 2009; Rubert *et al*., 2011).

Tiger nut milk, popularly called "atadwe" milk by Ghanaians and "horchata de chufa" by the Spaniards is a very popular drink in Ghana, Spain and some South American countries (Dokosi, 1998; Cortés *et al*., 2004). The refreshing non-alcoholic beverage of milky appearance has high nutritional quality with great market potential (Cortés *et al*., 2005). It is high in starch glucose and proteins. It is also rich in minerals like potassium, phosphorus, as well as vitamins E and C (Tigernuts Traders S.L., 2010 ) Tiger nut milk is also reported to contain myristic, linolenic and large amounts of oleic acid (Belewu and Belewu, 2007) It is commonly produced from dried tubers which are pre-treated by soaking in water.

The hydrated tubers are then milled in water to extract the milk. Pre-treatment of plant materials is a major process step in the extraction of non dairy milk. It is an important activity that affects the milk yield, quality, sensory characteristics, solids extracted and shelf life (liener 1994; Liu, 1997; Aidoo, *et al*., 2010). Soaking and boiling have extensively been used to pre-treat tiger nut tubers before extraction of milk and solids (Mosquera *et al*., 1996; Ndjouenkeu and Djomdi Ejoh, 2007; Sanful, 2009). There is however a dearth of information on the effect of other cooking methods on the yield of solids and acceptability of milk. The objective of this study therefore was to determine the effect of soaking and different cooking methods of tiger nut tubers on the yield of milk, solids and sensory quality of two varieties of tubers from eight different sites in Ghana.



## 4.2. Materials and Methods

### 4.2.1 Material samples

Six samples each of two varieties of tiger nut tubers (*Cyperus esculentus* L.) grown at eight different sites in Ghana (Ampenyi, Bawjiase, Danyameso, Ebu, Kwahu Aduamoa, Tampiong, Tanoso and Twifo Praso) were studied. The tubers were sorted, washed and dried for 5 days in a Sanyo oven (Model MOV-212, Japan) at 55$^{o}$C till moisture was between 7 to 10 % prior to evaluation.

### 4.2.2 Tiger nut milk extraction procedures

The procedure for screening different varieties of tubers from different sites for milk and solids extraction is shown in Figure. 4.1.

One hundred grams (100g) of oven dried nuts were soaked in covered vessels

with 600 ml of water for 12 hours. The water was discarded and the tubers milled in a Waring blender (Model 38BL41, USA) with fresh water (twice the new weight of tubers after soaking) at high speed for 5 minutes. The milk was pressed through a cheese cloth until no more extract was obtained to receive the milk and the weight (g $kg^{-1}$) determined for analysis.

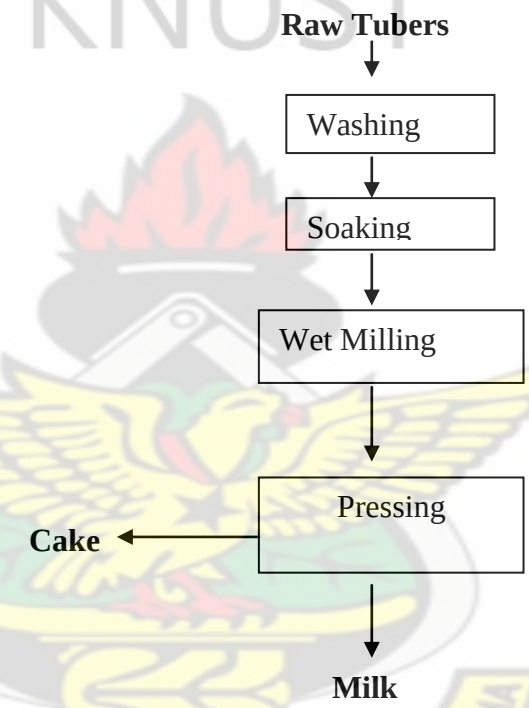



**Figure 4.1: Flow Diagram for milk production from different varieties of tiger nut tubers (Ofori-Anti, 2000)**

The method used for pre-treating plant materials before milk extraction is known to affect the yield and quality of milk and solids extracted (Quansem *et al*., 2009). The effect of different pre-treatment and its sequence of combination were evaluated in this study. The different pre-treatment methods (soaking and cooking) used for tubers from selected sites after screening are shown in Figure

4.2. Three main extraction procedures were evaluated. A: soaking only, B: soaking of tubers before cooking and C: cooking of tubers before soaking. One hundred grams (100 g) oven dried tubers were used for the milk extraction. In processing method A, the tubers were soaked in covered vessels for 6 hours in 600ml of water; and then wet milled for milk extraction. In process B, the tubers were also soaked in covered vessels for 6 hours in 600ml of water then tied in a piece of muslin cloth and boiled for 10 minutes. Another set of 100g of tubers were soaked for 6 hours in 600ml of water and steamed on a sieve over boiling water for 10 minutes. Yet another set of 100g tubers were soaked for 6 hours and roasted on trays in a hot air oven pre-heated to $180^{o}$ C for 10 minutes. Each of the samples of the three sets was milled in a Waring blender (Model 38BL41, USA) with fresh water (twice the new weight of tubers after soaking) at high speed for 5 minutes to facilitate milk extraction.

The first milk extract was obtained by pressing the extract through a cheese cloth until virtually no more liquid was available for extraction. Re-extraction of milk was done by adding same volume of water used for initial extraction to the cake. The mixture was stirred and pressed again through a cheese cloth until virtually no more extract was available to obtain the second milk extract. In pre-treatment method C, nuts were cooked (boiling, steaming and roasting) before soaking in 600ml of water (Figure 4.2).

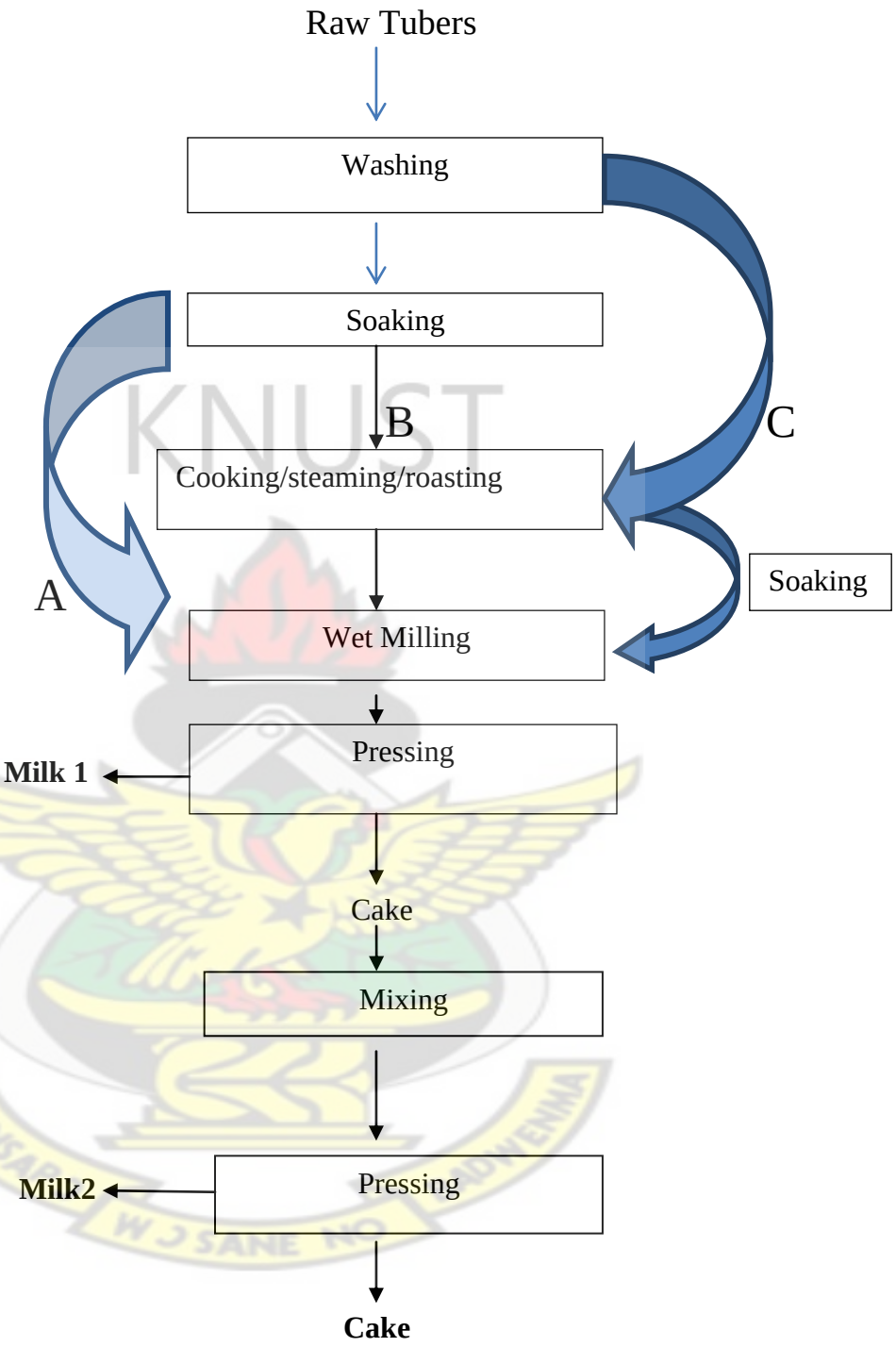



**Figure 4.2: Flow diagram for extracting milk and solids from different pre-treated tubers**

The milk and solids indices for the combined first and second extracts were determined for the tubers from the different sites and expressed as g $kg^{-1}$;

Milk Extracted (ME) = W2/W1 (Cai *et al*., 1997)

W2 = weight of extracted milk and W1 = weight of tubers

The total solids in milk were determined by drying a known volume in a calibrated dish in an oven at 105$^{o}$C. Milk solid (MS) was expressed as W3/W2 where W3 = Weight of dry powder (Akintunde and Akintunde, 2002)

Expected milk solid (EMS) was determined as;

EMS = MS x ME (see Appendix H 7)



4.2.3 Evaluation of sensory quality of tiger nut milk after pre-treatments

The seven samples obtained were evaluated using a balanced incomplete block (BIB) design (t=7, k =4, r =20, b =35 λ = 10) (Cochran & Cox, 1957) with a trained panel of 35 persons. All the samples were sweetened with the same amount of sugar per weight (20 g kg-$^{1}$ milk) to improve upon the taste of milk and were evaluated for flavor, after-taste and overall acceptability on a nine point hedonic scale with 1 representing least liked and 9 most liked (Appendix B2). Water was available for panellists to rinse their mouths and they were allowed to re-taste samples.

4.2.4 Statistical analysis

The General Linear Model was used to determine the differences in treatments using MINITAB 14 statistical package (MINITAB Inc., U.S.A.). Tukey's test

($p$ <0.05) was used to identify significant differences among treatment means.

## 4.3. Results and discussion

The yield indices of tiger nut milk are shown in Table 4.1. There were significant differences in milk extracted (ME), milk solids (MS) and expected milk solids (EMS) between the varieties and the sites investigated.

### 4.3.1 *Effect of Site on Yield of Milk Solids from Different Varieties of Tubers*

The effect of site on yield of milk characteristics are shown in Table 4.1. The results show significant differences in some of the indices measured for the same variety but planted at different site.

**Table 4.1: Yield of milk and solids of tubers from different sites and varieties**

| VARIETY | SITE | MILK CHARACTERISTICS | | |
|---|---|---|---|---|
| | | ME (g kg-$^{1}$) | MS (g kg-$^{1}$) | EMS (g kg-$^{1)}$ |
| BLACK | Ampenyi | 2706.3(4.2)$^{c}$ | 159.4(1.3)$^{a}$ | 431.4(4.0)$^{a}$ |
| | Bawjiase | 2587.2(9.8)$^{d}$ | 155.2(0.1)$^{b}$ | 401.6(1.5)$^{b}$ |
| | Danyameso | 2275.0(12.4)$^{e}$ | 150.1(1.4)$^{c}$ | 341.5(2.2)$^{c}$ |
| | Ebu | 2661.3(3.5)$^{f}$ | 157.1(0.7)$^{ab}$ | 418.1(1.8)$^{d}$ |
| | Tampiong | 2856.7(2.1)$^{g}$ | 162.4(0.7)$^{d}$ | 464.0(2.3)$^{e}$ |
| | Tanoso | 2991.2(1.3)$^{h}$ | 145.9(0.9)$^{e}$ | 436.5(2.5)$^{a}$ |
| BROWN | Ampenyi | 2333.0(2.3)$^{i}$ | 145.7(1.0)$^{f}$ | 340.0(2.3)$^{f}$ |
| | Bawjiase | 2652.2(12.5)$^{a}$ | 147.2(0.9)$^{g}$ | 390.4(3.0)$^{g}$ |
| | Danyameso | 2721.8(19.3)$^{j}$ | 137.6(1.1)$^{h}$ | 374.5(5.5)$^{h}$ |
| | Ebu | 2627.7(10.8)$^{ab}$ | 157.2(0.7)$^{i}$ | 399.7(3.3)$^{i}$ |
| | Kwahu Aduamoa | 2788.0(2.5)$^{k}$ | 164.0(1.0)$^{j}$ | 457.1(2.2)$^{j}$ |
| | Twifo Praso | 2612.8(2.6)$^{b}$ | 171.8(1.4)$^{k}$ | 449.0(4.1)$^{j}$ |

Key: ME (g kg-$^{1}$), Milk Extraction; MS (g kg-$^{1}$), Milk Solids; EMS (g kg-$^{1}$), Expected Milk Solids. Tukey's test was used to locate differences in means.

*Means in the same column but with different superscripts differ significantly ($p<0.05$).*

This observation could be due to the differences in the composition of the tubers. Tanoso black recorded the highest milk yield of (2991.2 g $kg^{-1}$) whereas Danyameso black recorded the lowest milk yield of (2275.0 g $kg^{-1}$). The highest milk solids of (162.4 g $kg^{-1}$) was recorded by Tampiong black making it the sample with the potential to yield the highest expected milk solids of 464.0 g $kg^{-1}$ with Ampenyi brown expected to yield the lowest expected milk solids of 340.0 g $kg^{-1}$.



4.3.2 *Effect of pre-treatments on milk characteristics*

The six sites in each block were reduced to two each for this phase of the study due to convinience. Though milk extracted from Tanoso black tubers was the highest, and the expected milk solids the second highest, Ampenyi black was selected alongside Tampiong black, Kwahu brown and Twifo Praso brown tubers for the next phase of the study. This was because these tubers gave higher milk solids. The results on milk yield, milk solids and expected milk solids after different combinations and sequence of soaking and cooking are shown in figures 4.3, 4.4 and 4.5.

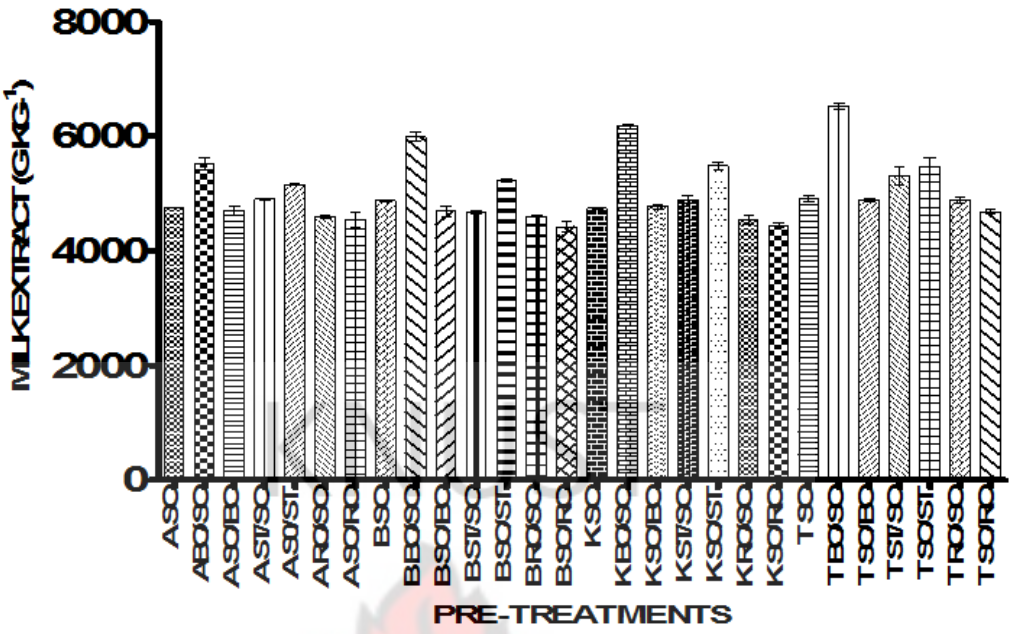



**Figure 4.3: Effect of pre-treatments on milk yield**

Key; A, Ampenyi: B, Tampiong; K, kwahu; T, Twifo Praso; SO, soaking; BO/SO, boiling and soaking; SO/BO, soaking and boiling; ST/SO, steaming and soaking; SO/ST, soaking and steaming; RO/SO, roasting and soaking; SO/RO, soaking and roasting.

The data revealed significant variations in characteristics of milk extracted after different pre-treatments and significant yield of solids in re-extractions. The findings are similar to milk extraction studies conducted on other plant materials (Tunde-Akintunde and Souley, 2009; Quasem *et al.*, 2009, Ye and Jiang, 2011).

Soaking and heat treatment of plant materials is an important step in the production of vegetable milk because it eliminates anti-nutritional factors, facilitates ease of heat penetration and consequent rapturing of tisues during of milling for release of materials, makes milk more palatable and digestible, and tenderizes plant materials to facilitate milling and extraction (Liu, 1997; Enneking and Wink, 2000; Adekanmi *et al*., 2009). Figure 4.3 shows that for all sites and

varieties, boiling before soaking of tubers yielded the highest milk with tubers (brown variety) obtained from Twifo Praso recording the highest milk extract of 6530.4 g kg-$^{1}$. Soaking before roasting of tubers however gave the lowest milk yield with black tubers from Tampiong recording the lowest of 4395.5 g kg-$^{1}$. Higher milk yield from boiling and then soaking could be attributed to better opening of cellular pores and weakening of cell walls after milling of tubers (Djomdi Ejoh and Ndjouenkeu, 2006; Ndjouenkeu and Djomdi Ejoh, 2007). Though boiling before soaking gave the highest milk yield, it recorded the lowest milk solids for both varieties and all sites with the lowest of 101.0 g kg-$^{1}$ recorded for black tubers from Tampiong as shown in Figure 4.4.

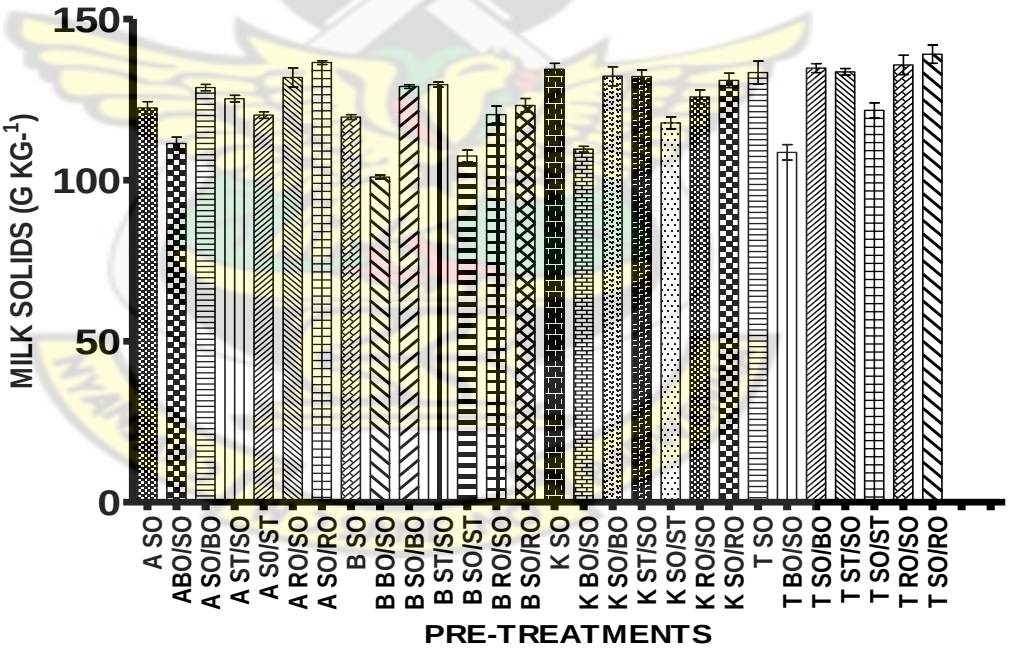



**Figure 4.4: Effect of pre-treatments on milk solids content**

Key; A, Ampenyi: B, Tampiong; K, kwahu; T, Twifo Praso; SO, soaking; BO/SO, boiling and soaking; SO/BO, soaking and boiling; ST/SO, steaming and soaking; SO/ST, soaking and steaming; RO/SO, roasting and soaking; SO/RO, soaking and roasting.

This observation could be due to the gelling of starch from boiling resulting in the trapping of solids in the filter cloth. Figure 4.5 shows the expected milk solids for all pre-treatments. A range of 541.4 g kg-$^{1}$ to 619.4 g kg-$^{1}$ was recorded for black tubers from Ampenyi and Tampiong with the lowest recorded for soaked and roasted black tubers from Tampiong and the highest recorded for soaked and boiled black tubers from Ampenyi.



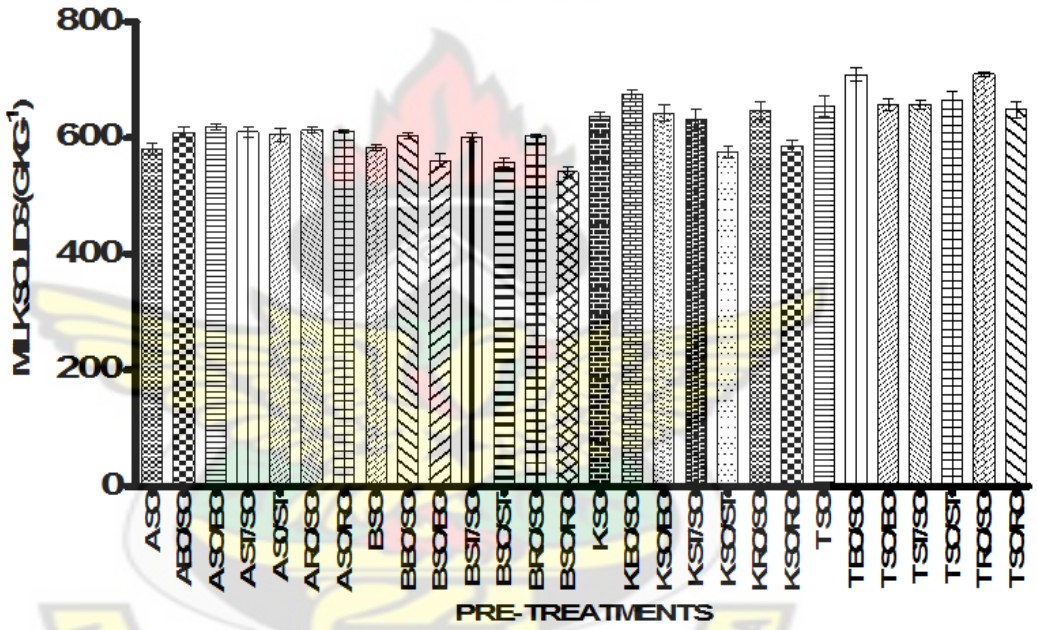



**Figure 4.5: Effect of pre-treatments on expected milk solids**

Key; A, Ampenyi: B, Tampiong; K, kwahu; T, Twifo Praso; SO, soaking; BO/SO, boiling and soaking; SO/BO, soaking and boiling; ST/SO, steaming and soaking; SO/ST, soaking and steaming; RO/SO, roasting and soaking; SO/RO, soaking and roasting.

A range of 571.4 g kg-$^{1}$ to 710.2 g kg-$^{1}$ was however recorded for brown tubers from Kwahu and Twifo Praso with the lowest recorded for steamed and soaked brown tubers from Kwahu and the highest recorded for roasted and soaked brown tubers from Twifo Praso . This was however not significantly different from the

yield obtained from soaked and boiled brown tubers fromTwifo Praso.

4.3.3 *Sensory Quality of Milk from Pre-treated Tubers*

The milk of Ampenyi black and Twifo Praso brown tubers were selected for sensory quality evaluation because of the potential of the tubers from these sites to yield higher milk solids (based on the data from Table 4.1). The results in Table 4.2 show that the different pre-treatments of Ampenyi black tubers did not result in any significant differences in the flavor, after-taste and overall acceptability.

**Table 4.2: Sensory quality of milk from different pre-treated tubers**

| PRE-TREATMENT | BLACK TUBERS (AMPENYI) | | | BROWN TUBERS (TWIFO PRASO ) | | |
|---|---|---|---|---|---|---|
| | Flavor | After-taste | Overall acceptability | Flavor | After-taste | Overall acceptability |
| SO | 7.1[a] | 6.8[a] | 7.0[a] | 6.0[a] | 5.8[a] | 5.8[a] |
| BO/SO | 6.4[a] | 6.4[a] | 6.1[a] | 5.9[a] | 5.8[a] | 6.0[a] |
| SO/BO | 6.4[a] | 6.4[a] | 6.4[a] | 5.5[a] | 5.5[a] | 5.7[a] |
| ST/SO | 5.8[a] | 6.0[a] | 5.8[a] | 5.9[a] | 5.6[a] | 5.9[a] |
| SO/ST | 6.5[a] | 6.3[a] | 6.7[a] | 5.6[a] | 5.6[a] | 5.7[a] |
| RO/SO | 6.9[a] | 6.4[a] | 6.4[a] | 7.1[b] | 6.4[a] | 6.5[a] |
| SO/RO | 7.0[a] | 6.5[a] | 7.0[a] | 6.2[a] | 6.3[a] | 6.4[a] |

SO; soaking; BO/SO, boiling and soaking; SO/BO, soaking and boiling; ST/SO, steaming and soaking; SO/ST, soaking and steaming; RO/SO, roasting and soaking; SO/RO, soaking and roasting

*Means in the same column with the same superscript are not significantly different ($p < 0.05$)*

All the pre-treatments were acceptable at varying degrees. The flavor of milk produced from roasted and soaked brown tubers from Twifo Praso was however most preferred to the milk from other pre-treatments. The observation that roasting before soaking give more acceptable milk is similar to studies reported for Sesame (Quasem *et al*., 2009). It was to be expected since roasting is known to change the flavor of foodstuffs because of the mailliard reaction that takes

place between sugars and proteins (Fox and Cameron, 1989). There was however no significant differences in the after-taste and overall acceptability of the milk.

### 4.4. Conclusion

Tiger nut tubers grown at different sites do not have the same potential for milk and solids extraction. Pre-treating tubers by soaking and cooking improved the expected yield of milk solids. The boiled and soaked as well as roasted and soaked tubers gave the most milk solids. Though the different cooking methods in combination with soaking had no effect on the sensory quality of milk from the black tubers, it did have an effect on milk from the brown tubers. Milk from tubers that were roasted before soaking had the most acceptable flavor.

*The next chapter reports on the study to determine the optimal levels of the pre-treatment method selected together with tuber: water ratio and milling time for the extraction of milk solids from two varieties of tiger nuts*

# CHAPTER 5

## 5.0 MODELLING OF MILK SOLIDS EXTRACTION PROCESS FROM TIGER NUT (*Cyperus Esculentus* L) TUBERS USING RESPONSE SURFACE METHODOLOGY

### 5.1. Introduction

Tiger nuts (*Cyperus esculentus* L) have been cultivated extensively in Africa, Asia and some European countries for centuries (Arranz, *et al*., 2006; Glew *et al.*, 2006). It is popularly eaten (raw or roasted) because of its delicious taste as a snack. An aqueous extract is also made from it as tiger nut milk and used for other food products. A popular beverage called *chufa de horchata* in Spain and *atadwe milk* in Ghana is a common product made from the tubers of tiger nuts (Abbiw, 1990; Dokosi, 1998; Cortés, *et al.*, 2004; Yeboah *et al.*, 2011). The nutritional composition of this drink has been reported as 25.0 g $kg^{-1}$ fat, 18.0 g $kg^{-1}$ protein, 22.0 g $kg^{-1}$ starch, and 4.0 g $kg^{-1}$ ash (Belewu and Belewu, 2007). Tiger nut fat has also been reported to be rich in myristic acid, oleic acid and linoleic acid (Dubois *et al*., 2007). It has also been suggested in several reports that tiger nuts probably have some health benefits, which include reducing the risk of colon cancer, helping to prevent heart attacks, thrombosis and activating blood circulation (Dokosi, 1998; Belewu and Belewu, 2007). Consequently, the application of tiger nuts in food products will have consumer appeal for its nutritional and perceived nutraceutical benefits. Though considerable studies have been conducted into its nutritional, quality, sensory characteristics, preservation and shelf life, (Mosquera *et al*., 1996; Pascual *et al*., 2000; Cortés, *et al.*, 2005; Belewu and Belewu, 2007; Mordi *et al.*, 2010; Rubert *et al*., 2011; Corrales *et al.*,

2012) more systematic studies need to be explored for its successful use as an ingredient in some food applications. To achieve this, a clear and efficient extraction protocol of the tiger nuts solids needs to be established. A number of studies have considered various extraction variables for tiger nut milk to include tuber size, soaking, roasting, toasting temperatures, and milling time (Djomdi Ejoh and Ndjouenkeu, 2006, Adekanmi *et al*., 2009, Sanful, 2009; Mordi *et al.,* 2010). Several other studies have shown that milling time, cooking time and meal: water ratios are very important processing factors in the aqueous extraction of solids from plant materials (Rhee *et al.*, 1972; Kim, 1989; Arifin *et al.,* 2009; Milani *et al.,* 2011; Ye and Jiang, 2011). The objective of this study therefore was to optimize boiling time, milling time and tuber: water ratio for the production of milk solids from two varieties of tiger nut tubers using response surface methodology (RSM).

**5.2. Materials and methods**

Black and brown varieties of tiger nut tubers were purchased from farmers in Ampenyi in the Komenda Edina Eguafo District and Twifo Praso in the Twifo Hemang Lower- Denkyira District all in the Central Region of Ghana. The tubers were sorted, washed and dried in a Sanyo oven (Model MOV-212, Osaka, Japan) at 55$^{o}$ C till moisture was between 7 and 10%. The samples were cooled, packaged and stored in jute sacks at room temperature of 27$^{o}$C until used for the study.

### 5.2.1 Experimental design and data analysis

Soaking of tubers after boiling was not investigated as an additional variable because intial trials showed that its combination with the others did not significantly affect the yield of solids. The independent extraction variables therefore considered were milling time ($x_1$), tuber: water ratio ($x_2$) and boiling time ($x_3$). The variables and their levels are as shown in Table 5.1. A Face Centered Central Composite Rotatable Design (Montgomery, 2001) was employed in the designing of experiments using MINITAB 14 statistical package (MINITAB Inc., USA). Twenty sample combinations were generated (Table 5.2).

### 5.2.2 Tiger Nuts Milk extraction procedure

One hundred grams (100 g) of the oven dried tubers with the corresponding volume of water according to the experimental design (Table 5.2) were used to boil the tiger nuts at the specified time. During boiling, between 20-50ml of water was used to top up in runs where water was lost due to evaporation. After the required time of boiling the nuts were drained and weighed. The boiled tiger nuts were then submerged in a volume of water that was twice its weight and then wet milled in a Logik blender (model RSH-005494-018, China) at high speed for the appropriate time according to the experimental design (Table 5.2). The blended tiger nuts were filtered through a cheese cloth to obtain tiger nuts milk (W2) which was used for the determination of the milk indices. The effects of the independent variables on extracted milk was determined as yield of milk from tubers and expressed as g $kg^{-1}$.

Milk Extracted (ME) = W2/W1 (Cai *et al*., 1997)

W2 = weight of extracted milk W1 = weight of tubers

The milk solids were determined by drying a known volume in a calibrated dish in an oven at 105$^{o}$C. Milk solid (MS) was expressed as W3/W2where W3 = Weight of dry powder (Akintunde and Akintunde, 2002) Expected milk solid (EMS), an estimate of the total obtainable milk solids that can be extracted was then expressed as MS x ME. (Appendix H7)

Each analysis was conducted in triplicate and the results averaged. Response surface regression analysis was used to model the effect of the independent variables on the expected milk solids (g kg-$^{1}$) of milk solids (*Y*) using MINITAB 14 statistical package. The quadratic polynomial model proposed for the response *(Y)* was as follows:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x^2_1 + b_{22}x^2_2 + b_{33}x^2_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3$$

The adequacy of the model was checked based on $R^2$ and adjusted-$R^2$ as well as the lack of fit error.

**Table 5.1: Independent process variables and their corresponding levels**

| MILK SOLIDS EXTRACTION | | | | |
|---|---|---|---|---|
| Independent variable | Levels | | | |
| | Symbol | -1 | 0 | 1 |
| Milling time (min) | $X_1$ | 5 | 17.5 | 30 |
| Tuber:Water (g ml-$^{1}$) | $X_2$ | 2 | 5 | 8 |
| Boiling Time (min) | $X_3$ | 10 | 125 | 240 |

**Table 5.2: Matrix of experimental runs and yields of milk solids from tiger nut tubers**

| VARIABLES AND THEIR LEVELS | | | | BLACK TUBERS (g kg-$^{1}$) | | | BROWN TUBERS (g kg-$^{1}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| Run Order | $X_1$ | $X_2$ | $X_3$ | ME | MS | EMS | ME | MS | EMS |
| | -1 (5.0) | -1 (2) | -1 (10) | 2038.7 | 239.5 | 488.3 | 2075.8 | 242.6 | 503.6 |
| 2 | -1 (5.0) | 0 (5) | 0 (125) | 5336.6 | 109.9 | 586.5 | 5128.3 | 116.4 | 596.9 |
| 3 | 0 (17.5) | 0 (5) | 0 (125) | 5763.7 | 122.7 | 707.2 | 5685.7 | 126.3 | 718.1 |
| 4 | 1 (30.0) | -1 (2) | 1 (240) | 1486.4 | 245.7 | 365.2 | 1687.7 | 253.6 | 428.0 |
| 5 | 0 (17.5) | 0 (5) | -1 (10) | 5549.1 | 119.3 | 662.0 | 5720.8 | 128.7 | 736.3 |
| 6 | 0 (17.5) | 0 (5) | 0 (125) | 5723.8 | 121.0 | 692.6 | 5800.2 | 126.0 | 730.8 |
| 7 | 0 (17.5) | 0 (5) | 0 (125) | 5554.4 | 118.7 | 659.3 | 5602.3 | 127.6 | 714.9 |
| 8 | 1 (30.0 | 1 (8) | -1 (10) | 7685.7 | 81.4 | 625.6 | 8991.1 | 88.2 | 793.0 |
| 9 | 0 (17.5) | 0 (5) | 0 (125) | 5488.1 | 123.0 | 675.0 | 5558.8 | 127.6 | 709.3 |
| 10 | -1 (5.0) | 1 (8) | -1 (10) | 8640.9 | 75.6 | 653.2 | 8855.2 | 79.4 | 703.1 |
| 11 | -1 (5.0) | -1 (2) | 1 (240) | 1434.9 | 237.1 | 340.2 | 1596.6 | 238.0 | 380.0 |
| 12 | 0 (17.5) | 1 (8) | 0 (125) | 8727.1 | 82.0 | 715.6 | 8913.9 | 84.5 | 753.2 |
| 13 | 0 (17.5) | 0 (5) | 0 (125) | 5428.2 | 123.0 | 667.7 | 5595.8 | 125.9 | 704.5 |
| 14 | -1 (5.0) | 1 (8) | 1 (240) | 8700.8 | 74.7 | 650.0 | 8873.7 | 76.0 | 674.4 |
| 15 | 1 (30.0) | 1 (8) | 1 (240) | 8811.3 | 83.5 | 735.7 | 8856.3 | 86.3 | 764.3 |
| 16 | 1 (30.0) | 0 (5) | 0 (125) | 5318.1 | 127.3 | 677.0 | 5467.4 | 128.8 | 704.2 |
| 17 | 0 (17.5) | 0 (5) | 1 (240) | 5441.7 | 127.0 | 691.1 | 5674.1 | 128.3 | 728.0 |
| 18 | 0 (17.5) | 0 (5) | 0 (125) | 5289.3 | 122.6 | 648.7 | 5276.1 | 125.8 | 663.7 |
| 19 | 0 (17.5) | -1 (2) | 0 (125) | 1795.7 | 247.2 | 443.9 | 1843.7 | 249.5 | 460.0 |
| 20 | 1 (30.0) | -1 (2) | -1 (10) | 1883.0 | 244.3 | 460.2 | 1958.0 | 247.4 | 484.4 |

Table represents coded value (and real value)

### 5.3. Results and discussion

From the data in Table 5.2, the black tiger nut tubers recorded 1434.9 g $kg^{-1}$ to 8811.3 g $kg^{-1}$ extracted milk and 74.7 g $kg^{-1}$ to 247.2 g $kg^{-1}$ milk solids while the brown tubers recorded 1596.6 g $kg^{-1}$ to 8991.1 g $kg^{-1}$ for milk extracted and 76.0 g $kg^{-1}$ to 253.6 g $kg^{-1}$ for milk solids. The observed values of milk extracted were higher than those reported by Djomdi Ejoh and Ndjouenkeu, (2006). This can be attributed to the lower volume of water used for the extraction in their study. The ranges of milk solids recorded however were similar to values determined by Er *et al*, (2009) for tiger nut tubers planted at different periods and that determined by Cortés *et al* (2004) for non sweetened *Chufa de horchata*. The data obtained for the extracted milk yield and milk solids were fitted to regression models, with the extraction variables (milling time, tuber: water ratio and boiling time) as the predictors. The fit statistics of extraction yield (Y) for the selected quadratic predictive model is shown in Table 5.3.

Analysis of variance of the full regression models show that in all cases, the models had no significant lack of fit (Table 5.3) and had adjusted R-squared values of 95.2 and 96.4 for the black and brown samples respectively. The models were therefore adequate to be used to predict the effects of the independent variables milling time ($X_1$), meal: water ratio ($X_2$) and boiling time ($X_3$) on the expected milk solids (EMS).

**TABLE 5.3: Coefficients of variables in regression models for expected milk solids (EMS) from black and brown tiger nut tubers**

| SOURCE | d.f. | BLACK TUBERS | | BROWN TUBERS | |
|---|---|---|---|---|---|
| | | **Coefficient** | **P - Value** | **Coefficient** | **P - Value** |
| **Parameter** | 9 | 237.2750 | 0.000 | 207.9020 | 0.000 |
| **Linear** | | | 0.000 | | 0.000 |
| $x_1$ | 1 | 7.8541 | 0.110 | 10.6104 | 0.002 |
| $x_2$ | 1 | 128.5440 | 0.000 | 138.4080 | 0.000 |
| $x_3$ | 1 | -1.0199 | 0.225 | -1.1243 | 0.008 |
| **Square** | | | 0.000 | | 0.000 |
| $x_{11}$ | 1 | -0.2721 | 0.023 | -0.3237 | 0.005 |
| $x_{22}$ | 1 | -10.5232 | 0.000 | -10.5035 | 0.000 |
| $x_{33}$ | 1 | 0.0002 | 0.897 | 0.0023 | 0.055 |
| **Interaction** | | | 0.003 | | 0.073 |
| $x_{12}$ | 1 | 0.2040 | 0.428 | 0.5033 | 0.048 |
| $x_{13}$ | 1 | 0.0145 | 0.049 | 0.0058 | 0.339 |
| $x_{23}$ | 1 | 0.1268 | 0.001 | 0.0444 | 0.097 |
| Residual | | | | | |
| *Error* | 10 | | | | |
| **Lack-of-fit** | 5 | | 0.243 | | 0.451 |
| Pure Error | 5 | | | | |
| **Total** | 19 | | | | |
| **Adj-$R^2$** | | 95.20 % | | 96.40 % | |

5.3.1 *Effect of milling time, meal: water and boiling time on the aqueous extraction of milk solids from tubers*

The expected milk solid (EMS), an estimate of the total obtainable milk solids that can be extracted was calculated from the milk and solids extracted from the tubers. From the regression models for black and brown tubers, the expected milk solids were positively influenced by milling and tuber: water ratio but negatively by boiling time. Table 5.3 also shows that while the interaction of tuber: water ratio and boiling time was significant ($P < 0.05$) for black tubers; it was not significant for brown tubers. Significant interaction suggests that the EMS for black tubers at any tuber: water ratio depended on the time period for which it was boiled. At low tuber: water ratio, increased boiling time decreased EMS, while at high tuber: water ratio longer boiling times increased the expected milk solids (EMS) for the black tubers. This is very well illustrated in the surface plot in Figure 5.1.

The model for brown tubers showed that the effects of tuber: water ratio on EMS were independent of the boiling time, but were significantly influenced by the milling time. It therefore seems that particle size and the tuber: water ratios are very important factors in the extraction of milk solids. Indeed for the brown samples, the effects of increasing milling time on EMS were marginal at low tuber: water ratio, while it was profound at high tuber: water ratio (Figure 5.3). Similar trends were observed for the plots of the black samples. The combined effect of milling time and tuber: water ratio for the tubers (Figure 5.2 and 5.3) showed that, EMS increased slightly with milling time from 5 minutes for both

tubers till 20.6 minutes and 23.4 minutes for black and brown respectively when maximum yield of 720.5 g $kg^{-1}$ and 769.4 g $kg^{-1}$ were obtained. The observed increases in EMS after increasing milling time were similar to observations by Djomdi Ejoh and Ndjouenkeu, (2006). The subsequent decrease in EMS could be attributed to the finer particles generated from continuous milling plugging the pores of the cheese cloth thus slowing down the passage of more solids. Increases in EMS were however more pronounced at higher tuber: water ratio with maximum yield obtained at tuber: water ratio of 1:7.1 and 1:7.4 for black and brown tubers respectively. This phenomenon has also been observed in aqueous extraction studies predicting yield quality of soymilk, extracting reducing sugar from cashew apple bagasse and for crude polysaccharide from *Plantango asiastica* (Akintunde and Akintunde, 2002; Kuila *et al*, 2011; Ye and Jiang, 2011). It has been attributed to the availability of more liquid which increases the driving force of solids out of the plant material (Milani *et al*, 2011). The coefficient of the reduced model EMS after re-evaluation of the full model with respect to the significance of parameters at $p \leq 0.05$ is shown in table 5.3. The reduced model statements for EMS for black and brown varieties are as follow;

$$Y_{black} = 237.27 + 128.55X_2 - 0.03X^2_1 - 10.25X^2_2 + 0.12X_2X_3$$

$$Y_{brown} = 207.90 + 10.61X_1 + 138.40X_2 - 1.12X_3 - 0.32X^2_1 - 10.50X^2_2 + 0.50X_1X_2$$

Where $Y_{black}$ is the predicted (EMS) for black tubers, $Y_{brown}$ is the predicted (EMS) for brown tubers. $X_1$, is milling time (min), $X_2$ is tuber: water ratio and $X_3$ boiling time (min).

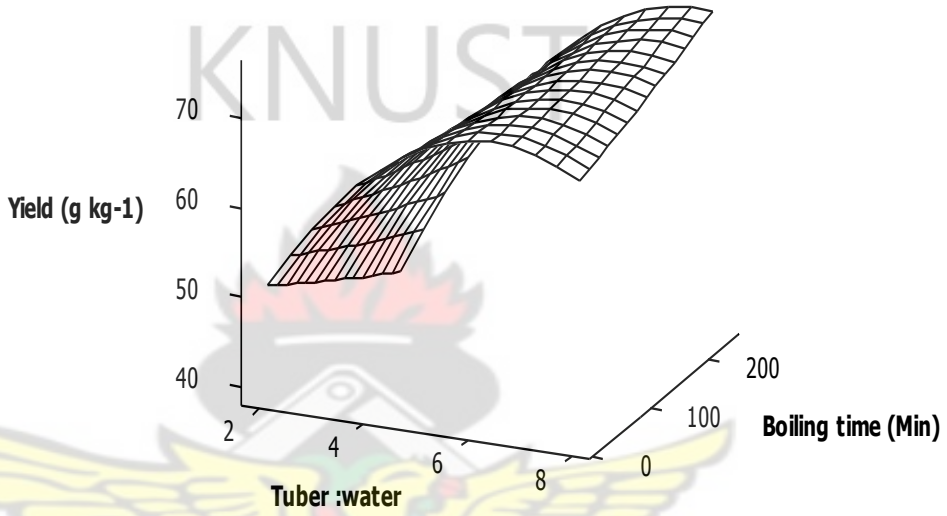



**Figure 5.1: Surface plot of EMS (g kg-$^{1}$) as a function of tuber: water and boiling time for black tubers**

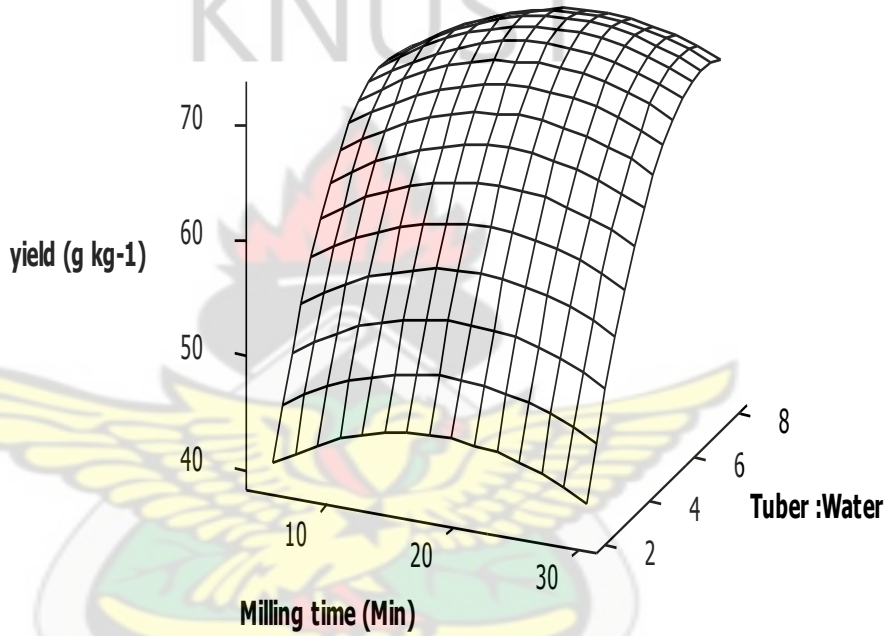



**Figure 5.2: Response surface plot of EMS (g kg-$^{1}$) as a function of milling time and tuber: water for black tubers**

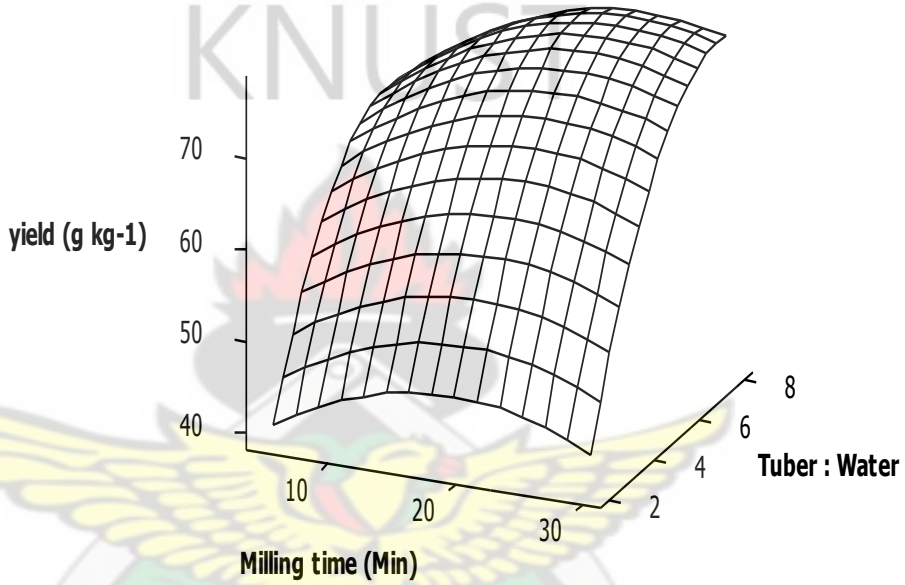



**Figure 5.3: Response surface plot of EMS (g kg-$^{1}$) as a function of milling time and tuber: water for brown tubers**

Boiling also seemed to have significant effects on the solids extracted from the tiger nut tubers (Table 5.3). Increasing the boiling time increased the EMS for both samples. For black tubers, maximum EMS of 687.7 g $kg^{-1}$ was obtained after 10.7 minutes of boiling time and 16.0 minutes of milling time while for brown tubers, increasing boiling time increased milk solids till maximum yield of 762.3 g $kg^{-1}$ at 10.7 minutes of boiling time and 20.6 minutes of milling time. The initial increase could be attributed to the softening of hard tissues of the tuber to facilitate milling into finer particles (Ndjouenkeu and Djomdi Ejoh, 2007).

Figure 5.4 shows that there were significant interaction effects of milling time and boiling time on EMS. The figure also shows that there is an optimum milling time at which maximum EMS were obtained. Boiling time on the other hand decreased EMS at low milling times (i.e. larger particles) while it increased EMS at longer milling times (smaller particle size)

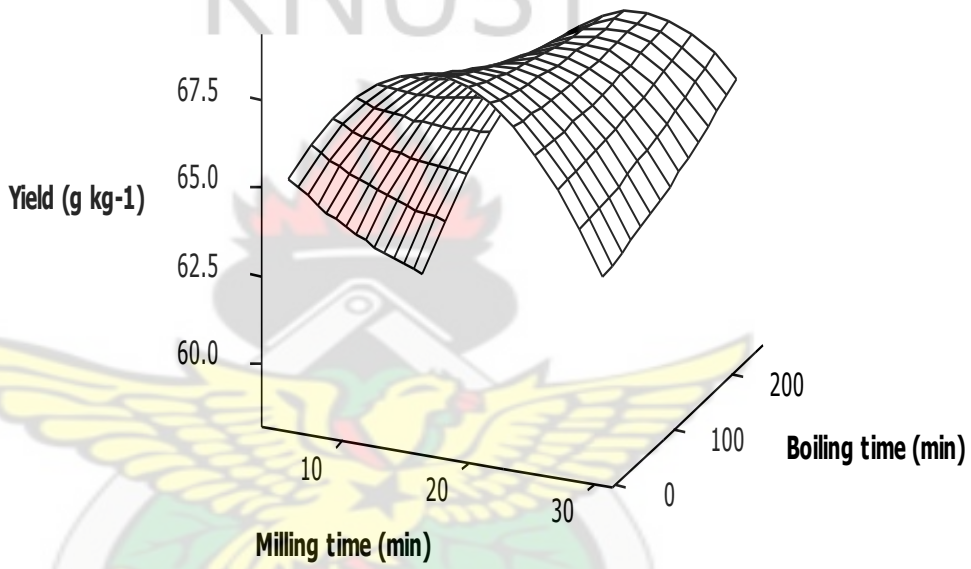



**Figure 5.4: Response surface plot of EMS (g kg-$^{1}$) as a function of milling time and boiling time for black tubers**

5.3.2. *Determination of the factor settings for optimum yield of milk solids*

Contour plots obtained from regression models were overlaid to determine the region of the variable combinations that yielded the optimum EMS, using MINITAB (version 14). The optimum region was where the combinations of the variables settings of milling time, tuber: water ratio and boiling time met the minimum criteria of 700 g $kg^{-1}$ EMS (Figure 5.5). The minimum criteria for EMS were derived from preliminary extractions of black and brown tiger nut tubers. Various combination of factors that resulted in EMS yields above 700 g $kg^{-1}$ are shown in Table 5.4

The table shows that optimum extraction of milk solids can be achieved at a minimum boiling time of 10 minutes for both black and brown tubers, when tuber: water is averagely 1:7 and at about 20-24 minutes of milling time.

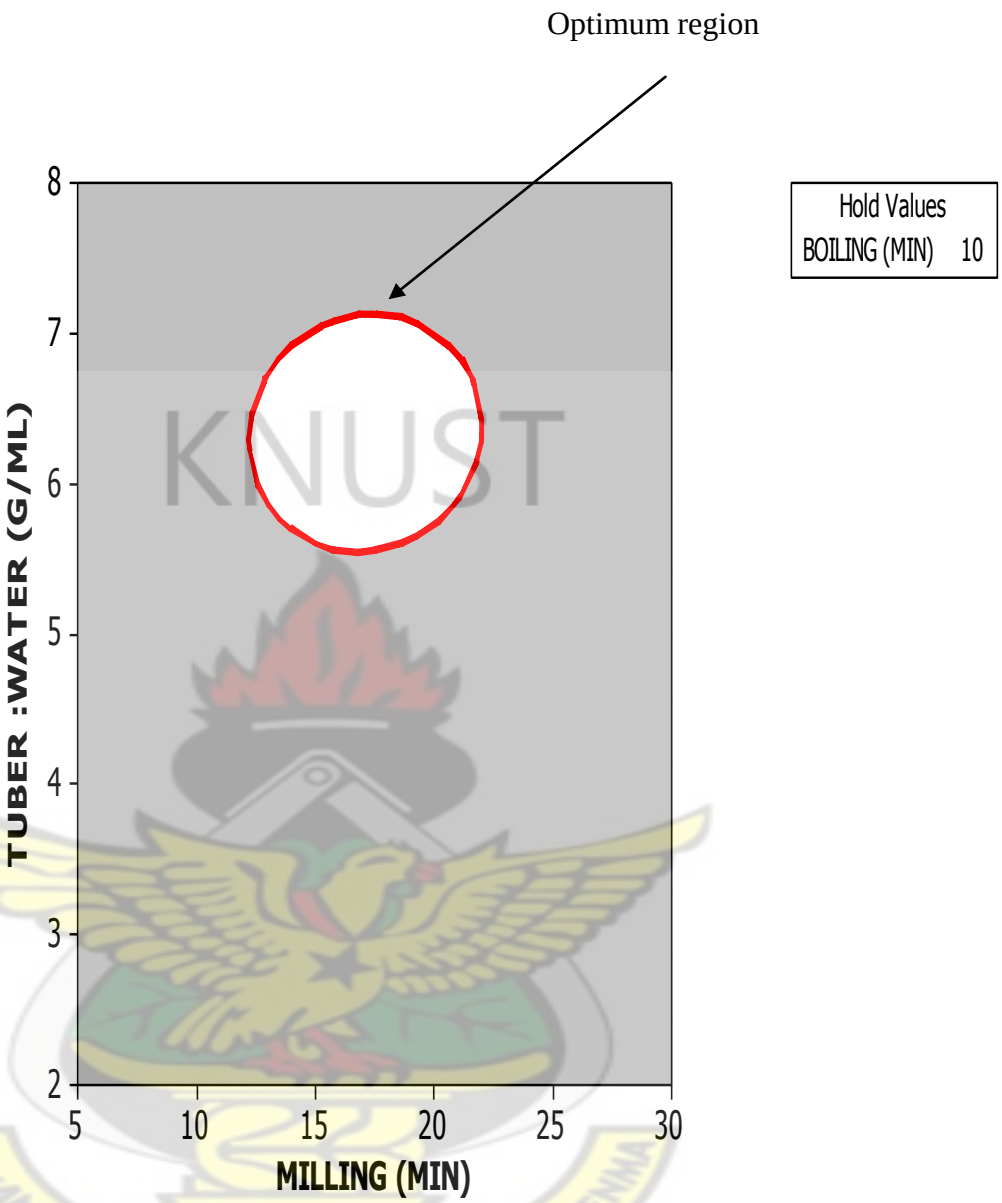



**Figure 5.5 Overlaid contour plot of EMS for black tubers, showing settings of the process variables to obtain optimum yield of milk solids**

**Table 5.4: Summary of optimum EMS (g kg-$^{1}$) of tiger nut solids as a function of treatment variables**

| TUBERS | EXTRACTION FACTORS | | | |
|---|---|---|---|---|
| | Milling time ($X_1$) | Tuber: water ratio ($X_2$) | Boiling time ($X_3$) | EMS (g kg-$^{1}$) |
| Brown | 20.6 | 1:5 | 10.7 | 762.3 |
| Brown | 22.0 | 1:7.2 | 10 | 809.9 |
| Brown | 5.0 | 1:7.9 | 239.3 | 717.5 |
| Brown | 23.4 | 1:7.4 | 125 | 769.4 |
| Brown | 17.5 | 1:7.0 | 10.2 | 803.3 |
| Brown | 22.6 | 1:8 | 10.4 | 802.0 |
| Brown | 24.2 | 1:7.6 | 240 | 793.1 |
| Brown | 30.0 | 1:7.4 | 10.2 | 789.6 |
| Black | 17.2 | 1:6.4 | 10 | 706.6 |
| Black | 20.6 | 1:7.1 | 125 | 720.5 |
| Black | 17.5 | 1:7.7 | 239.9 | 744.3 |
| Black | 23.6 | 1:8 | 239.1 | 754.0 |
| Black | 23.8 | 1:7.8 | 240 | 754.9 |
| Black | 30.0 | 1:7.9 | 239.3 | 743.9 |



5.3.3 *Verification of predictive model*

The reliability of the model in predicting EMS of tiger nut milk solids was verified in separate extraction runs, using the extraction variable combinations

within the optimum region in Figure 5.5. The two data sets (i.e. data obtained from using the models to predict the EMS and experimental data obtained to verify the model adequacy) are shown in Table 5.5

**Table 5.5 Predicted and experimental EMS (g kg-$^{1}$) of tiger nut tubers at the selected factor levels**

| Tiger Nut Variety | Milling time (min) | Tuber: water (g ml-$^{1}$) | Boiling (min) | Milk Extracted (g kg-$^{1}$ ) | Milk Solids (g kg-$^{1}$) | Predicted Value (g kg-$^{1}$) | Experimental Value (g kg-$^{1}$ ) |
|---|---|---|---|---|---|---|---|
| Black | 17.0 | 1:6.4 | 10.0 | 689.56 (2.93) | 94.4 (0.3) | 706.6 | 650.7 (4.0) |
| Brown | 22.0 | 1: 7.0 | 10.0 | 785.75 (3.36) | 95.2 (1.9) | 809.9 | 748.4 (18.4) |

The results indicate that the experimental values obtained for the black and brown tubers were 92.09 % and 92.41% of the predicted values respectively, thus confirming the adequacy of the predictive models.

### 5.4. Conclusion

The yield of milk solids extracted from tiger nut tubers was determined by variables such as the degree to which the nuts were boiled before crushing, the extent to which the crushed nuts were milled, and the tuber: water ratio during extraction. The interactions of the variables influenced the yield of milk solids. Optimization of the processing conditions using Response Surface Methodology techniques showed estimated boiling time of 10 minutes, crushing and milling for about 17-22 minutes and then extracting using a 1: 7, tuber: water ratio will yield

optimum EMS. These findings could be used as a basis for establishing scale up criteria for the extraction of tiger nut milk solids for industrial applications.

*The next chapter reports on the study to determine the chemical and functional properties of tiger nut milk and solids extracted with the determined variables above as well as the milk cake*

# CHAPTER SIX

## 6.0 CHEMICAL COMPOSITION AND FUNCTIONAL PROPERTIES OF TIGER NUT (*Cyperus Esculentus* L) MILK, POWDER AND CAKE

### 6.1. Introduction

The search for new food ingredients from underutilized crops has been ongoing in developing countries to support food and nutrition security. One crop that has gained attention is tiger nut (Belewu and Belewu, 2007; Sanful, 2009; Adekanmi, *et al.,* 2009; Adejuyitan, 2011). It is commonly known as earth almond, tiger nut, chufa, yellow nut sedge, Zulu nuts and *atadwe* by the Akans in Ghana (Dokosi, 1998; Pascual *et al*., 2000). In Ghana the black and brown varieties are mainly cultivated. Harvested tubers are first dried and distributed through intermediaries, mostly women who buy them in bulk and retail from almost all the major and minor markets in the country.

The tubers are used as food by several countries around the Mediterranean, especially Egypt and Spain as well as countries in West Africa especially Cameroon, Nigeria and Ghana (Dokosi, 1998; Pascual *et al*., 2000). Tiger nut can be eaten raw, roasted or dried. It is also baked and milled into powder for use as food additives, spices or be made into a refreshing beverage called "Horchata De Chufas" or tiger nut milk (Mosquera *et al*., 1996; Umeri and Enebeli, 1996). Natural horchata de chufa has a pH in the range 6.3-6.8 and is rich in starch, glucose and proteins. It is also rich in minerals like potassium, phosphorus, and has appreciable quantities of vitamins E and C. (Tigernuts Traders S.L., 2009; Belewu and Abodunrin, 2008). In Ghana tiger nut remains classified as a minor crop and not captured in official records (Wills, 1962; SRID, 2011).

Though considerable studies have been done on the proximate composition and mineral content of tiger nuts tubers, (Eyeson and Ankrah, 1975, Fatoki *et al*., 1995; Pascual *et al.*, 2000; Coskuner *et al*., 2002; Oladele and Aina, 2007; Adejuyitan, 2011) very little has been done on tiger nut milk, its solids and cake. The objectives of this study were to determine the chemical and functional properties of tiger nut milk, its powder and cake in order to explore its potential in food formulations.



## 6.2 Materials and methods

### 6.2.1 Materials

The Brown variety of tiger nut tubers (*Cyperus Esculentus* L.) grown at Twifo Praso, in Ghana was harvested in the main planting period (April to July) and used for the study. The tubers were sorted, washed and dried in a Sanyo oven (Model MOV-212, Osaka, Japan) at 55$^{o}$C for 5 days till moisture was between 7 and 10 %.

### 6.2.2 Milk extraction

The process for the extraction of milk from tiger nut tubers was as shown in Figure 6.1. The tubers were milled in a Stephan universal machine industrial blender (Type VCM 12, Hameln, Germany). The first and second extracts were mixed together and stored in an Ocean freezer (Model, NJ55 TB ECO, Italy) at -18$^{o}$C till freezed dried.

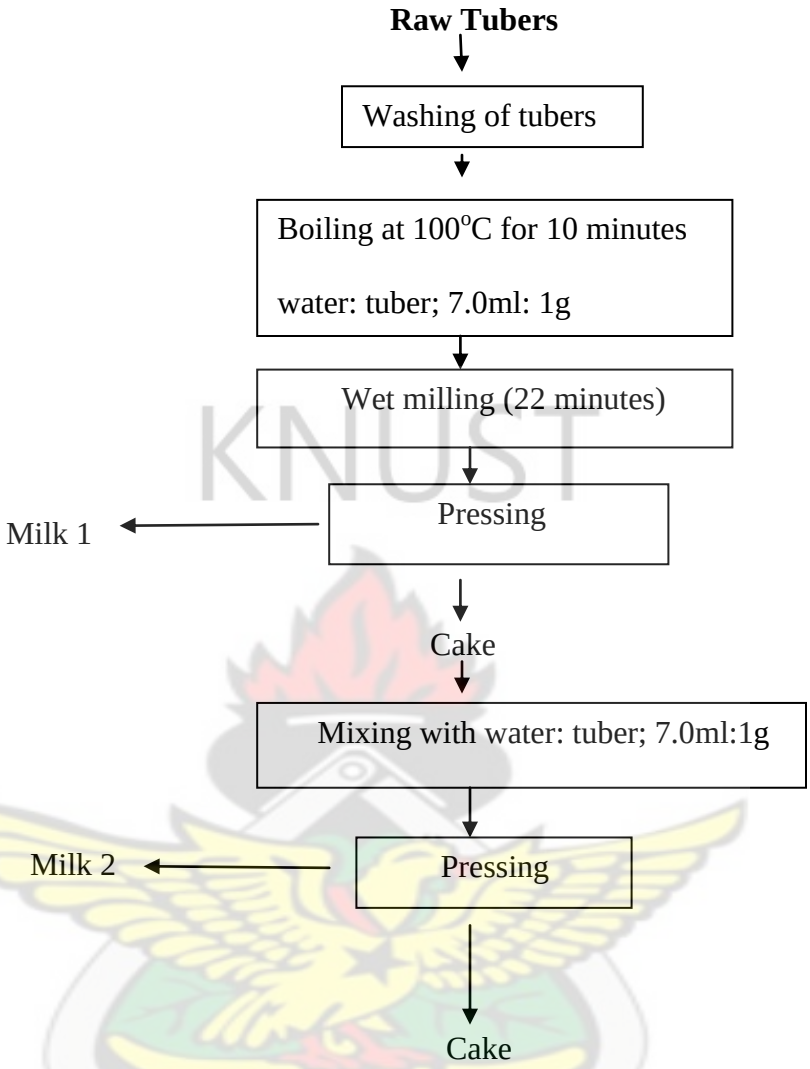



**Figure 6.1: Flow diagram for extracting milk from tubers**

6.2.3 Freeze drying of milk

The milk was freeze dried to obtain tiger nut milk solids for the determination of its functional properties. A True Ten Industrial Company vacuum freeze dryer (Model YK-118-50, Taichung City, Taiwan) was used to freeze dry extracted milk. About 50 g of milk was poured into polyethylene bags and frozen in an Ocean freezer (Model, NJ55 TB ECO, Italy) at -18°C for 12 hours. The frozen samples were then placed on a metal tray and then loaded into the vacuum freeze

dryer, which had been pre-conditioned for 10 minutes. The freeze dried samples were milled using a Thomas Scientific laboratory mill (Model 3383-L70, Swesdesboro, New Jersey, USA) through a 0.4 µm sieve to obtain the milk powder. The milled sample was packed in a polyethylene bag, wrapped in aluminum foil and stored in an Ocean freezer (Model, NJ55 TB ECO, Italy) at -18$^{o}$C for future use.

6.2.4. Tiger nut cake flour

Tiger nut cake obtained after milk extraction was oven dried at 55$^{o}$C using Sanyo, Japan; oven (Model MOV-212, Osaka, Japan) till moisture was between 7 and 10 % w/w. The dried cake was milled using a laboratory mill (Christy and Norris ltd, Chelmsford England) through a 0.8 µm sieve to obtain the flour. The tiger nut cake flour was packed in a polyethylene bag, wrapped in aluminum foil and stored in an Ocean freezer (Model, NJ55 TB ECO, Italy) at -18$^{o}$C for future use.

6.2.5. Chemical Analysis

Moisture and crude protein (N x 6.25) contents were determined in triplicate using the air oven drying method at 105$^{0}$C (AOAC 925.10, 2000) and (AOAC 960.52, 2000) respectively. Total ash and crude fat, were also determined in triplicate according to the AOAC official methods (AOAC 923.03, 2000; AOAC 920.39, 2000). Crude fibre was determined according to methods described in Pearson's Composition and Analysis of Foods (Egan *et al.*, 1981) with modifications (Appendix A1). Crude protein (N x 6.25) was determined by the Macro-Kjeldahl method using 1.0 g samples. Ash was determined by the incineration of a 1.0 g sample placed in a muffle furnace maintained at 550$^{0}$C for

6 hours (until ash was obtained). Crude fat (ether extract) in cake and powder were determined by exhaustively extracting 5.0 g of the sample with petroleum ether by use of a Soxhlet apparatus. The oil in the milk was obtained by the Gerber's method (Nielson, 1998). The level of carbohydrate was obtained by the difference method, that is, by subtracting the sum of the protein, fat (lipid), fiber and ash from the total dry matter. The calorific value was calculated by multiplying the mean values of the crude protein, fat and carbohydrates by the Atwater factors of 4, 9 and 4 respectively (FAO, 2006). pH was determined by mixing 10.0 g of sample with 90 ml distilled water, allowing it to stand for 30 minutes, filtering with Whatman filter paper number 1 and determining the pH on filtrate using a Jenway pH meter (Model 3505, Essex, UK).

Sucrose was determined by polarimetry using the Bellingham Stanley polarimeter (Model ADP 220, Kent, UK). Fourty gram (40 g) of sample, dissolved in 100ml of water was filtered using a whatman filter paper number 1 and analysed for sucrose using the polarimeter. Reducing sugars were determined by the titrimetric method of Lane and Elyon (Eghan *et al*., 1981). Total Antioxidant Capacity was measured in samples by using Ferric Reducing Antioxidant Power (FRAP assay) (Benzie and Strain, 1999) and the results expressed as mM frap/100g (Appendix A2). Total Phenol Content was determined by the method of Mojca-Skerget *et al.* (2005) and the results expressed as mg gallic acid equivalent/ ml (mg GAE/ml) (Appendix A3).

6.2.6 Mineral analysis

A wet digestion method using nitric acid (AOAC, 999.11, 2000) was used to eliminate all organic matter from the samples before samples were analyzed for the individual minerals. One gram (1.0 g) of the sample was weighed into a 250 ml beaker. Twenty five millilitres (25 ml) of conc. $HNO_3$ was added and the beaker was covered with a watch glass. The sample was digested with care on a hot plate in a fume chamber until all the organic matter had been oxidized (20-30 min.). The pale yellow solution was cooled and 1 mL of 70 % $HClO_4$ was added with care. Digestion was continued until the solution was almost colorless (until all the $HNO_3$ was removed). The solution was then cooled slightly after the digestion process, and about 30 ml distilled water was added and allowed to boil for about 10 minutes using a Jenway hotplate and stirrer (Model, 1103.2, Essex UK) then filtered when hot through No. 4 Whatman filter paper into a 100 ml volumetric flask. The beaker was washed well with distilled water and filtered. The flask was then cooled and made up to the 100 ml mark. This solution was used for all the mineral analysis.

The following minerals; Sodium (Na), Potassium (K), Magnesium (Mg), Calcium (Ca), Zinc (Zn), Iron (Fe), Phosphorus (P), Manganese (Mn) and Copper (Cu) were all determined in triplicate with the use of the Perkin Elmer Atomic Absorption Spectrophotometer (Model AA 220FS, Massachussetts, USA).

6.2.7. Functional Properties

The bulk density was determined in triplicate by the method of Narayana and Narasimga (1982). A 50 g flour sample was put into 100 ml measuring cylinder.

The cylinder was gently agitated continuously until a constant volume was obtained. The bulk density ($g/cm^3$) was calculated as weight of flour (g) divided by flour volume ($cm^3$).

The water absorption capacity was determined in triplicate according to the method described by Gbeddy (2001). 5.0 g of the sample was weighed and mixed with 20 ml distilled water and allowed to stand for 30 min. at a room temperature of 28$^oC$. The mixture was then centrifuged at 1512 relative centrifugal force (RCF) for 15 min. using Denley centrifuge (Model BS400, Sussex, England). The excess water was decanted from the centrifuge tubes into a measuring cylinder and the volume determined. The water absorption capacity was calculated as (ml) of water absorbed per gram of flour.

The oil absorption property was determined in triplicate according to the method described by Gbeddy, (2001). 2.0 g of the sample was mixed with 5 ml of vegetable oil (Frytol vegetable oil with a density of 0.89 g/ml), and allowed to stand for 15min. at a room temperature of 28$^oC$. The mixture was centrifuged at 1512 relative centrifugal force (RCF) for 15min. using Denley centrifuge (Model BS400, Sussex, England). The oil was decanted from the centrfuge tubes into a measuring cylinder and the volume determined. The oil absorption capacity was calculated as (ml) of oil absorbed per gram of flour.

6.2.8. Statistical analysis

The data obtained was subjected to analysis of variance (ANOVA) using MINITAB 14 statistical package (MINITAB Inc., U.S.A.).

## 6.3 Results and Discussions

### 6.3.1 Physical and chemical composition of tiger nut milk, solids and cake

The physical and chemical composition of tiger nut milk, solids and cake are summarized in Table 6.1

The milk had very high moisture content whilst the milk powder was high in protein, ash, fat, carbohydrate, anti-oxidants and total phenols. The milk cake was however high in total dietary, insoluble and soluble fibers.



**Table 6.1: Physical and chemical composition of Twifo Praso brown tiger nut milk and solids**

| COMPONENT | MILK[a] | MILK POWDER[b] | MILK CAKE[c] |
|---|---|---|---|
| Moisture (g kg-$^{1}$) | 904.8 ± 1.9 | 56.1 ± 0.6 | 71.0 ± 1.0 |
| Total dry matter (g kg-$^{1}$) | 95.2 ± 1.9 | 943.9 ± 0.1 | 929.0 ± 1.1 |
| Protein (g kg-$^{1}$) | 2.3 ± 0.2 | 76.9 ± 0.8 | 46.1 ± 1.8 |
| Ash (g kg-$^{1}$) | 0.2 ±0.1 | 24.4 ± 3.2 | 5.7 ± 0.5 |
| Fat (g kg-$^{1}$) | 7.8 ± 0.3 | 395.6 ± 3.3 | 242.7 ± 3.6 |
| Carbohydrate (g kg-$^{1}$) | 83.8 | 465.9 | 145.8 |
| Total dietary fiber (g kg-$^{1}$) | 1.1 ± 0.2 | 37.2 ± 0.1 | 559.7 ± 2.4 |
| Insoluble fiber (g kg-$^{1}$) | 1.1 ± 0.0 | 37.2 ± 0.1 | 556.3 ± 2.9 |
| Soluble fiber (g kg-$^{1}$) | 0.0±0.0 | 0.0±0.0 | 3.4 ± 0.2 |
| Anti oxidant level (mM frap/100g) | 140.7 ± 1.6 | 4634 ± 17.0 | 1169 ± 19.5 |
| Total phenol (mg GAE/ml) | 5.7 ± 0.8 | 187.7 ± 1.8 | 57.70 ± 0.4 |

Milk[a] = tiger nut milk; Milk Powder[b] = tiger nut milk powder; Milk Cake[c] = tiger nut milk cake

The total dry matter, an indication of solids in the milk was 95.2±1.9 g kg-$^{1}$. This was lower than the 144.0 g kg-$^{1}$and 217.0 g kg-$^{1}$determined for cow and goat milk respectively by Belewu and Azeez (2008) but was comparable to that determined by same for coconut. The protein content recorded for the milk, 2.3 g kg-$^{1}$ was lower than the 3.84 g kg-$^{1}$ determined in unsweetened tiger nut drink by Cortes *et al*., (2004). This could be due to the differences in the solids content of the milk. It was also relatively lower than the 25.2 g kg-$^{1}$ determined for sesame seeds and the range of 30.9 - 36.7 g kg-$^{1}$ recorded for soymilk extracted from different varieties of soy beans (Harjai and Singh, 2007). This can be attributed to the relatively higher content of protein in the seeds. It was also significantly lower than the protein content of 29.0 g kg-$^{1}$ recorded for cow milk (Chen, 1989). The protein content of the milk powder, 76.9 g kg-$^{1}$ was significantly lower than the 381.8 g kg-$^{1}$ determined for skimmed milk powder by Aidoo *et al*, (2010). It was also lower than the range of 268.1 g kg-$^{1}$ -314.0 g kg-$^{1}$ recorded by same authors in the composite milk powder products developed from cowpea and peanuts. This could be attributed to the relatively higher levels of protein in cowpea and peanut compared to tiger nut tubers. The protein content recorded for the cake, 46.1g kg-$^{1}$ was relatively lower than the range of 79.2 to 93.1 g kg-$^{1}$ for protein content in okara determined for varieties of soybeans by Hajai and Singh (2007). The oil content of 395.6 ± 3.3 g kg-$^{1}$, 242.7 ± 3.6 g kg-$^{1}$in the milk powder and cake respectively were significantly higher than the 7.8 ± 0.3 g kg-$^{1}$ recorded for the milk. This was also higher than the 176.6 g kg-$^{1}$ recorded for the raw tubers in table 3.1. The 7.8 ± 0.3 g kg-$^{1}$ of fat in the milk compared to the 77.1 g kg-$^{1}$ for

peanut milk produced by Isanga and Zhang (2007) can be attributted to the fact that their peanut milk was prepared as slurry from whole nuts. The high levels of fat in the powder and cake implies that these can also be exploited for its oil which is known to have a good fatty acid profile similar to olive oil (Fatoki, *et al*., 1995). The high level of oil in the tiger nut milk powder however reduces its potential as an ingredient in bar chocolate because many vegetable oils have been found to reduce the formation of stable ß crystals resulting in reduced gloss, texture and resistance to heat and fat bloom. The carbohydrate content determined was 83.8 g $kg^{-1}$, 465.9 g $kg^{-1}$and 145.8 g $kg^{-1}$ for milk, powder and cake respectively. The carbohydrate content in the milk was relatively higher than the range of 19.9 - 27.8 g $kg^{-1}$ determined in different varieties of soybeans (Tunde-Akintunde and Souley, 2009). The total dietary fiber value of 559.7 g $kg^{-1}$ recorded for the cake was significantly higher than the 66.6 g $kg^{-1}$ recorded for soy okara by Wickramarathna and Arampath (2003). It was also higher than the range of 10.9 g $kg^{-1}$ to 141.7 g $kg^{-1}$ recorded for rice, barley and wheat by Azizah and Zainon (1997). The high total dietary fiber values in the cake however compared favorably with the 597.1 g $kg^{-1}$ obtained by Sánchez-Zapata *et al*., (2010) in their study on tiger nut cake. Dietary fiber has well documented beneficial effects on human health and body functions thus the need to increase its consumption. The high amount suggests that the cake can be added to foods to increase the fiber content.

Several anti-oxidants are known to have free radical scavenging activity leading to their beneficial implications in human health. The anti-oxidant capacities of

aqueous extracts of wild plants including tiger nuts by Cook *et al* (1998) have indicated that tiger nut tubers have a relatively high total anti-oxidant capacity. The results of the determination of the anti-oxidant levels and total phenol in the processed tubers are summrized in Table 6.1. The results indicate that the dehydration of the milk improved the levels significantly. The anti-oxidant level of 4634 mM frap/100g for the milk powder was comparable to the 4645 mM frap/100g and 4666 mM frap/100g determined in raw potatoes and pumpkin seeds by Kuyanga *et al*. (2011) but was significantly lower than the 7102 mM frap/100g determined by same authors for groundnuts which is a popular nut in Ghana. Though the results for the total phenols 187.7 mg of GAE/ml was significantly lower than the 611 mg of GAE/ml determined for raw cocoa by Lee *et al*., (2003). It was however higher than the range of 7.66 to 63.20 mg GAE recorded for different types of natural and alkalized cocoa powder by Miller *et al.,* (2008).

6.3.2 Composition of sugars and minerals in processed tiger nut products.

The total sugars, sucrose and reducing sugars content of the processed tiger nut tubers are presented in table 6.2. Among the products, the milk powder had significantly higher contents of sugars.

**Table 6.2: Composition of sugars in processed Twifo Praso brown tiger nut tubers**

| COMPONENT (g kg-$^{1}$) | MILK[a] | MILK POWDER[b] | CAKE[c] |
|---|---|---|---|
| Total sugars | 8.9 ± 0.1 | 353.7 ± 1.2 | 8.4 ± 0.2 |
| Sucrose | 8.2 ± 0.9 | 316.7 ± 1.5 | 6.5 ± 0.2 |
| Reducing sugar | 0.7 ± 0.0 | 36.3 ± 1.0 | 1.9 ± 0.0 |

Milk[a] = tiger nut milk; Milk Powder[b] = tiger nut milk powder; Milk Cake[c] = tiger nut milk cake



The reducing sugar content of the dehydrated tiger nut milk was within the range 7.0 g kg-$^{1}$ to 75.0 g kg-$^{1}$ determined for dairy milk substitutes developed from dehydrated peanut-cowpea blend by Aidoo *et al* (2010). It was however lower than the 357.0 g kg-$^{1}$ determined for skimmed power (Aidoo *et al*., 2010) a major dairy milk product used for the manufacturing of chocolate products.

The mineral content of liquid milk, powder and cake are shown in Table 6.3. The results show that the materials are a potential source of the following macro elements, phosphorus, potassium, magnesium and the trace element zinc needed for good nutrition. It also shows that the dehydration of the liquid milk had also resulted in the improvement of the levels of all the elements in the milk.

**Table 6.3: The mineral content of liquid milk, milk cake and milk powder from processed tiger nut tubers**

| | MINERAL CONTENTS (mg $kg^{-1}$) IN TIGER NUT SAMPLES | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Sample** | **Phosphorus (P)** | **Potassium (K)** | **Magnesium (Mg)** | **Calcium (Ca)** | **Sodium (Na)** | **Iron (Fe)** | **Copper (Cu)** | **Zinc (Zn)** | **Manganese (Mn)** |
| **MILK**[a] | 116.89 ±4.50 | 410.75 ±0.00 | 74.00 ±0.00 | 12.75 ±0.00 | 129.25 ±0.00 | 24.50 ±0.25 | 5.50 ±0.00 | 20.33 ±0.14 | 7.33 ±0.14 |
| **MILK POWDER**[b] | 1227.80 ±1.84 | 4253.00 ±47.20 | 770.80 ±22.40 | 133.90 ±0.50 | 1357.65 ±0.45 | 257.35 ±1.60 | 56.60 ± 0.60 | 213.50 ±0.20 | 77.60 ±0.400 |
| **MILK CAKE**[c] | 319.20 ±0.00 | 761.50 ±00 | 591.00 ±0.00 | 14.00 ±0.00 | 66.25 ±0.25 | 22.00 ±0.43 | 11.75 ±0.00 | 72.25 ±0.00 | 44.50 ±0.00 |

Milk[a] = tiger nut milk; Milk Powder[b] = tiger nut milk powder; Milk Cake[c] = tiger nut milk cake

6.3.3 The pH and functional properties of processed tiger nut products

The pH values of the milk and the powder were 6.80 ± 0.05 and 6.87 ± 0.02. The acidity of the products were within the range of 6.0 – 6.9 reported for other vegetable milk like coconut and soy (Belewu and Belewu, 2007; Harjai and Singh, 2007). The water absorption capacity (WAC) and oil absorption capacity (OAC) for the milk powder and cake were 0.87 ± 0.03 (ml/g); 1.09 ± 0.03(ml/g) and 15.27 ± 0.16; 3.51 ± 0.21 respectively.

**Table 6.4: The pH and functional properties of milk and powder from processed tiger nut tubers**

| CHARACTERISTICS | MILK[a] | MILK[b] POWDER | MILK CAKE[c] |
|---|---|---|---|
| pH | 6.87 ± 0.02 | 6.80± 0.05 | 6.60±0.01 |
| Bulk density (g/cm$^3$) | N.D | 0.80 ± 0.02 | 0.26 ± 0.02 |
| Water absorption capacity (ml/g) (WAC) | N.D | 0.87 ± 0.03 | 15.27 ± 0.16 |
| Oil absorption capacity (ml/g) (OAC) | N.D | 1.09 ± 0.03 | 3.51 ± 0.21 |

N.D not determined
Milk[a] = tiger nut milk; Milk Powder[b] = tiger nut milk powder; Milk Cake[c] = tiger nut milk cake

While the WAC of milk powder was lower than the 1.37 ml/g and 1.26 ml/g determined for yellow and brown tiger nut flour the OAC of the milk powder was comparable to 1.07 ml/g and 1.13 ml/g determined for same by Oladele and Aina (2007). It was however also lower than the 3.70 ml/g determined for conophor nut flour by Odoemelam, (2003). The WAC and OAC for the milk cake was 15.27 ± 0.16 (ml/g) and 3.51 ± 0.21 (ml/g) respectively. The OAC for the milk cake was significantly different from the 1.50 ± 0.03 (ml/g) recorded for OAC by Appiah *et al*., (2011) for *Artocarpus altilis* pulp flour.

The higher WAC and OAC may be attributed to the opening up of fibers as well as break down of carbohydrates and starch molecules during pre-treatment of tubers for milk extraction. The WAC of flour represents its ability to associate with water under conditions where water is limited such as dough and pastes (Giami, 1993). The results therefore suggest that the tiger nut milk cake can find useful application in food systems that will require some moistness and water holding. The Lower WAC for the milk powder however suggests it will be desirable for making thinner gruels like porridge. Fat absorption is an important property in food formulation because fat improves flavor and mouth feel of foods. The higher OAC for the milk cake suggests that the cake will be a better flavor retainer and mouth feel improver (Okezie and Bello, 1988) than the tiger nut milk powder with OAC of 1.09 ± 0.03(ml/g). Basically, the mechanism of oil absorption capacity is mainly due to the physical entrapment of oil by capillary action (kinsella, 1976). More over the hydrophobicity of proteins also plays a major role in fat absorption (Voutsinas and Nakai, 1983) as well. The significantly higher figures for fiber in milk cake than milk powder may account for the differences in the figures recorded. This may be due to the opening up of the fibers during the cooking of the tubers to entrap more oil rather than exposing hydrophobic sites on proteins.

The bulk density of the tiger nut milk powder was 0.80 g/cm$^3$ compared to 0.26 g/cm$^3$ recorded for the milk cake and the 0.62 g/cm$^3$ and 0.55 g/cm$^3$ determined for the yellow and brown tiger nut flour respectively (Oladele and Aina, 2007). Generally bulk density is very important in deciding the packaging requirement, material handling and application in wet processing in

food industry. The higher figure for the milk powder suggests that more space and materials will be required to handle the milk powder than the tiger nut flour. The bulk density of 0.26 ± 0.02 g/ml obtained for the milk cake makes the cake relatively less dense than 0.80 ± 0.02 g/ml.

### 6.4. Conclusion

The processed tiger nut products had desirable nutritional quality as well as functional properties. They were rich in carbohydrates and fat but low in proteins. Sodium, potassium magnesium and phosphorus which are important for normal physiological functions were the major macro elements in the tubers. The tubers can be said to have a high potential for the production of ingredients for healthy spreads and beverages as well as the formulation of high fiber recipes.

*The next chapter reports on the study of the performance of tiger nut milk and cake in some chocolate products and the shelf life of tiger nut milk-skimmed milk chocolate beverage*

# CHAPTER SEVEN

## 7.0 PRODUCT FORMULATION, SENSORY QUALITY AND SHELF LIFE OF CHOCOLATE PRODUCTS USING TIGER NUT (*Cyperus Esculentus* L) MILK AND CAKE

### 7.1 Introduction

Non dairy milk is an emulsion obtained by water extraction of plant materials such as oil seeds and legumes (Edwards, 1998). It has attracted a lot of interest because of its potential as a low cost and healthy alternative to cow milk (Wang, 1980; Diarra *et al*., 2005). In comparison to cow milk, they have no significant cholesterol or lactose, which makes them an attractive alternative to dairy milk for people conscious of their health and/or diet, vegetarians, and the lactose intolerant. Tiger nut milk, called *horchata de chufa* is a very popular drink in Spain and some South American countries (Cortes *et al*., 2004). It is commonly produced from dried nuts which are ground and extracted with water. In West African countries, the tubers are also used to prepare a milky beverage called "atadwe milk" in Ghana after which the main by-product (cake) is fed to fowls (Umerie *et al*., 1997; Ofori-Anti, 2000).

Dietary fibre intake has been reported to improve serum lipid concentrations, lowering of blood pressure, improving blood glucose control in diabetes, aiding weight loss and improving immune function (Anderson *et al.,* 2009). Sánchez-Zapata *et al*. (2009) has reported that tiger nut milk cake (TNMC) has a high proportion of total dietary fiber (TDF: 59.71 g/100 g), composed mainly of insoluble dietary fiber (IDF: 99.8%). They have also reported that TNMC can be used as a source of dietary fiber in food products. The success of such products however will depend largely on their acceptability by consumers as well as their shelf life stability. The main objective of this study

therefore was to develop acceptable tiger nut milk and cake chocolate products, and to assess the storage stability of the beverage using accelerated shelf life tests.

## 7.2 Materials and methods

The protocol for the optimum extraction of milk from the tiger nut tubers (boiling time of 10 minutes, tuber: water ratio of 1:7 and 20 minutes of milling time) was used to extract he milk. The cake residue obtained from the milk extraction was dried for 24 hours at 55$^{o}$C in a Sanyo oven (Model MOV-212, Osaka, Japan) and together with the milk used to develop products which can be readily commercialized because they are widely consumed.

### 7.2.1 Formulation and evaluation of tiger nut milk -dairy milk beverage

This formulation was done to determine the boundaries for skimmed dairy and tiger nut milk in the mixture design. The beverage formulations in Table 7.1 were pasteurized at 70 ˚C for 30 minutes before evaluation. A Rank Test using a randomized complete block design involving 45 untrained panelists made up of 60 % males and 40 % females and aged between 30-60 years (mainly staff of Cocoa Processing Company Ltd.) were used (Appendix B3). Each panelist was asked to rank the acceptability of samples on a scale of 1-5. The most acceptable was ranked one (1) and the least five (5). Water was available for panelists to rinse their mouth and they were allowed to re-taste samples.

The ranks assigned to the samples were then analyzed using the Friedman Test, and the combination, which gave the most acceptable beverage, was selected.

**Table 7.1: Evaluation of level of substitution of skimmed milk tiger nut milk beverages**

| INGREDIENTS | RECIPES | | | | |
|---|---|---|---|---|---|
| | **A** | **B** | **C** | **D** | **E** |
| Icing sugar (g) | 141.02 | 141.02 | 141.02 | 141.02 | 141.02 |
| Cocoa powder (g) | 57.48 | 57.48 | 57.48 | 57.48 | 57.48 |
| Soy lecithin (g) | 1.40 | 1.40 | 1.40 | 1.40 | 1.40 |
| Vanillin (g) | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Liquid skimmed milk 9.5 % w/w (g) | 800.00 | | | 400.00 | 400.00 |
| Brown variety tiger nut milk 9.5 % w/w (g) | | 800.00 | | 400.00 | |
| Black variety tiger nut milk 9.4 % w/w (g) | | | 800.00 | | 400.00 |
| Total (g) | 1000 | 1000 | 1000 | 1000 | 1000 |



### 7.2.2 Formulation of the tiger nut milk chocolate beverage

Design of Experiment

A 4-component Constrained Mixture Design (CMD) (Cornell, 2002) was used for the recipe formulations using MINITAB (version 14) Statistical Package by MINITAB Inc (PA, USA). The mixture components consisted of sugar ($X_1$), cocoa powder ($X_2$), skimmed milk powder ($X_3$), and tiger nut milk ($X_4$). The lower and upper bound constraints for each mixture component, determined from the above trials are presented in Table 7.2.

**Table 7.2: lower and upper bound constraints for each mixture component**

| Component/Ingredient | Lower bound constraint (%) | Upper bound constraint (%) |
|---|---|---|
| Sugar | 11.50 | 37.50 |
| Cocoa Powder | 3.50 | 29.50 |
| Skimmed Milk | 5.00 | 31.00 |
| Tiger nut Milk | 54.00 | 80.00 |



These were used to generate the fifteen (15) formulations shown in table 7.3 using the MINITAB software. All the ingredients (ie the four components of each formulation) were blended at high speed for 20 minutes. The blender was intermittently stopped every 5 minutes for 1 minute. The homogenized product was poured into a Buchi rotary evaporator (Model R-215, Switzerland) and pasteurized for 30 minutes at 70$^{o}$C. The product was quickly poured into PET bottles pre-sterilized with 70 % ethanol and quickly dropped into cold water. They were stored at 4$^{o}$C till ready for evaluation.

**Table 7.3: Design matrix of the 15 formulations/recipes in proportions**

| Recipe/ Formulations | Sugar ($X_1$) | Cocoa powder ($X_2$) | Skimmed milk ($X_3$) | Tiger nut milk ($X_4$) | Total |
|---|---|---|---|---|---|
| 1 | 11.5000 | 3.5000 | 31.0000 | 54.0000 | 100.0000 |
| 2 | 11.5000 | 12.1667 | 13.6667 | 62.6667 | 100.0000 |
| 3 | 20.1667 | 3.5000 | 13.6667 | 62.6667 | 100.0000 |
| 4 | 37.5000 | 3.5000 | 5.0000 | 54.0000 | 100.0000 |
| 5 | 20.1667 | 12.1667 | 13.6667 | 54.0000 | 100.0000 |
| 6 | 11.5000 | 16.5000 | 5.0000 | 67.0000 | 100.0000 |
| 7 | 11.5000 | 29.5000 | 5.0000 | 54.0000 | 100.0000 |
| 8 | 11.5000 | 3.5000 | 5.0000 | 80.0000 | 100.0000 |
| 9 | 24.5000 | 3.5000 | 5.0000 | 67.0000 | 100.0000 |
| 10 | 11.5000 | 3.5000 | 18.0000 | 67.0000 | 100.0000 |
| 11 | 20.1667 | 12.1667 | 5.0000 | 62.6667 | 100.0000 |
| 12 | 24.5000 | 3.5000 | 18.0000 | 54.0000 | 100.0000 |
| 13 | 18.0000 | 10.0000 | 11.5000 | 60.5000 | 100.0000 |
| 14 | 11.5000 | 16.5000 | 18.0000 | 54.0000 | 100.0000 |
| 15 | 24.5000 | 16.5000 | 5.0000 | 54.0000 | 100.0000 |



7.2.3 Formulation of high fiber chocolate spread

Oven dried tiger nut cake obtained after milk extraction, was used as one of the ingredients in chocolate spread recipes as outlined in Table 7.4. The chocolate spread was prepared according to the procedure for spread production in cocoa Processing Company, Tema using a laboratory ball mill, Wieneroto Attrition Ball Refiner (Wiener & Co. Apparatenbouw B.V. The Netherlands). The process flow diagram for the chocolate spread is outlined in

Figure 7.1. The spread samples were stored in plastic containers for sensory evaluation.

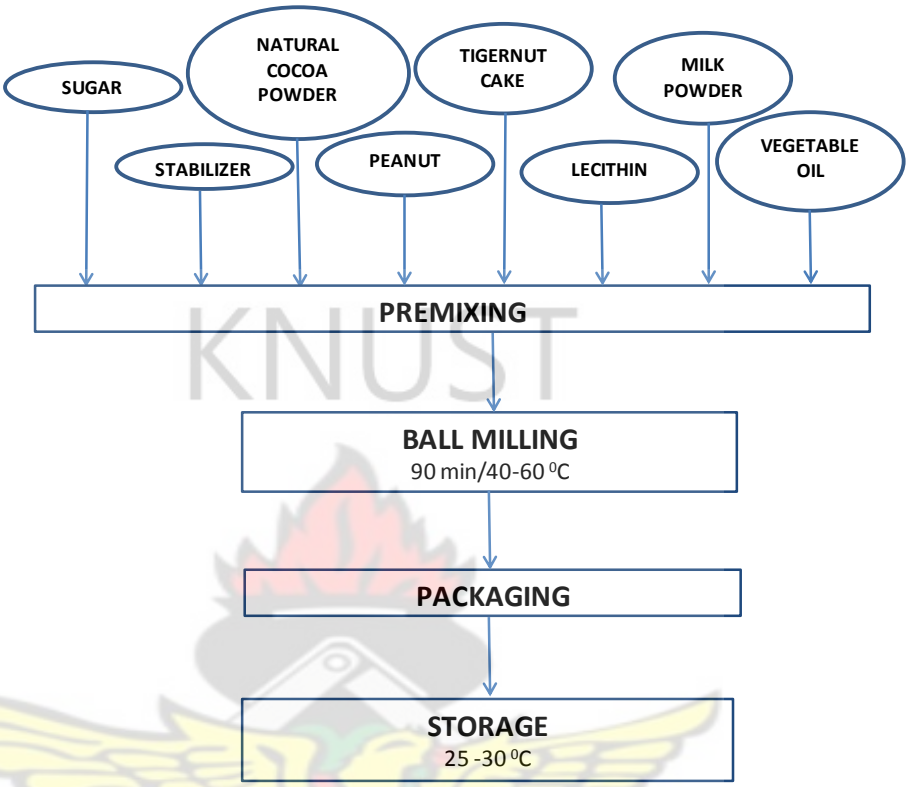



**Figure 7.1: Flow diagram for tiger nut milk cake chocolate spread process in the laboratory**

**Table 7.4: Recipes for tiger nut milk cake chocolate spread**

| INGREDIENTS | RECIPE 1 | RECIPE 2 | RECIPE 3 | RECIPE 4 | RECIPE 5 |
|---|---|---|---|---|---|
| Sugar (g) | 35.93 | 30.93 | 25.93 | 35.93 | 30.93 |
| Refined vegetable oil (g) | 28.62 | 28.62 | 28.62 | 28.62 | 28.62 |
| Natural Cocoa Powder (g) | 9.98 | 9.98 | 9.98 | 9.98 | 9.98 |
| Peanut (g) | 12.97 | 12.97 | 12.97 | 12.97 | 12.97 |
| Skimmed Milk Powder (g) | 10.98 | 10.98 | 10.98 | 5.98 | 5.98 |
| Tiger Nut Milk Cake (g) | 0.00 | 5.00 | 10.00 | 5.00 | 10.00 |
| Stabilizer (Cessa Powder) (g) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Lecithin (g) | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Vanillin (g) | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |



7.2.4 Formulation of high fiber chocolate bar

Oven dried tiger nut cake was used as one of the ingredients in preparing high fiber, low milk and sucrose free chocolate targetted at the wellness market using the recipes outlined in Table 7.5. The chocolate was prepared according to the procedure for making chocolate in cocoa Processing Company, Tema using a laboratory ball mill, Wieneroto Attrition Ball Refiner (Wiener & Co. Apparatenbouw B.V. The Netherlands); conched with a laboratory conch; a

mini conch specially built for use by the Research & Development Laboratory of Cocoa Processing Company, Tema; and tempered and molded manually as outlined in the process flow diagram in Figure 7.2. The chocolate samples were wrapped in aluminum foil for storage and sensory evaluation.

**Table 7.5: Recipes for tiger nut milk cake chocolate**

| INGREDIENTS | RECIPE 1 | RECIPE 2 | RECIPE 3 | RECIPE 4 | RECIPE 5 |
|---|---|---|---|---|---|
| Cocoa Liquor (g) | 28.10 | 28.10 | 28.10 | 28.10 | 28.10 |
| Maltitol (g) | 30.00 | 27.50 | 25.00 | 22.50 | 20.00 |
| FCM (g) | 9.00 | 9.00 | 9.00 | 9.00 | 9.00 |
| Cocoa Butter (g) | 23.56 | 23.56 | 23.56 | 23.56 | 23.56 |
| SMP (g) | 9.00 | 9.00 | 9.00 | 9.00 | 9.00 |
| Tiger Nut milk Cake (g) | 0.00 | 2.50 | 5.00 | 7.50 | 10.00 |
| Vanilin (g) | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 |
| Lecithin (g) | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |

FCM = Full milk powder, SKM = Skimmed milk powder

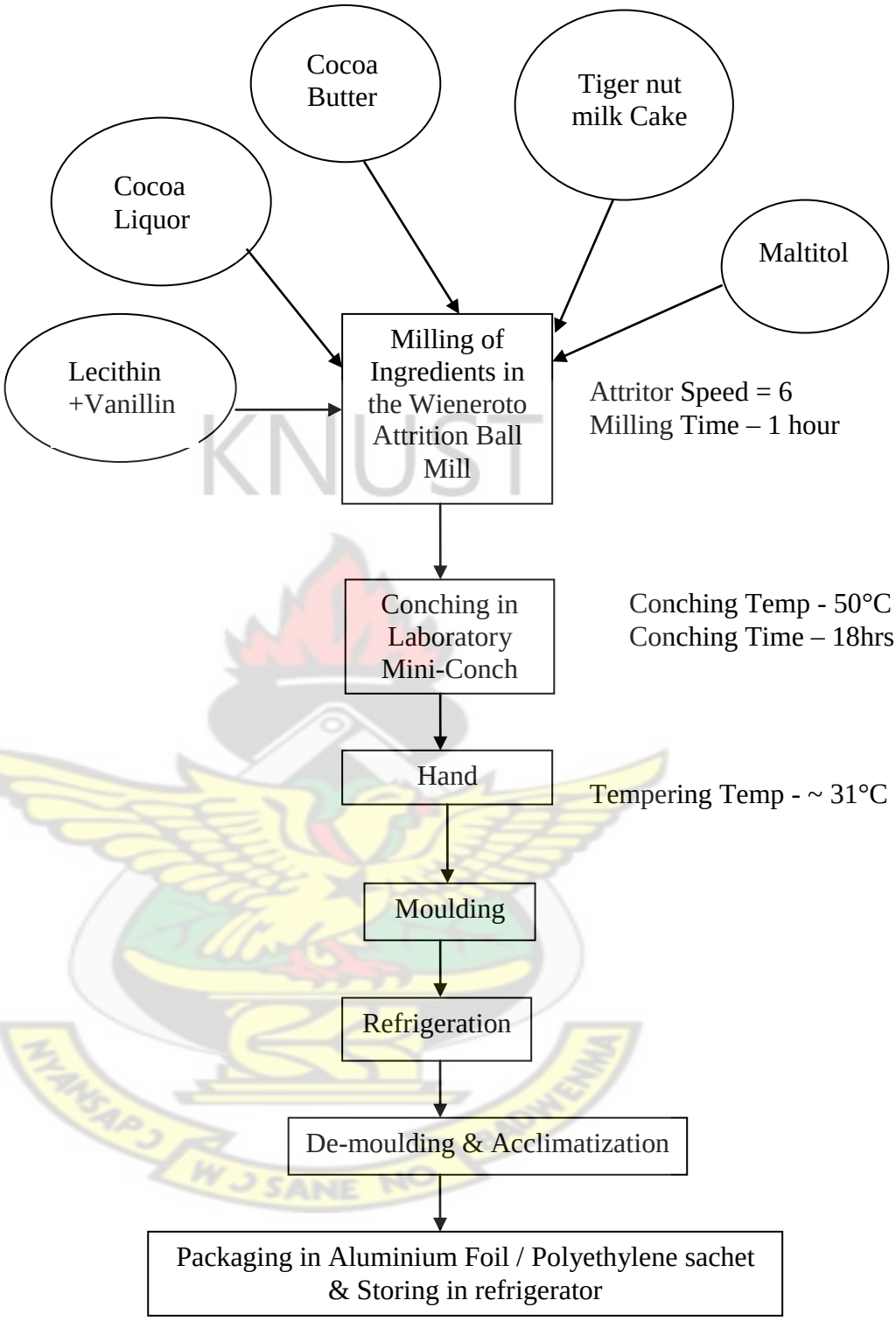



**Figure 7.2: Process Flow chart of the chocolate making process in the laboratory**

7.2.5 Sensory evaluation of tiger nut milk chocolate products

A Hedonic Rating Test (Carpenter *et al*.,2000) involving 35 trained assessors comprising of students from the Department of Nutrition and Food Science of the University of Ghana was used (Appendix B4). The formulations were rated on five attributes (mouth feel, flavor, appearance, after-taste and overall acceptability) which had been determined to be important sensory attributes in chocolate products. Tests were conducted in a room illumined with fluorescent light and kept at a temperature of 25 ˚C. The panelists were asked to allow 1-minute interval between tasting of samples and then score for the attributes of each sample based on the agreed numbers. 15ml of each formulation was presented to panelists in a transparent plastic cup coded with three-digit random numbers. Panelists were provided with water and were allowed to re-taste samples. The sensory assessment followed a Balanced Incomplete Block Design (Plan 11.24, t=15, k=3, r- 7, b=35, λ=1, E=.71, Type I, Cochran and Cox, 1957) where t was number of treatments, k the number of experimental units per block, r, was the number of replications of each treatment, b, the number of blocks; λ, the number of blocks in which each pair of treatment occured together and E, efficiency factor. This design allowed each assessor to evaluate 3 samples out of a total of 15. Each of the 15 formulations was evaluated 7 times by different assessors according to the design. Each of the panelists was randomly assigned one block of three samples from the design. The order within each block was randomized.

7.2.6 Evaluation of high fiber chocolate spread

A Rank sum Test involving 45 untrained panellists made up of 60 % males and 40 % females and aged between 30-60 years (mainly staff of Cocoa

Processing Company Limited. Tema, Ghana) was used (Appendix B5). A randomized complete block design was used for the 5 samples. Each panelist was asked to rank the acceptability of samples on a scale of 1 – 5. The most acceptable was ranked first and the least fifth. Panelists were provided with water to rinse their mouth and were allowed to re-taste samples.

7.2.7 Evaluation of sensory quality of high fiber bar chocolates

A Rating Test (Descriptive Analysis with Scaling) involving 35 trained panelists in Cocoa Processing Company, Limited, Tema, Ghana was used to evaluate the sensory characteristics of the recipes (Appendix B6). The formulations were rated on 6 attributes (flavor, mouth feel, appearance, taste, after-taste and overall acceptability) which are known to be important sensory attributes of chocolate on a nine point hedonic scale with 1 representing disliked extremely and 9 liked extremely. The objective of this sensory evaluation was to determine the feasibility of using tiger nut cake to produce high fiber chocolate acceptable to consumers. The assessors agreed on the testing procedure, the sensory attributes and use of the ballot sheet. Tests were conducted in a room illuminated with fluorescent light. The temperature in the test room was controlled at 25$^{o}$C and the doors to the room closed to prevent any outside influence that might interfere with normal perception.

7.2.8 Accelerated shelf life studies

Tiger nut milk chocolate drink was prepared according to figure 7.3. Using the recipe derived from the optimum region obtained in section 7.2.2.

The main ingredients (all expressed in % w/w) were sugar, 22.75; cocoa powder, 11.75; skimmed milk powder, 5.00 and tiger nut milk 60.50. The pastuerized beverages were stored at 18$^{o}$C (the temperature in most out door

vending refridgerators) and 28$^{o}$C (room temperature) for the accelerated shelf life study. Two (2) sets of 15 bottles of Pasteurized and bottled beverages were stored at 18$^{o}$C and 28$^{o}$C for 4 weeks in a Tamashi cooling cabinet (Model TXFV-X55D, China.) and Hach colony incubator (Model 153, Loveland, USA) respectively. The initial pH, colour and microbial levels were determined after which 3 bottles each from the 2 different temperatures under study were sampled and analyzed at the end of every week for the next 4 weeks and analyzed for these selected parameters. Kinetic models were fitted to the experimental data collected as a function of time in order to determine the kinetic constants.

7.2.9 Chemical and Physical analysis

The pH was determined by mixing 10.0 g of sample with 100 ml distilled water, allowing it to stand for 30 minutes, filtering with Whatman filter paper number 1 and determining the pH on filtrate using a Jenway pH meter (Model 3505, Essex, UK).

The colour (L*a*b*) of the beverages were determined using the Minolta Chroma Meter (Minolta CR 300 series). The Chroma meter was calibrated with a standard white tile (L* = 97.95, a*= -0.12, b* = +1.64). The total color difference [$\Delta E = (L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2]^{1/2}$ was calculated from values for L* - (lightness), a* - (redness), and b* - (yellowness).

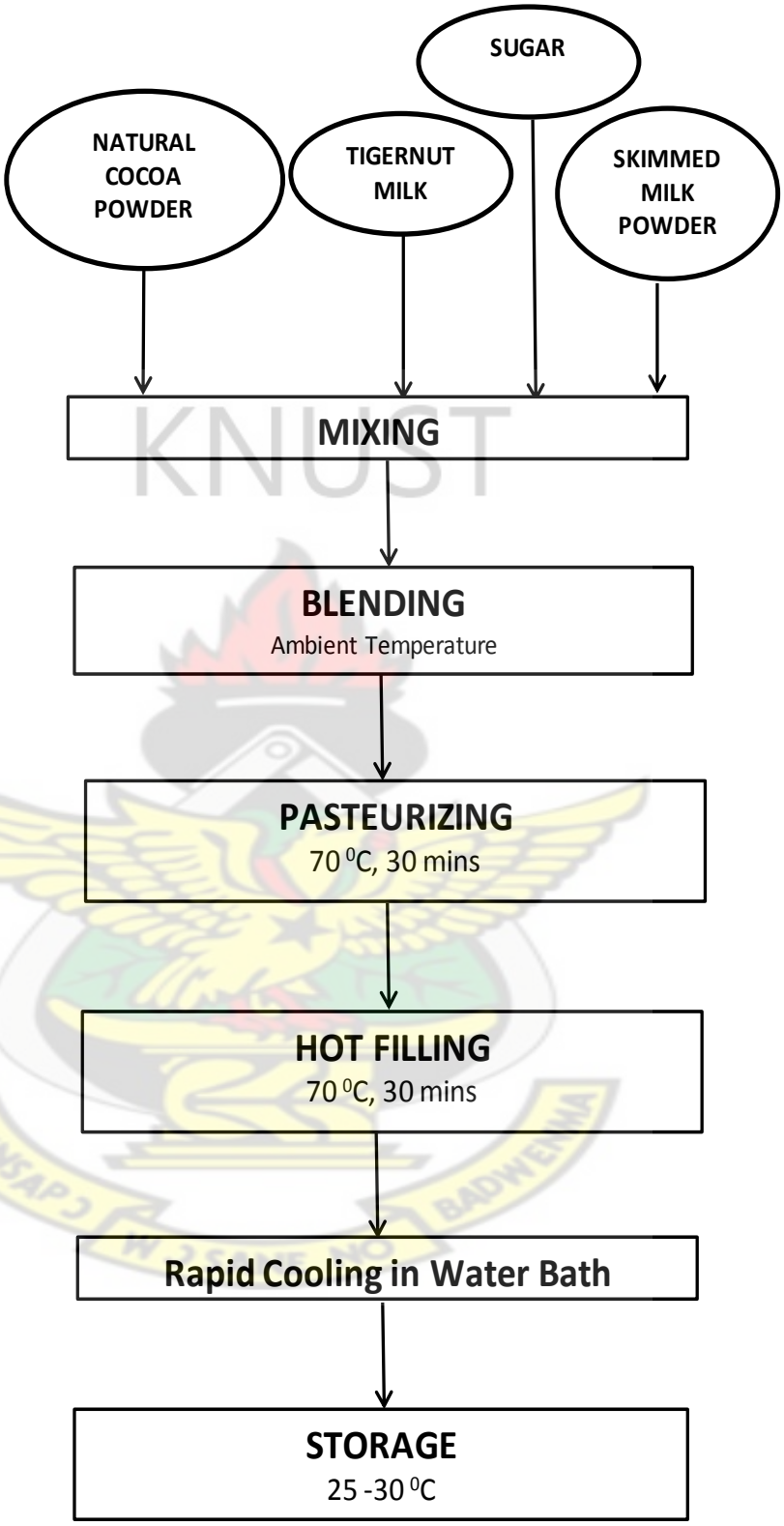



**Figure 7.3: Process Flow diagram for tiger nut milk cocoa beverage**

7.2.10 Microbial analysis

Samples for analysis were prepared by adding 10 g of material to 90 ml of Ringers solution and the mixture shaken. Following this 1.0 ml of the mixture was used for the subsequent determinations.

*Total plate count (TPC) determination*:

The total population counts of the meso-philic bacteria were determined in triplicate using the plate count agar (pH 7.0 from Oxoid Ltd., Basingstoke, Hampshire - England) method of Frazier and Westhoff (1978). Using micropipettes and sterilized pipette tips, 1ml of the dilutions was transferred into sterilized petri-dishes in duplicates. About 15ml of a setrilized plate count agar were added and swirled thouroghly for 1 minute and allowed to solidify. The plates were incubated at 35 °C for 48hrs. The number of colonies developed were counted and recorded as colony forming units per gram of sample (cfu/g).

*Yeasts and moulds determination*:

Malt Extract Agar (pH 5.4 from Oxoid Ltd., Basingstoke, Hampshire - England) method (Frazier and Westhoff, 1978) was used to determine the yeasts and moulds population in the samples in triplicates. Sterilized petri-dishes were inoculated with 1 ml. of the sample solution after which 15ml of the malt extract agar were added and swirled to mix.The plates were incubated at 25 °C for 5 days. The number of colonies developed were counted and recorded as colony forming units per gram of sample (cfu/g).

*Coliforms determination (presumptive test)*

Lauryl Tryptose Broth (pH 6.8 from Oxoid Ltd., Basingstoke, Hampshire - England) was used to determine the presence of coliforms. Test tubes

containing broth of concentration 35.6 g/l were inoculated with samples in triplicates and incubated at 35 °C for 48 ± 2 hr. The presence of gas trapped in the Durham tubes would indicate a positive test for coliforms.

7.2.11 Data analysis

The means of treatments for Physical and microbiological characteristics of the samples were compared using ANOVA procedures. The data for sensory tests were analyzed using non parametric tests (i.e. Friedman's rank tests). All Statistical analysis were done using MINITAB 14 statistical package (MINITAB Inc., U.S.A.).



## 7.3 Results and Discussion

7.3.1 Evaluation of tiger nut milk-dairy milk in drinking chocolates beverage

A summary of the rank sums of skimmed milk and a combination of skimmed milk and tiger nut from different varieties of tubers is shown in Table 7.6. The results showed that a combination of tiger nut milk from brown variety tubers and skimmed milk was the most acceptable milk for the development of the beverage. This could be due to the improvement in the taste of beverage with the incorporation of the dairy milk (Kashid, *et al*., 2007)

**Table 7.6: Rank sums of tiger nut /skimmed milk drinking chocolate beverages**

| Milk in recipe | Sum of Ranks | Order of Ranks |
|---|---|---|
| Tiger nut milk (brown variety) | 171[a] | 4th |
| Tiger nut milk (black variety) | 185[b] | 5th |
| Tiger nut milk (brown variety)-skimmed milk | 117[c] | 2nd |
| Tiger nut milk (black variety)-skimmed milk | 140[d] | 3rd |
| Skimmed milk | 87[e] | 1st |

*Scale: 1 – Most Acceptable 5 – Least Acceptable*

*Mean values having different superscript letters in columns are significantly different ($p<0.05$)*

7.3.2 Mean ratings of sensory attributes from tiger nut milk-skimmed milk chocolate drink

A summary of the mean scores for the sensory attributes is shown in Table 7.7. Tiger nut milk-skimmed milk chocolate drink prepared from mixtures containing 11.5 – 37.5 % sugar, 3.5 – 12.1667 % cocoa powder, 5.0 – 18.00 % skimmed milk and 54.00 – 80.00 % tiger nut milk had ratings for all attributes above 5.0. Ratings for mouth feel, flavor, after-taste and over all acceptability however were below 5.0 for all mixtures that cocoa powder content was higher than 16.50 %.

**Table 7.7: Formulation and mean ratings of sensory attributes**

| Recipe/ Formul-ations | Sugar ($X_1$) | Cocoa powder ($X_2$) | Skimmed milk ($X_3$) | Tiger nut milk ($X_4$) | Appearance | Mouth Feel | Flavor | After-taste | Overall Aaccept-ability |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 11.5000 | 3.5000 | 31.0000 | 54.0000 | [a]6.57 ±2.76 | [ab]6.14 ±2.12 | [ab]7.71 ±0.95 | [ab]6.43 ±1.82 | [ab]7.00 ±1.00 |
| 2 | 11.5000 | 12.1667 | 13.6667 | 62.6667 | [a]5.71 ±1.98 | [ab]6.43 ±1.81 | [ab]6.57 ±0.53 | [ab]5.86 ±1.68 | [ab]6.14 ±1.57 |
| 3 | 20.1667 | 3.5000 | 13.6667 | 62.6667 | [a]6.57 ±1.72 | [ab]6.86 ±1.57 | [ab]6.71 ±1.70 | [ab]6.71 ±2.21 | [ab]6.86 ±1.77 |
| 4 | 37.5000 | 3.5000 | 5.0000 | 54.0000 | [a]5.86 ±2.54 | [ab]6.29 ±2.56 | [ab]6.86 ±1.07 | [ab]6.71 ±2.29 | [ab]6.00 ±2.31 |
| 5 | 20.1667 | 12.1667 | 13.6667 | 54.0000 | [a] 5.71 ±2.43 | [ab]6.00 ±2.00 | [ab]7.00 ±0.82 | [ab]5.00 ±2.71 | [ab]6.00 ±2.65 |
| 6 | 11.5000 | 16.5000 | 5.0000 | 67.0000 | [a]6.29 ±1.70 | [b]3.00 ±1.53 | 3.71 ±1.89 | 2.57 ±0.98 | 3.29 ±2.06 |
| 7 | 11.5000 | 29.5000 | 5.0000 | 54.0000 | [a]4.00 ±2.45 | [b]3.57 ±2.23 | 3.14 ±1.57 | 2.86 ±1.86 | 2.86 ±2.12 |
| 8 | 11.5000 | 3.5000 | 5.0000 | 80.0000 | [a]7.00 ±1.63 | [a]6.57 ±1.98 | [ab]6.57 ±1.98 | [ab]6.57 ±2.22 | [ab]6.00 ±2.64 |
| 9 | 24.5000 | 3.5000 | 5.0000 | 67.0000 | a6.86 ±1.68 | ab6.29 ±2.56 | ab6.00 ±2.24 | ab5.86 ±3.13 | ab6.57 ±2.70 |
| 10 | 11.5000 | 3.5000 | 18.0000 | 67.0000 | a5.71 ±2.21 | ab6.71 ±1.50 | ab6.57 ±2.70 | ab6.86 ±2.04 | ab7.00 ±1.60 |
| 11 | 20.1667 | 12.1667 | 5.0000 | 62.6667 | a5.43 ±2.23 | ab5.00 ±2.83 | ab6.57 ±2.70 | ab6.71 ±2.75 | ab5.29 ±2.43 |
| 12 | 24.5000 | 3.5000 | 18.0000 | 54.0000 | a6.71 ±0.49 | ab7.00 ±1.91 | ab5.43 ±2.44 | ab5.43 ±2.15 | ab6.57 ±2.37 |
| 13 | 18.0000 | 10.0000 | 11.5000 | 60.5000 | a4.86 ±2.97 | ab6.00 ±2.65 | ab4.71 ±2.36 | ab5.14 ±2.54 | ab6.00 ±3.32 |
| 14 | 11.5000 | 16.5000 | 18.0000 | 54.0000 | [a]4.71 ±1.25 | [ab]4.43 ±2.44 | [ab]4.43 ±2.44 | [ab]4.00 ±1.41 | [ab]4.86 ±2.04 |
| 15 | 24.5000 | 16.5000 | 5.0000 | 54.0000 | [a]4.71 ±1.89 | [ab]3.86 ±2.12 | [ab]4.14 ±2.19 | [ab]3.71 ±2.50 | [ab]3.86 ±2.12 |

Mean values of sensory attributes in a column having different superscript letters are significantly different ($p<0.05$)

Mixture regression analysis was used to fit the response data (of sensory attributes) to quadratic polynomial models. The predictive models are summarized in Table 7.8. The $R^2$ values of the models ranged from 0.59 to 0.78 with the adjusted $R^2$ ranging from 0.55 to 0.76.

**Table 7.8: Regression models for sensory attributes**

| PREDICTOR VARIABLE | COEFFICIENTS | | | | |
|---|---|---|---|---|---|
| | **Appearance** | **Mouth feel** | **Flavor** | **After-taste** | **Overall Acceptability** |
| ($X_1$) Sugar | -35.9 | -75.24 | -69.98 | -71.64 | -62.26 |
| ($X_2$) Cocoa powder | -104.9 | -69.79 | -50.81 | -62.02 | -45.68 |
| ($X_3$) Skimmed milk powder | 8.4 | -15.22 | -14.21 | -5.95 | -23.01 |
| ($X_4$) Tiger nut milk | -4.3 | -10.03 | -8.73 | -8.62 | -6.82 |
| *($X_1$) ($X_2$)* | 286.3* | 326.32* | 308.63* | 326.02* | 337.93* |
| *($X_1$) ($X_3$)* | 38.1 | 44.81 | 59.59* | 66.97* | 66.10* |
| *($X_1$) ($X_4$)* | 78.9* | 162.27* | 149.80* | 149.50* | 130.72* |
| *($X_2$) ($X_4$)* | -74.4* | -37.16 | -49.07 | 157.26* | -28.89 |
| *($X_2$) ($X_3$)* | 152.4* | 88.75* | 58.04* | 73.54* | 35.73 |
| *($X_3$)($X_4$)* | 22.6 | 69.28* | 62.60* | 51.18* | 76.67* |
| $R^2$ | 0.5887 | 0.7412 | 0.7313 | 0.7787 | 0.7749 |
| $R^2$ (adjusted) | 0.5497 | 0.7167 | 0.7059 | 0.7577 | 0.7536 |

**Significant coefficient at $p \leq 0.05$*

7.3.3 Effect of components on appearance

Appearance is an important visual attribute of a new product being considered for consumer acceptability. Evaluation of appearance was mainly based on the colour of the drink. The appearance of a beverage largely depends on the composition of chromatic components and the processing parameters especially the severity of heat treatment. There were significant differences between the samples. A mean score of 7.00 was obtained for the recipe with the highest proportion of tiger nut

milk and the lowest proportion of cocoa powder (Table 7.7). Figure 7.4 shows the Cox response trace plot for appearance generated using the regression model. It indicated that increasing proportion of cocoa powder and tiger nut milk continuously affected the appearance of the drink negatively. Cocoa powder showed a sharp drop in the value for appearance of the beverages. This is understandable since unlike tiger nut milk which was off white in color, cocoa powder was coloured with a more intense dark colour.

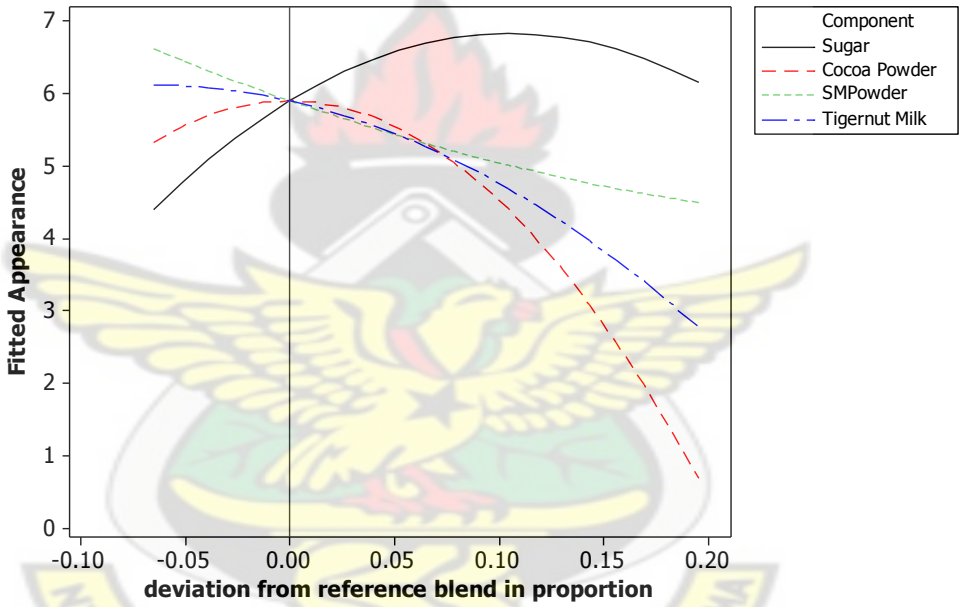



Figure 7.4: Cox response trace plots for appearance

### 7.3.4 Effects of components on after-taste

The lingering taste after eating foods is an important attribute for judging the acceptability of foods. In this study, high mean score of the recipe meant low lingering after-taste. The data shows that the lower the proportions of cocoa powder in the recipes, the lower the after-taste and, hence the higher the score (Table 7.7). This is understandable since cocoa powder is known to contain some

bitter principles (mainly flavanoids and other complex compounds) which are developed during the roasting of cocoa in beans processing (Cacao De Zaan, 1993).

7.3.5 Effect of components on mouth feel

Mouth feel is a measure of the feel of smoothness, grainy, chalky, heat and coolness among others on the tongue. It is a property of great importance for the sensory appreciation of many foods thus associated with consumer acceptability (Folkenberg *et al.*, 1999). The Mouth feel of instant cocoa beverages has been found to be correlated with cocoa properties (such as cocoa content and particle size) and the viscosity properties of the beverage (Folkenburg *et al*., 1999). From table 7.4, mouth feel was significantly affected by the different components in the recipe. This could be attributed to the increasing proportion of insoluble particles from the different components. Very low mean scores were recorded for beverages with cocoa powder proportions above 16.5 % and proportions of tiger nut milk between 54 % and 67 %.

7.3.6 Effect of components on flavor

One of the important attributes of chocolate milk beverage is its chocolaty flavor which has been attributed to the presence of milk solids and milk fat in the product. The highest mean score of 7.71 was attained in the recipe with the highest proportion of skimmed milk. Normally the gradual increase of cocoa powder in cocoa/chocolate recipes contributes positively to flavor of the products till an optimum level beyond which its bitter components begin to mask its flavor. The mean scores showed that increases above the proportion of 12.17 % lead to

reduction in flavor and overall acceptability. This could be because beyond this level the bitter principles in cocoa masks the acceptable flavor

7.3.7 Effect of components on overall acceptability

The highest mean scores of 7.00 was obtained for two recipes with the lowest content of sugar and cocoa powder (sugar 11.50; cocoa powder, 3.50; skimmed milk 31.00; tiger nut milk, 54.00 and sugar 11.50; cocoa powder, 3.50; skimmed milk 18.00; tiger nut milk, 67.00) The Cox response trace plots showed that while an increase in sugar improved acceptability, increases in the proportions of tiger nut milk did not. Very sharp decreases in overall acceptability however were recorded for tiger nut milk and cocoa powder increases.

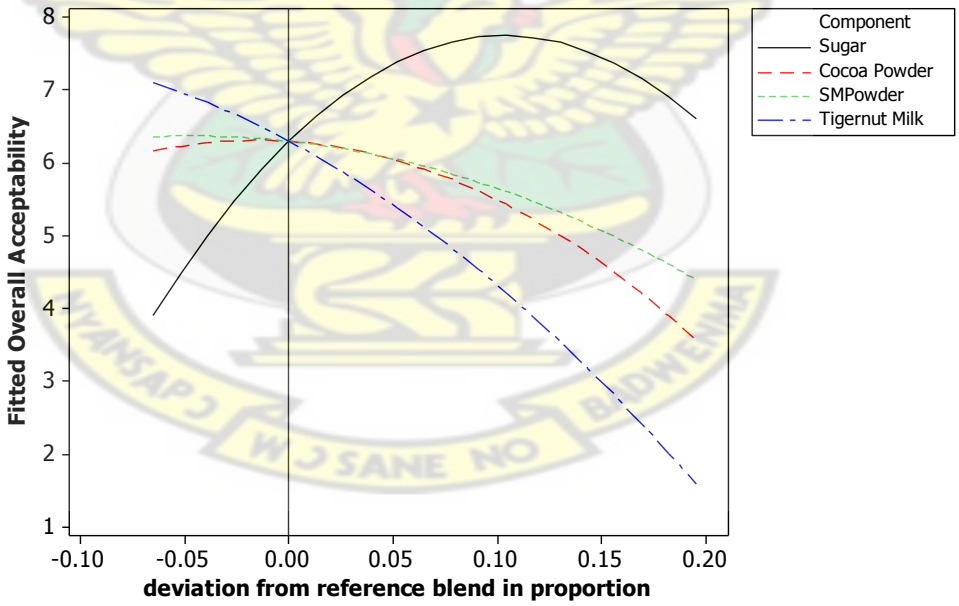



Figure 7.5: Cox response trace plots for overall acceptability

7.3.8 Determination of optimum formulation proportions based on sensory attributes

Contour plots of the individual attributes were overlaid, using MINITAB (version 14) and the optimum region (of the component formulations) was where the criteria for all the sensory attributes were satisfied. In this case, the criteria were based on the mean scores that suggested very acceptable sensory attributes. Over laid contour plots of different combination suggested that holding skimmed milk at 5 % will result in a more acceptable beverage as shown in figure 7.6.



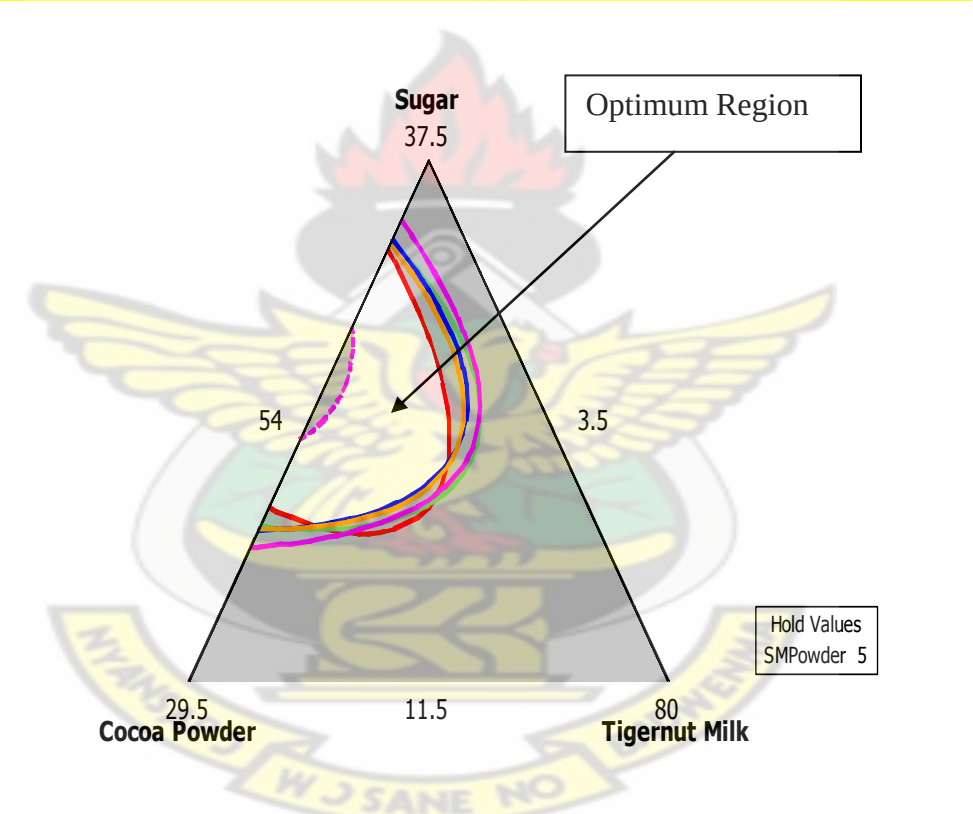



Figure 7.6: Over laid plots of sensory attributes showing the optimum region of formulation

7.3.9. Model Validation

To determine whether the predictive models obtained were sufficient and adequate, three and two formulations were selected from within and outside the region respectively (Table 7.9).



**Table 7.9: formulations for validation experiments**

| Validation Sample No. | Sugar | C. Powder | SM Powder | TN Milk |
|---|---|---|---|---|
| 1* | 28.58 | 8.73 | 5.00 | 57.69 |
| 2* | 22.75 | 11.75 | 5.00 | 60.50 |
| 3* | 20.56 | 19.67 | 5.00 | 54.77 |
| 4 | 16.80 | 20.92 | 5.00 | 57.28 |
| 5 | 33.42 | 4.84 | 5.00 | 56.74 |

Sample 1*, 2* and 3* were samples from the optimum, region.

Samples 4 and 5 from outside the optimum region

The five formulations were prepared and subjected to sensory evaluation by the same panel of judges. Results from the verification study (Table 7.10) showed that the ratings for the beverage from the formulations within the optimum region compared well with predicted ratings

**Table 7.10: Predicted and validated ratings for sensory attributes within and outside optimum region**

| | ATTRIBUTE | | | | |
|---|---|---|---|---|---|
| | Appearance | Mouth feel | Flavor | After-taste | Overall accept ability |
| Predicted Ratings | 7.00-7.90 | 7.30-8.40 | 7.00-8.20 | 7.10-8.10 | 7.50-8.40 |

| | | | | | |
|---|---|---|---|---|---|
| Sample 1* | 7.03[a] | 7.33[a] | 7.30[a] | 7.33[a] | 7.56[a] |
| Sample 2* | 7.03[a] | 7.56[a] | 7.06[a] | 7.10[a] | 7.83[a] |
| Sample 3* | 7.13[a] | 7.63[a] | 7.46[a] | 7.56[a] | 8.83[b] |
| Sample 4 | 5.63[b] | 5.96[b] | 5.63[b] | 5.66[b] | 6.46[c] |
| Sample 5 | 5.93[b] | 6.00[b] | 5.63[b] | 5.40[b] | 4.36[d] |

Sample 1*, 2* and 3* were samples from the optimum, region.

Samples 4 and 5 from outside the optimum region



7.3.10. The nutritional characteristics of the formulated high fiber chocolate and spread are presented in Table 7.11 and 7.12.

**Table 7.11: Chemical composition of high fiber chocolate spread products**

| COMPONENT | RECIPES | | | | |
|---|---|---|---|---|---|
| | Recipe 1 | Recipe 2 | Recipe 3 | Recipe 4 | Recipe 5 |
| Moisture (g kg-$^{1}$) | 15.4± 0.2[a] | 14.2 ± 0.2[b] | 16.5± 0.5[c] | 15.6 ± 0.3[a] | 14.2 ± 0.2[b] |
| Protein (g kg-$^{1}$) | 23.3 ± 1.5[a] | 26.3 ± 1.5[bc] | 28.0± 1.0[bc] | 22.3 ± 0.5[a] | 24.0 ± 1.0[ab] |
| Ash (g kg-$^{1}$) | 14.6 ± 0.2[a] | 15.5 ± 0.2[b] | 15.6 ±0.2[cd] | 14.8 ±0.2[a] | 15.8 ±0.1[cd] |
| Fat (g kg-$^{1}$) | 403.7 ± 1.0[a] | 415.7± 1.4[b] | 435.4 ±1.0[c] | 428.6 ±0.8[d] | 430.4 ±0.6[d] |
| Carbohydrate (g kg-$^{1}$) | 506.4 | 470.1 | 417.6 | 460.9 | 421.8 |
| Total dietary fiber (g kg-$^{1}$) | 35.4 ± 0.2[a] | 64.2 ± 0.2[b] | 99.5 ±0.5[c] | 66.2 ±0.6[d] | 98.7 ±0.5[c] |
| Reducing sugar (g kg-$^{1}$) | 32.3 ± 0.5[a] | 32.7 ± 0.5[a] | 28.0 ±0.8[b] | 21.7± 0.5[c] | 23.3 ±0.5[c] |
| Sucrose (g kg-$^{1}$) | 348.3±1.2[a] | 301.0 ±1.0[b] | 254.7±0.6[c] | 293.3± 1.2[d] | 339.0 ±1.0[e] |
| Total sugar (g kg-$^{1}$) | 371.0 ±1.0[a] | 334.0±1.0[b] | 282.7 ±0.5[c] | 315.7 ±0.6[d] | 352.3± 0.6[e] |

Mean values in the row having different superscript letters are significantly different ($p<0.05$)

**Table 7.12: Chemical composition of high fiber bar chocolate products**

| COMPONENT | RECIPES | | | | |
|---|---|---|---|---|---|
| | Recipe 1 | Recipe 2 | Recipe 3 | Recipe 4 | Recipe 5 |
| Moisture (g kg-$^{1}$) | 21.5 ±0.2$^{a}$ | 21.5 ±0.3$^{ab}$ | 20.8 ±0.1$^{abc}$ | 20.6 ±0.2$^{cd}$ | 20.5 ±0.3$^{cd}$ |
| Protein (g kg-$^{1}$) | 100.3 ±0.1$^{a}$ | 101.5± 0.2$^{b}$ | 102.5 ±0.2$^{c}$ | 103.3 ±0.2$^{d}$ | 104.6± 0.2$^{e}$ |
| Ash (g kg-$^{1}$) | 14.6 ±0.2$^{a}$ | 15.5 ±0.1$^{b}$ | 15.8 ±0.1$^{c}$ | 16.0 ±0.1$^{d}$ | 16.3 ±0.1$^{d}$ |
| Fat (g kg-$^{1}$) | 324.0 ±3.8$^{a}$ | 335.7 ±1.1$^{b}$ | 346.0 ±3.1$^{c}$ | 353.3 ±3.5$^{d}$ | 361.8 ±1.9$^{d}$ |
| Carbohydrate (g kg-$^{1}$) | 529.9 | 514.3 | 502.2 | 492.3 | 481.4 |
| Total dietary fiber (g kg-$^{1}$) | 9.7 ±0.2$^{a}$ | 11.5 ±0.3$^{b}$ | 12.7 ±0.1$^{c}$ | 14.5 ±0.2$^{d}$ | 15.4 ±0.2$^{e}$ |

Mean values in the row having different superscript letters are significantly different ($p<0.05$)

The moisture content of the cocoa products ranged from 14.2±0.2 to 16.5±0.4 g kg-$^{1}$ and 20.5±0.3 to 21.5±0.3 g kg-$^{1}$ for chocolate spread (Table 7.11) and bar chocolate (Table 7.12) respectively and their protein content from 22.5±0.2 to 28.0±0.8 g kg-$^{1}$ and 100.3±0.1 to 104.6±0.2 g kg-$^{1}$ for same i.e chocolate spread (Table 7.11) and bar chocolate (Table 7.12) respectively. There were also significant differences ($P< 0.05$) between the moisture and protein contents of the control samples (recipe1) in Tables 7.11 and 7.12 and the other samples in both tables which had the tiger nut cake.The results show that increasing the substitution of tiger nut cake for sugar in the recipes resulted in slight but significant increases in the protein content of the product. This can be attributed to relatively low content of protein in the cake. Ash contents ranged from 14.6±0.2 to 15.8±0.1 g kg-$^{1}$. Fat and fiber contents in the products ranged from 403.7 to 435.4 g kg-$^{1}$; 35.4 to 98.7 g kg-$^{1}$ and 322.3 to 360.8 g kg-$^{1}$, 9.7 to 15.4 g kg-$^{1}$ for

chocolate spread and bar chocolate respectively. The differences of fat and fiber contents as the proportion of tiger nut cake increased in the recipes were significant ($P< 0.05$) for all products developed. The significant increases in chemical composition due to the incorporation of ingredients with relatively higher nutrient composition has also been reported by other researchers for utilization of okara in bread, tiger nut flour in wheat based cake and distillers spent grain in snack food *kokoro* (Wickramarathna and Arampath, 2003; Chinma *et al.*, 2010; Awoyale *et al.*, 2011). The formulated high fiber chocolate products were evaluated for preference and sensory attributes and the results are presented in Table 7.13 and 7.14.

**Table 7.13: Preference ranking of high fiber chocolate spread prepared with tiger nut milk cake**

| Spread samples | Sum of ranks | Order of ranks |
|---|---|---|
| Spread without milk cake (control) | 112[a] | 3rd |
| Spread with 5 % (w/w) sugar substituted with milk cake | 68[b] | 1st |
| Spread with 10% (w/w) sugar substituted with milk cake | 126[a] | 5th |
| Spread with 5 % (w/w) skimmed milk substituted with milk cake | 77[b] | 2nd |
| Spread with 5% (w/w) sugar and 5% (w/w) skimmed milk substituted with 10% (w/w) milk | 116[a] | 4th |

cake

Mean values in the column having different superscript letters are significantly different ($p<0.05$)

**Table 7.14: Sensory analysis of high fiber bar chocolate prepared tiger nut milk cake**

| | Mean ratings of sensory attributes | | | | | |
|---|---|---|---|---|---|---|
| Sample No. | Appearance | Taste | Mouth feel | Flavor | After-taste | Acceptability |
| Recipe 2 | $7.00\pm1.19^{a}$ | $6.00\pm0.75^{a}$ | $7.12\pm1.24^{a}$ | $6.50\pm0.75^{a}$ | $7.00\pm0.92^{a}$ | $7.25\pm1.03^{a}$ |
| Recipe 3 | $5.25\pm0.46^{b}$ | $5.87\pm0.99^{a}$ | $5.37\pm0.74^{b}$ | $5.75\pm0.46^{a,b}$ | $6.50\pm1.06^{a}$ | $6.12\pm0.64^{b}$ |
| Recipe 4 | $5.25\pm0.70^{b}$ | $5.50\pm1.19^{a,b}$ | $4.50\pm1.30^{b,c}$ | $5.37\pm1.18^{b}$ | $5.25\pm1.03^{b}$ | $4.62\pm0.74^{c}$ |
| Recipe 5 | $4.50\pm 0.75^{b}$ | $4.75\pm1.03^{b}$ | $4.12\pm1.12^{c}$ | $4.00\pm0.92^{c}$ | $4.62\pm1.06^{b}$ | $4.62\pm1.18^{c}$ |

Mean values in the column having different superscript letters are significantly different ($p<0.05$)

The level of preference of chocolate spread with sugar and milk substituted with tiger nut milk cake as well as the control was obtained through simple ranking test. The rank sums indicating the preference are shown in Table 7.13. The Friedman's rank test showed that chocolate spread with 5% sugar substituted with tiger nut milk cake was the most acceptable (having least rank sum). By the rank sums it was followed by chocolate spread with 5% skimmed milk substituted with tiger nut milk cake even though there were no significant differences between acceptability. The control was third in ranking with the 10 % substitution of skimmed milk with tiger nut milk cake following in ranking. This indicates that a

combination of tiger nut and peanut paste is more acceptable than the use of only peanut in recipes. Furthermore substitution of 5 % skimmed milk or sugar with 5% of tiger nut milk cake is more preferred than 10% substitution of same while a minimum of 30 % sugar ratio in recipes is preferred for acceptability of products.

The observed trend from the sensory analysis of the bar chocolate was; for all the sensory attributes, increasing tiger nut proportion resulted in a corresponding decrease in all the sensory attributes. However, there was no significant difference in taste, flavor and after-taste between 2.5 and 5.0 % substitution of maltitol with tiger nut milk cake.

7.3.11 Shelf life studies

Colour, pH and microbiological growth in the chocolate drink was monitored to determine the estimated shelf life of the beverage

7.3.11.1 Colour

Color may provide an indication of the effects of ingredient, storage or processing on how the final product conforms to standards. It is influenced by some chemical changes in foods, such as microbial activity, change in temperature, enzymatic or non-enzymatic reactions (maillard reaction and caramelization), anthocyanins and fat-soluble plant pigments among others (Belitz *et al.,* 2009). Figures 7.7, 7.8 and 7.9 shows the effect of the different storage temperatures on the L*, a* and b*colour parameters of the beverages. Colour measurements on cocoa drinks

showed a consistent increase in the L-value of the cocoa drinks and it ranged from 60.05±0.93 to 76.62± 0.21 and 60.05±0.93 to 75.76±0.17 for the drink stored at 18$^{o}$C and 28$^{o}$C respectively. This indicates that the drinks generally became lighter in colour (increasing L) with increasing storage time (weeks) at both temperatures.

The a$^{*}$ value of the drink stored at 18$^{o}$C increased gradually from 9.44±0.01 to 17.57±0.10 from beginning of study to end of week 2 and declined to 13.67± 0.10 on week 4 while the beverage stored at 28$^{o}$C increased from 9.44 ± 0.01 to 17.57±0.10 for the same period. The beverage stored at 18$^{o}$C recorded an increase in b value from 7.99±0.25 at the start to 23.23±0.10 at week end 2 before dropping and remained fairly constant at end of weeks 3 and 4. The b value of the beverage stored at 28$^{o}$C however increased from 7.99± 0.25 at the start till 17.28±0.10 at the end of week 2, remained fairly constant till the end of week 3 before rising to 23.23±0.10 at the end of week 4. The figures recorded for the a and b values suggest formation of water soluble colored pigments from the polyphenolic compounds due to enzymatic and non enzymatic reactions during storage (McEvily *et al*., 1992). The relatively higher values at 28$^{o}$C compared to 18$^{o}$C can be attributed to the storage temperature which accelerated these reactions.

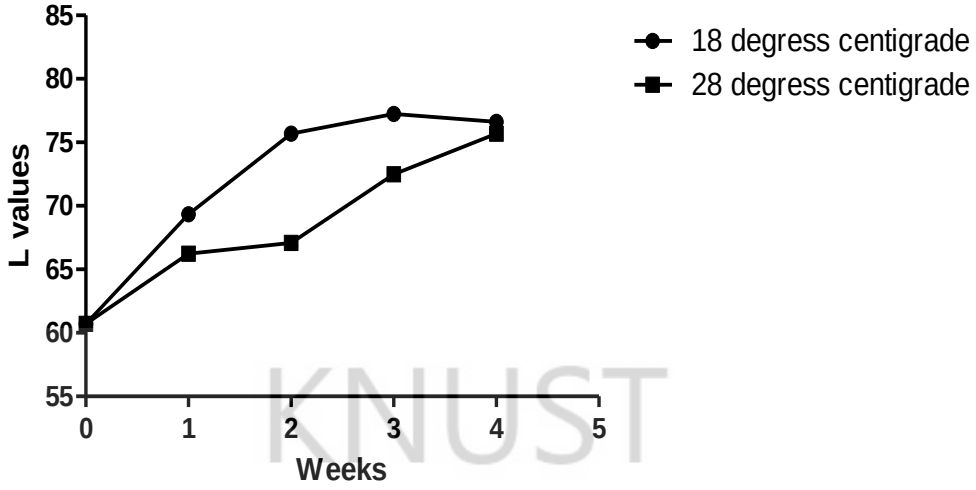



**Figure 7.7: Colour (L-value) of Cocoa Drink Stored at 18$^{o}$C and 28$^{o}$C**

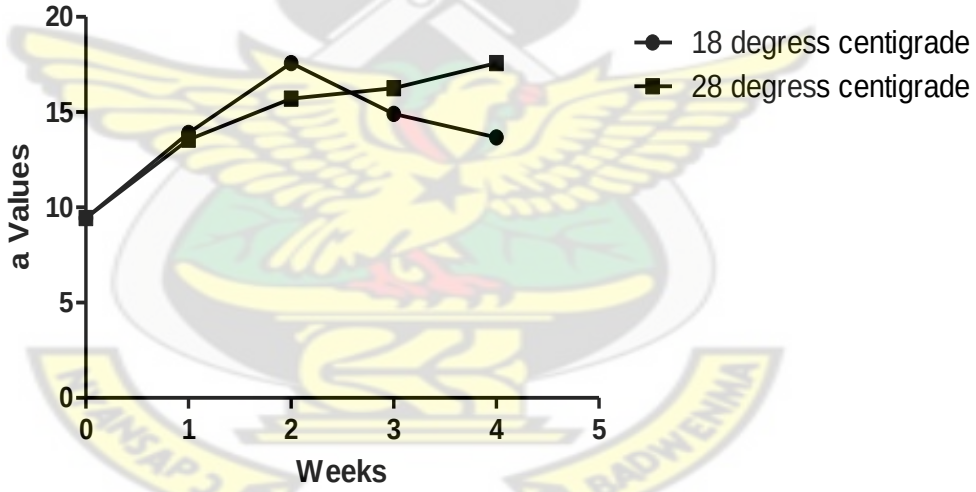



**Figure 7.8: Colour (a-value) of Cocoa Drink Stored at 18$^{o}$C and 28$^{o}$C**

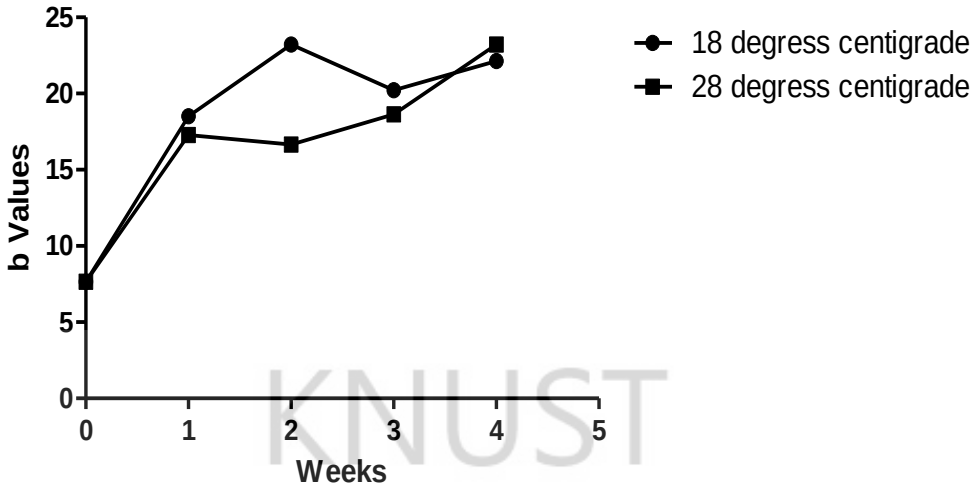



**Figure 7.9: Colour (b-value) of Cocoa Drink Stored at 18$^{o}$C and 28$^{o}$C**

7.3.11.2 pH

Organic acids have a pronounced impact on food flavor and quality. The effect of different temperatures on the pH of the beverages during storage is presented in figure 7.10. The results show that storage at both temperatures affected the pH significantly. It also showed that at both temperatures, the pH decreased for a period before rising with the beverage stored at 28$^{o}$C decreasing more sharply. The sharper decline in the pH of the beverage stored under conditions that support microbial growth has also been reported in similar studies (Sampedro *et al.*, 2009). This could be attributed to the faster growth of microorganisms that produced lactic acid.

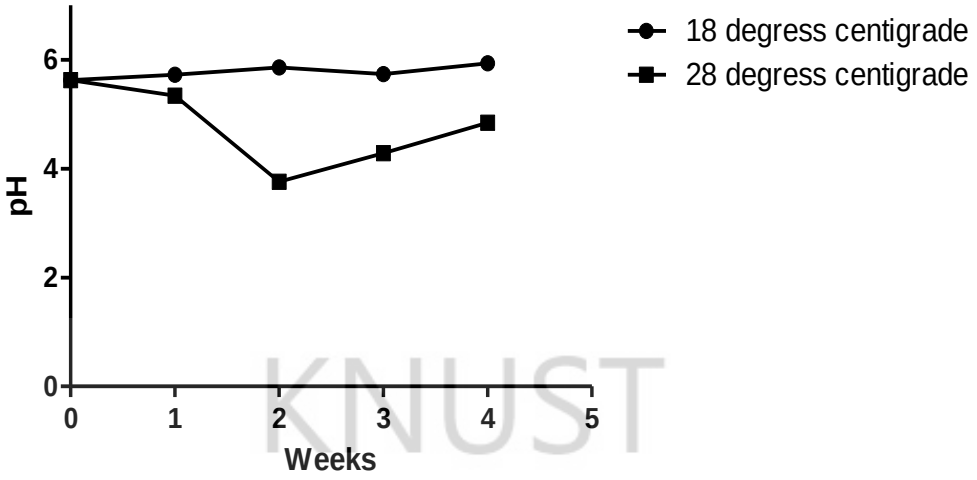



**Figure 7.10: Changes in pH of Cocoa Drink Sored at 18$^{o}$C and 28$^{o}$C**

7.3.11.3 Microbiological quality

Microbiological growth in foods result in food spoilage with development of undesirable sensory characteristics and in certain cases, the food becomes unsafe for consumption. The effect of storage at different temperatures on the bacterial counts and the yeast and moulds in the beverages are shown in Table 7.15.

**Table 7.15: Changes in microbiological count of cocoa drink at different temperatures**

| | 18°C | | | | 28°C | | | |
|---|---|---|---|---|---|---|---|---|
| Weeks | TPC (cfu/g) | Yeast (cfu/g) | Moulds (cfu/g) | Coli-forms | TPC (cfu/g) | Yeast (cfu/g) | Moulds (cfu/g) | Coli-forms |
| 0 | 47 | 0 | 0 | negative | 47 | 0 | 0 | negative |
| 1 | 115 | 0 | 0 | negative | 4901 | 0 | 0 | negative |
| 2 | 553 | 0 | 0 | negative | 30000 | 0 | 0 | negative |
| 3 | 988 | 0 | 0 | negative | TNTC | 0 | 0 | negative |
| 4 | 1688 | 0 | 0 | negative | TNTC | 0 | 0 | negative |



The microbiological counts of 47 cfu/ ml, 0 and 0 for total plate count, yeast and moulds respectively at the start of the study was within standards for chocolate products (GSA, 2007). This is an indication that the temperature and time for pasteurizing the beverage made it microbiologically safe for consumption. Increases in the microbial population in the beverage at the different storage temperatures (47 cfu/ g to 1688 cfu/ g at 18°C and 47 cfu/ g to TNTC at 28°C) is an indication that the storage temperatures were not low enough to inhibit microbiological growth thus making the drinks susceptible to spoilage at both temperatures over time with the one stored at 28°C losing wholesomeness faster.

7.3.12 Accelerated storage studies

The sharp increase in total plate count and drop in pH at the end of week 1 of beverage stored at 28°C compared to beverage stored at 18°C means that the reaction rate constant is influenced to a large extent by a change in temperature.

The influence of temperature on the reaction rate may be described by using the Arrhenius relationship as follows;

$k = ko\ exp^{EA/RT}$

Where

ko is the pre exponential factor,

EA is the activation energy,

R is the ideal gas constant

T is the temperature in $^{o}C$

Based on the Arrhenius equation the data obtained was fitted into the model for quality degradation with temperature. In was transformed into a log model from which the Arrhenius model parameters were calculated and fitted into a linear model.The slope factor representing the 18°C was determined (see appendix J for calculation). The shelf life at $18^{o}C$ was estimated to be 24.55 weeks, approximately 6 months.

**7.4 Conclusion**

Tiger nut milk can be combined with skimmed milk powder to produce chocolate beverages. Tiger nut milk cake can also be used to formulate acceptable bar chocolate and spread products. Flavor, mouth feel, appearance and after-taste were very important in determining the overall acceptability of the beverage. The optimum ingredient formulations for acceptable tiger nut milk skimmed milk powder beverage was determined to be sugar, 20.56-28.58 %; cocoa powder,

8.73-19.67 %; skimmed milk powder, 5.0 % and tiger nut milk 54.77-60.50 %. At 18$^{o}$C the beverage can be stored for 6 months.

## OVERALL CONCLUSION

The variety of tiger nut tubers, site and planting period of tubers have significant effects on some of its chemical properties such as protein, fat and energy content, minerals as well as acceptability of its milk.

Tiger nut tubers grown at different sites do not have the same potential for milk and solids. Pre-treating tubers by soaking and cooking improved the expected milk solids with the boiled and soaked as well as roasted and soaked tubers expected to yield the most milk solids.

The yield of milk solids extracted from tiger nut tubers is determined by variables such as the degree to which the nuts are boiled before crushing, the extent to which the crushed nuts are milled, and the meal to water ratio during extraction. The interactions of the variables also influence the yield of milk solids. Optimization studies using response surface techniques estimated that boiling time of 10 minutes, crushing and milling for about 17-22 minutes and then extracting using a 1:7 meal to water ratio will yield optimum milk solids.

The processed tiger nut products had desirable nutritional quality as well as functional properties. The products have a high potential as ingredients for the production of healthy chocolate bars, beverages, and spreads.

Tiger nut milk can be combined with skimmed milk powder to produce chocolate beverages. The optimum ingredient formulations for acceptable tiger nut milk-skimmed milk chocolate beverage was determined to be sugar, 20.56-28.58 %; cocoa powder, 8.73-19.67 %; skimmed milk powder, 5.0 % and tiger nut milk 54.77-60.50 % . At 18$^{o}$C the beverage can be stored for 6 months.

## RECOMMENDATION

The growth of microbiological organisms and fatty acid deterioration of tubers during washing and drying of tubers should be evaluated in the growing communities to ensure consistency in quality of products and milk.

The use of spray and drum drying techniques for the dehydration of milk should be explored to enhance the use of the milk.

## REFERENCES

**Abbiw, D.** (1990). Useful Plants in Ghana, Intermediate Technology Publications Ltd., Southampton Row, London, pp 30.

**Abd El- Khair, A.A**., (2009). Optimization of a new version of chocolate milk for endurance performance and post exercise recovery. *Research Journal of Agriculture and Biological Sciences* 5(4): 482-489.

**Ablordeppey, S.D.** (2004). Tiger nut takes rightful place. *Daily Graphic*, 72: 13.

**Adam, W.B., Hardy, F. and Nierenstein, M.** (1931). The catechin of the cocoa bean. *Journal of American Chemical Society* 53(2), 727-728.

**Addo-Quaye, A. A., Darkwa, A. A and Ampiah M. K. P**. (2011). Performance of three cowpea (Vigna Unguiculata (L) Walp) varieties in two agro-ecological zones of the Central Region of Ghana I: Dry matter production and growth analysis ARPN *Journal of Agricultural and Biological Science* 6, (2), 1990-6145,

**Adejuyitan, J.A., Otunola, E.T., Akande, E.A., Bolarinwa, I.F., and Oladokun FM.** (2009). Some physicochemical properties of flour obtained from fermentation of tiger nut (Cyperus esculentus) sourced from a market in Ogbomoso, Nigeria. *African Journal of Food Science* 3, 51-55.

**Adejuyitan, J.A.** (2011). Tiger nut processing: its food uses and health benefits. *American Journal of Food Technology* 6(3): 197-201

**Adekanmi, O. K., Oluwatooyin, O. F. and Yemisi A. A**. (2009). Influence of processing techniques on the nutrients and antinutrients of tiger nut

(Cyperus esculentus L.) *World Journal of Dairy and Food Sciences* 4 (2): 88-93

**Adjei-Nsiah, S.** (2010). Yield and nitrogen accumulation in five cassava varieties and their subsequent affects on soil chemical properties in the forest/savanna transitional agro-ecological zone of Ghana. *Journal of Soil Science and Environmental Management* 1, 015-020

**Ah-Leung, S., Bernard, H., Bidat, E., Paty, E., Rance, F., Scheinmann, P. and Wal, J.M.** (2007). Allergy to goat and sheep milk without allergy to cow's milk. *Allergy* 61(11), 1358-1365.

**Aidoo, H., Sakyi-Dawson, E., Tano-Debrah, K and Saalia, F.K.** (2010). Development and characterization of dehydrated peanut-cowpea milk powder for use as a dairy milk substitute in chocolate manufacture. *Food Research International* 43, 79-85

**Akintunde, T.Y.T. and Akintunde, B.O.** (2002). Development of models for predicting the yield quality of soymilk. *The Journal of Food Techenology Africa* **7**, 55-58

**Alesson-Carbonell, L., Fernandez-Lopez, J., Perez-Alvarez, J.A., and Kuri, V.** (2005a). Functional and saensory effects of fibre-rich ingrident on breakfast fresh sausages manufacture. *Food Science & Technology International* 11, 89-97

**Alesson-Carbonell, L., Fernandez-Lopez, J., Perez-Alvarez, J.A., and Kuri, V.** (2005b). Characteristics of beef burger as influenced by various types of

lemon albedo. *Innovation in Food science & Emerging Technologies* 6,247-255.

**Anderson, J. W., Baird, P., Davis, R. H., Jr., Ferreri, S., Knudtson, M. and Koraym, A.** (2009). Health benefits of dietary fiber. *Nutrition Reviews* 67, 188–205.

**Anyidoho, I.E.** (2006). Nutritional Quality of Tiger nut Porridge. Thesis presented to the Science Laboratory Technology Department, School of Applied Sciences. Accra Polytechnic, Accra, Ghana.

**AOAC** (2000). Official Methods of Analysis. Association of Official analytical Chemists 17th Edition, Washington D. C

**Appiah, F., Oduro, I. and Ellis, W. O.** (2011). Pasting properties of Treculia africana seed flour in Ghana and the production of a breakfast meal. *Agriculture and Biology Journal of North America* 2 (2), 325-329

**Arafat, S.M., Gaafar, A.M., Basuny A.M., and Nassef S.L.** (2009) Chufa Tubers (Cyperus esculentus L.): As a new source of food, *World Applied Sciences Journal* 7 (2), 151-156,

**Ariefdjohan, M.W, Savaiano, D.A.** (2005). Chocolate and cardiovascular health: is it too good to be true? *Nutrition Review* 63 (12), 427-30.

**Arifin, J., Bono, A., Farm, Y.Y., Ling, A.L.L. and Fui, S.Y.** (2009). Protein extraction from palm kernel meal. *Journal Applied Science* 9, 2996-3004

**Arranz, I., Stroka, J. and Neugebauer, M.** (2006) Determination of aflatoxin B, in tiger nut based soft drinks, *Food Additives and contaminants: Part A*, 23, 3, 305-308

**Asiamah, K.** (2005.) process optimization of cowpea- peanut milk. M. Phil. Thesis, Department of Nutrition and Food Science, University of Ghana.

**Attaie, H., Breitschuh, B., Braun, P. and Windhab E.J.** (2003). The functionality of milk powder and its relationship to chocolate mass processing, in particular the effect of milk powder manufacturing and composition on the physical properties of chocolate masses. *International Journal of Food Science and Technology* 38, 325-335

**Awoyale, W., Maziya-Dixon, B., Sanni L.O. and Shitu T.A.** (2011). Nutritional and sensory properties of a maize-based snack food (kokoro) supplemented with treated Distillers' spent grain (DSG). *International Journal of Food Science and Technology* 46, 1609-1620

**Awua P. K.** (2002). Cocoa Processing and chocolate Manufacture in Ghana, David Jamieson and Associates ltd. pp 14-18

**Azizah, A.H and Zainon, H.** (1997) Effect of processing on dietary contents of selected legumes and cereals *Malaysia Journal Nutrition* 3,131-136,

**Banzon, J.A., and Velasco, J.R.** (1982). Coconut Production and Utilization. Philippine Coconut Research and Development Foundation Inc. (PCRDF). pp. 213 – 215

**Barber, S.** (1981). Recent contributions to research in the Valencia region on local products in food science and technology. *Revista de Agroquca y Tecnologde Alimentos* 21, 175-184

**Barminas, J.T., Maina, H.M., Tahir, S., Kubmarwa D. and Tsware, K.** (2001). A preliminary investigation into the biofeul characteristics of Tiger nut (Cyperus esculentus) oil. *Bioresource Technology* 79, 87-89.

**Belewu, M.A. and Abodunrin, A.O.** (2008). Preparation of Kunun from unexploited rich food source: Tiger nut (Cyperus esculentus). *Pakistan Journal of Nutrition* 7: 109-111.

**Belewu, M.A. and Belewu, K.Y.** (2007). Comparative physico-chemical evaluation of tiger-nut, soybean and coconut milk sources. *International Journal of Agricultural Biology* 9, 785-787

**Belewu, M.A., & Azeez, T.A** (2008). Coconut Chocolate: Preparation, Composition and Organoleptic Qualities, *European Journal of Scientific Research* 1,144-152

**Belitz, H.D., Grosch, W. and Schieberle, P.** (2009). *Food Chemistry* (4th Edition). Springer-Verlag, Berlin. pp. 173,959-966.

**Benward, M. F., and Benward, W.** (2000). Healthy Baby Infant Formula Milk and Healthy Baby Toddler Formula Milk, United States Patent No., 6,063,433.

**Benzie, I.F. and Strain, J.J.** (1999). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry* 239, 70-76.

**Berry, D.** (2002). Healthful ingredients sell dairy foods. Dairy Foods, 103:54-56.

**Besbes, S. S., Attia, H., Deroanne, C., Makni, S. and Blecker, C.** (2008). Partial replacement of meat by pea fibre and wheat fibre: Effect on the

chemical composition, cooking characteristics and sensory properties of beef burgers. *Journal of Food Quality* 31, 480-489.

**Borderias, A.J., Sanchez-Alonso, I., and Perez-Mateos, A.** (2005). New applications of fibres in foods: Addition of fishery products *Trends in Food Science and Technology* 16, 458-465.

**Boloorforshan, M. and Markakis P.** (1979). Protein supplementation of navy beans with Sesame *Journal of Food Science* 44, 390-391;

**Bosch L, Alegría-Torán A, Farré-Rovira R.** (2005). RP-HPLC determination of tiger nut and orgeat amino acid contents. *Food Science and Technology International* 11, 33–40

**Bressani, R, and Elias, L.G.** (1983). Guidelines for the development of processed and packaged weaning foods. *Food and Nutrition Bulletin* 5, 57-63.

**Brown, G.A.** (2002). Characterization of enzyme hydrolyzed cowpea-peanut milk and the dehydrated residual meal. B.Sc. Project Report, Department Of Nutrition and Food Science, University Of Ghana.

**Cacao De Zaan** (1993). The Cocoa Manual. De Zaan publication.

**Cai T. D., Chang K. C., Shih M. C., Houa, H. J. and Ji, M.** (1997). Comparison of bench and production scale methods for making soymilk and tofu from 13 soybean varieties *Food Research International*, Vol. 30, 9, 659-668,

**Carpenter, R.P., Lyon, D.H. and Hasdell, T.A**. (2000). Guide lines for sensory analysis in food product development and quality control 2nd edition Aspen Publishers, Inc. Maryland

**Chaiseri, S. and Dimick, P.S.** (1989). Lipid and hardness characteristics of cocoa from different regions. *Journal of Americal Oil Chemist Society*. 66, 1771-1776.

**Chan, L., and Beuchat, L. R.** (1992). Chemical, physical and sensory characteristics of peanut milk as affected by processing conditions. *Journal of Food Science* 57, 401–405.

**Chen, S.** (1989). Preparation of fluid soymilk. In Proceedings of the World Congress on Vegetable protein Utulization in Human Foods ans Animal Feedstuffs Edited by Applewhite, T.N., American Oil Chemists Society, Champaign, Illinois, pp. 341-351

**Chevallier, A.** (1996). The Encyclopedia of Medicinal Plants. Dorling Kindersley publisher, London.

**Chinma, E.C., Abu, J.O. and Abubakar, Y.A.** (2010). Effect of tiger nut (Cyperus esculentus) flour addition on the quality of wheat- based cake.

**Cochran, W.G. and Cox, G.M.** (1957). Experimental designs. John Wiley & Sons, New York, 469

**Cook, J. A., Vanderjagt, D., Dasgupta, A., Mounkaila, G., Glew, R.S., Blacwell, W and Glew. R. H.** (1998). Use of the Trolox assay to estimate the antioxidant content of seventeen edible wild plants of Niger. *Life Sciences* 63,106-110

**Cornell, J.A.** (2002). Experiments with mixtures Dsigns, Models and Analysis of Mixtures Data. John Willey & Sons, New York.

**Corrales, M., de Souza, P. M., Stahl, M.R. and Fernandez, A.** (2012). Effect of the decontamination of a fresh tiger nuts' milk beverage (horchata) with short wave ultraviolet treatments (UV-C) on quality attributes. *Innovation Food Science and Emerging Technology* 13, 163-168

**Cortés, C., Esteve, M. J., Frgola, A., and Torregrosa, F.** (2004). Physical and chemical properties of different commercially available types of "horchata de chufa". *Italian Journal of Food Science* 1, 113-121.

**Cortés, C., Esteve, M.J., Frgola, A., and Torregrosa, F.** (2005). Quality characteristics of horchata (a Spanish vegetable beverage) treated with pulsed electric fields during shelf- life. *Food Chemistry* 91, 319-325

**Coskuner Y., Karababa E. and Nazlican A.A.** (2000) Nutritive value and technological eveluation of Chufa (Cyperus esculentus) 4th Int. Conf on food Physics Abstr. Book pg 75

**Coskuner, Y., Ercan, R., Karababa, E., and Nalizcan, A.N.** (2002). Physical and chemical properties of chufa (Cyperus esculentus L.) tubers grown in the Cukurova region of Turkey. *Journal of the Science of Food and Agriculture* 82, 625-631

**CRDO** (2012). Consejo Regulador de la Denominación de Origen Chufa de Valencia. Available from: http://www.chufadevalencia.org Accessed June 20, 2014.

**Cummings, J.H., Hill, M.J., Dja, J.R and Wiggins, H.S.** (1976). Changes in fecal composition and colonic function due to cereal fier. *American Journal Clinical Nutrition* 29, 1468-1473.

**Dakogre, T.** (2008). Morphological characteristics and evaluation of tiger nuts in Ghana. A thesis submitted to the Department of Crop Science. School of Agriculture, Univerity of Cape Coast

**De Venanzi, F.A.** (1991). Chufa composition and horchata quality. M.S. thesis, Univ. of Florida, Gainsville,

**Demirag, K., Elmaci, Y., & Altug, T.** (1999). Formulation and quality evaluation of reduced sugar and reduced calorie Kazandibi. *Journal of Food Quality* 22(1), 101–108

**Diarra, K. Nong, Z.G. and Jie, C.** (2005). Peanut Milk and peanut milk based products production: A review. *Critical Review of Food Science Nutrition* 45, 405-423

**Dickinson, E.** (1992). An introduction to food colloids. Melbourne: Oxford University Press.

**Dickinson, E. and Stainsby, G.** (1988). Emulsion stability in advances in emulsions and foams. London: Elsevier Applied Science.

**Djomdi Ejoh, R. and Ndjouenkeu, R.** (2006). Characteristics of tiger nut (Cyperus esculentus L.) tubers and their performance in the production of a milky drink. *Journal of Food Process and Preservattion* 30, 145-163

**Dokosi, O.B.** (1998) Herbs of Ghana. Ghana University Press, Accra, pp.548, 549

**Dubois, V., Breton, S., Linder, M., Fanni, J. and Parmentier, M.** (2007). Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential. *European Journal of Lipid Science and Technology*.109: 720–32

**Dukehart, M. R., Dutta, E. and Vaeth, J.** (1989). Dietary fiber supplementation: Effect on exocrine pancreatic secretion in man. *American Journal Clinical Nutrition* 50, 1023-1028

**Edwards, E.V.** (1998). Milk Recipes from Nut & Seeds. Brushton, New York, Teach Services, Inc.

**Egan, H., Kirk, R.S., and Sawyer, R.** (1981). Pearson's Chemical Analysis of Foods. (8th Edn.) Churchill Livingstone, London

**Ekeanyanwu R. C. and Ononogbu C. I.** (2010) Nutritive value of Nigerian Tiger nut (Cyperus esculentusL.) *Agricultural Journal* 5(5), 297-30

**El-Algamy, E. I.** (2007). The challenge of cow milk protein allergy. *Small Ruminants Research* 68 (1), 64-72

**Engler, M.B, and Engler, M.M.** (2006). The emerging role of flavonid-rich cocoa and chocolate in cardiovascular health and disease. *Nutrition Review* 109-118.

**Enneking, D., and Wink, M.** (2000). Toward the elimination of anti nutritional factors in grain legumes. In Knight, R. (ed.). Linking Research and Marketing Opportunities for Pulses in the 21st Century. Proceedings of the Third International Food Legume Research Conference, Adelaide 1997. Current Plant Science and Biotechnology in Agriculture. 34. (pp.375-384); London, Kluwer Academic Publishers,

**Er, F, Ozcan, M.M, Gumuscu, A, A. D, and Unver, A.** (2009). The influence of planting time and planting Closeness of ground almond (Cyperus esculentus L.) populations of some agronomic and quality properties. *World Applied Sciences Journal* 6 (5), 616-623,

**Eyeson, K.K, and Ankrah, E.K.** (1975). Composition of Foods commonly used in Ghana, CSIR, Ghana.

**Fatoki, O.S, Omode, A.A. and Olaogu, K.A.** (1995). Physicochemical Properties of some Underexploited and Non-conventional Oilseeds. *Journal of Agriculture and Food Chemistry* 43, 2850-2853

**FAO Corporate Document Repository** (2006) Calculation of the Energy Content of Foods- Energy Conversion Factors. Available from http://www.fao.org Accessed June 20, 2011.

**Fernandez-Lopez, J., Sayas-Barbera E., Navarro, C., and Perez- Alvarez., J.A.** (2008). Physico-chemical and microbiological profiles of salchichon (Spanish dry-fermented sausage) eneiched with orange fibre. *Meat Science* 80, 410-417.

**Fernandez-Lopez, J., Viuda-Martos, M., Sendra, E., Sayas-Barbera, E Navarro, C., and Fernandez-Lopez, J., A.** (2007). Orange fibre as potential functional ingredient for dry-cured sausage. *European Food Research and Technology* 226, 1-6.

**F.D.A.** (2009). Guidance for industry, a food labeling guide, [http://www.fda.gov/food/guidance]

**Folkenberg, D.M., Bredie, W.L.P. and Martens, M.** (1999). What is mouth feel? Sensory-rheological relationships in instant hot cocoa drinks. *Journal of Sensory Studies* 14,181-195

**Forsyth, W.G.C.** (1955). Cacao polyhenolic substances. Sepration and estimation on paper chromatograms. *Biochemical Journal* 60 (1), 108-11

**Fox, B.A. and Cameron, A.G.** (1989). Food Science, Nutrition and Health. pp 285-304, Kent,

**Frazier, W. O. and Westhoff, D. C.** (1978) Food Microbiology; AVI Publishing Co. Inc. Westport Connecticut

**Gacula, M.C. and Singh, J.** (1984). Statistical methods in food and consumer research. London: Academic press.

**Galvez, F.C.F., Resurreccion, A.V.A. and Ware G.O.** (1995). Formulation and process optimization of mungbean noodles. *Journal of Food Processing and Preservation* 19,191-205

**Garcia, M. L., Caceres. E. and Seigas, M. D.** (2007). Utilisation of fruit fibres in conventional and reduced-fat cooked-meat sausages. Journal of the Science of Food & Agriculture 87,624-631

**Gavin, M. and Wettstein, A.** (1990). Soymilk and other Soya Products – From the Traditional Method of Production to the New Manufacturing Processes. Buhler Ltd. Uzwil, Switzerland

**Gbeddy D.V.** (2001). Production of Miso-like Products using Local Grain Legumes. M.Phil. Thesis, Department of Nutrition and Food Science, University of Ghana

**Gensinde, A.F., Oyawoye, O.M. and Adebisi, A.** (2008).Comparative Studies on the Quality and Quality of Soymilk from different varieties of soybean *Pakistan Journal of Nutrition* 7(1), 157-160,

**Giami, S.Y.** (1993). Effect of processing on the proximate composition and functional properties of cowpea (Vigna unguiculata) flour. *Food Chemistry* 47,153-158

**Glew, R.H., Glew, R.S., Chuang, L.-T., Huang, Y.-S., Millon, M., Constans, D. and Vanderjagt, D.J.** (2006) Amino acids, minerals and fatty acids content of pumpkin seeds (Cucurbita spp) and Cyperus esculentus nuts in the Republic of Niger. *Plant Foods and Human Nutrition* 61, 51-55,

**GSA,** (2007). Ghana Standards, GS 741:2007 (2nd edition) Cocoa and Cocoa Products-Specification for Chocolate, Ghana Standards Authority, Accra

**Hajirostamloo, B.** (2009). Comparison of Nutritional and Chemical Parameters of Soymilk and Cow milk. *World Academy of Science, Engineering and Technology* 33, 436-438

**Han, I.K., Kin, S.W., Chae, B.J., Ha, J.K., Park, Y.J., and Lee Y.S.** (1988). Bio availability of amino acids in cereals and legumes Pg 351-355 In Proc 5th Asian congress of nutrition, October 26-29, Osaka Japan.

**Harjai, N. and Singh, G.** (2007). Evaluation of different soybean varieties for manufacture of soymilk. *International Journal of Food Technology and Nutrition* 2, 71-77

**Harper, W.J. and Hall, C.W.** (1976). Dairy Technology & Engineering, The AVI Publishing Company Inc. Connecticut, U.S.A. pp. 59

**Harris B.C.** (1973), Eat the weeds, Pivot Health p.197, 342

**Heath, H. and Reineccius, G.** (1986). Flavor Chemistry and Technology. AVI Publishing Company, Inc., Westport, CT.

**Hinds, M.J., Beuchat, L.R., and Chinnan, M.S.** (1997). Properties of a thermal-processed beverage prepared from roasted partially defatted peanuts. *International Journal of Food Science and Technology* 32,203-211

**Holland, B. Unwin, I.D. and Buss H.D.** (1992) The composition of foods the royal society of chemistry, Thomas Graham House, Science Park, Millton Road, the Bath Press, UK. pp 100-107

**Howeler, R.H.** (2002). Cassava mineral nutrition and Fertilization. In Cassava: Biology, Production and Utilization, Hilocks, R.J. Thresh J.M. and Bellotti A.C. (Eds): 115-147. CAB International, Wallingford, UK.

**Hoyer, R.W., Hoyer, B.Y., Crosby, P.B., and Deming, W.E.** (2001). What is quality? Quality Progress, Quality vocabulary Part 1; international terms 34 (7), 52-62

**Hu, G., Huang, S., Cao, S., and Ma, Z.** (2009). Effect of enrichment with hemicelluloses from rice bran on chemical and functional properties of bread. *Food Chemistry* 115, 839-842.

**Igoe, R.S., and Hui, Y.H.** (2001). Dictionary of food ingredient 4th edn Aspen Publishers, Inc. Gaithersburg, Maryland,

**ICCO,** (2005)**. International Cocoa Organization**, Inventory of the health and nutritional attributes of cocoa and chocolate. Promotion committee PRC/3/4/ Rev 1 West gate house Ealing London, W51YY

**IOCCC,** (1996). **International Office of Cocoa Chocolate and Sugar Confectionery**, Determination of the free fatty acid content of cocoa fat as a measure of cocoa nib acidity. Analytical Method 42.

**Irvine, J. R.** (1969). Chufa. West African agriculture, 3rd edition, Vol. 2, West African crops, London: Oxford University Press, pp. 272

**Isanga, J., and Zhang, G.** (2009). Production and evaluation of some physicochemical parameters of peanut milk yoghurt, *LWT -Food Science and Technology* 42, 1132-1138.

**Iserliyaska, D., Chinnan, M.S., and Ressurreccion, A.V.A.** (2012). Physicochemical and sensory properties of a peanut drink. Agricultural Engineering International 14, (2): 49-57

**Johnson, E. J. and Marlett, J. A.** (1986). A simple method to estimate neutral detergent fiber content of typical daily menus. *The American Journal of Clinical Nutrition* 44, 127-134.

**Kashid, U.B., Sontakke, A.T. and Shined, D.B.** (2007). Manufacture of golden milk shake from cow milk blended with safflower milk. *Journal of Dairying Foods and Home Science* 26: 159-163

**Kay, D.E.** (1987) Revised by E.G.B. Gooding Cwp and Product digest N0.2, London TDRI. Tropical Development and Research institute 127 clerkenwell Road, London ECIR 5DB.

**Kelley, J.R., and Fredrickson, L.H.** (1991). Chufa biology and management. United States Department of Interior, Fish and Wildlife Service, Fish and wildlife leaflet 134.18:1-6

**Kelly, J., Kelly, P.M. and Harrington, D.** (2002). Influence of processing variables on the physicochemical properties of spray dried fat-based milk powders *Lait* 82, 401-412

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

**Kim, M., No, S. and Yoon, S.H.** (2007). Stereospecific Analysis of Fatty Acid Composition of Chufa (Cyperus esculentus L.) Tuber Oil. *Journal of American Oil Chemists Society* 84:1079-1080

**Kinsella, J.E.** (1976). Functional Properties of Soy Proteins. *Journal of American Oil Chemists' Society* 56, 242-258

**Knapp, A, and Hearne, J.F.** (1939). The presence of Leuco-anthocyandins in Criolla cacao. *Analyst* 64,475-80.

**Kouane, D., Zhang, G., and Chen, J.** (2005). Peanut milk and peanut milk based products production. A review. *Critical Reviews in Food Science and Nutrition* 45:405-423

**Kris-Etherton P. M. and Yu, S.** (1997). Individual fatty acid effects on plasma lipids and lipoproteins: human studies. *American Journal of Clinical Nutrition* 65, 1628S-1644S

**Kris-Etherton, P.M, Taylor, D. S., Yu-Poth, S., Huth, P., Moriarty, K., Fishell, V. Hargrove, R. L Zhao, G. and Etherton T. D.** (2000). Polyunsaturated fatty acids in the food chain in the United States.*American Journal of Clinical Nutrition* 71(suppl):179S-188S.

**Krupa, H., Atanu, J.H. and Patel H. G.** (2011) Synergy of dairy with non dairy ingredients or product: a review. *African Journal of Food Science* 5 (16): 817-832

**Kuila, A., Singh, A., Mukhopadhyay, M., and Banerjee R.** (2011). Process optimization for aqueous extraction of reducing sugar from cashew apple bagasse: A potential, low cost substrate. *LWT – Food Science and Technology* 44, 62-66

**Kuntz, D.A., Nelson, A.I., Steinberg, M.P., and Wei, L.S.** (1978). Control of Chalkiness of Soymilk. *Journal of Food Science* 43, (4) 1279 – 1283

**Labuza, T.P. and Schmidl, M.K.** (1985). Accelerated shelf-life testing of foods. *Food Technology* 9: 57-64, 134.

**Lado C, Then M, Varga I, Szoke E and Szentmihályi, K.** (2004). *Journal of Biosciences*, 59(5-6):354-358

**Lampert, L.M.** (1970). Modern Dairy Products. London: Chemical publishers Inc, New York.

**Lattimer, J. M. and Haub M. D.** (2010). Effects of Dietary Fiber and Its Components on Metabolic Health *Nutrients*, 2, 1266-1289

**Lee, K., Kim, Y. and Lee, C.** (2003) Cocoa has more phenoloic phytochemicals and a higher anto oxidantscapacity than teas and red wine. *Journal of Agricultural and food Chemistry* 51:7272

**Lees, R. and Jackson E.B,** (1985). Sugar Confectionery and Chocolate Manufacturing. Great Britain, Thomson Litho Ltd. pp 66

**Liang, B. and Hartel, R. W.** (2004). Effects of Milk Powders in Milk Chocolate. *Journal of Dairy Science*. 87:20–31

**Liener, I.E.** (1994). Implications of Anti-nutritional Components in Soybeans Foods. *CRC Critical Reviews in Food Science and Nutrition* 34, 31- 67

**Linssen, J.P.H., Cozijnsen, J.L. and Pilnik, W.** (1989). Chufa (Cyperus esculentus): A new source of dietary fiber. *Journal of Science Food & Agriculture* 49, 291-296.

**Liu, K.** (1997). Soybean Chemistry, Technology & Utilization. New York, NY Chapman and Hall, pp 1000

**Lokhande, A.T., Deshmukh, V.S., Adangale, S.B., Khating, L.E. and Walkunde, T.R.** (2010). Formulation of Rasogolla from cow milk blended with safflower milk. *Journal of Dairying Foods and Home Science* 29, 185-188

**Lund, D.B.** (1982). Quantifying reactions influencing quality of foods: Texture, Flavor and Appearance. *Journal of Food Processing and Preservation* 6,133-153

**Maieves, H.A., Cardoso de Oliveira, D., Bernardo, C., de Oliveira Müller, C.M. and Amante, E.R.** (2012). Microscopy and texture of raw and cooked cassava (Manihot esculenta Crantz) roots. *Journal of Texture Studies* 43 (2), 164-73

**Makeri, M.U., Bala, S.M and Kassum, A.S.** (2011).The effect of roasting temperatures on the rate of extraction and quality of locally-processed oil

from two Nigerian peanut (Arachis hypogea L.) cultivars. *African Journal of Food Science* 5: 194-199

**Mani, S., Jaya, S. and Vadivambal, R.** (2007). Optimization of Solvent Extraction of Moringa (Moringa Oleifera) Seed Kernel Oil Using Response Surface Methodology. *Food and Bioproducts Processing Trans IChemE, Part C*, 85: (C4) 328-335

**Martensson, O., Oste, R., and Holst, O.** (2000). Lactic acid bacteria in an oatbased non dairy milk substitute: fermentation characteristics and exopolysaccharide formation. *Lebensmittel- Wissenschaft-und-Technologie* 33(8), 525–530

**McEvily, A.J., Iyengar, R, and Otwell, W.S.** (1992). Inhibition of enzymatic browning in foods and beverages, *Critical Review of Food Science and Nutrition*, 32, 253-273

**Milani, E., Koocheki, A. and Golimovahhed Q.A.** (2011). Extraction of inulin from Burdock root (Arctium Lappa) using high intensity ultrasound. *International Journal of Food and Technology* 46, 1699-1704

**Miller, K.B., Hurst, W.J., Payne, M.J., Stuart, D.A., Apgar, J., Sweigart, D.S. and Ou B.** (2008) Impact of alkalization on the antioxidant and flavanol content of commercial cocoa powders. *Journal of Agriculture and Food Chemistry* 56, 8527-8533

**Minifie, B.W**. (1989). Chocolate, Cocoa and Confectionery – Science and Technology. Chapman and Hall, London.

**Mitchell, D.C., Mcmaholm, K.E., Shively, C.A., Apgar, J.L. and Kris – Etherton, P.M., (1989)** Digestibility of cocoa butter and corn oil in human subjects: a prelimenary study. *American Journal of Clinical Nutrition* 50:983-6

**Mojca-Skerget, Kotnik, P., Hadolin, M., Hras, A.R., Simonic, M and Knez, Z.** (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chemistry* 89,191–198

**Montgomery, D.C**. (2001). *Design and Analysis of Experiments*. New York: John Wiley and Sons

**Mordi, J. I., Ozumba, A.U., Elemo, G.N. and Olantunji, O.** (2010). Physicochemical and Sensory evaluation of NigerianTiger-Nut Extract Beverage. *Bioscience Resource Communication* 22,203-207

**Morse, D. and Sedivic, K.** (1990). Know your forages. North Dakota State University Publication AS-991 http://www.ag.ndsu.edu Accessed 1st October 2011

**Mosquera, L.A., Sims, C.A., Bates, R.P and Okeefe, S.F.** (1996). Flavor and Stability of Horchata De Chufas. *Journal of Food Science* 61, 856-861

**Mulligan, G. A., and Junkins, B.E.** (1976). The biology of Canadian weeds. 17. Cyperus Ofori-Anti A.O., (2000) esculentus L. *Canadian Journal of Plant Science* 56,339-350

**Nadutey, Y.D.** (1999). Process characteristics of yoghurt-like product from cowpea and groundnut milk. B.sc Project Report, Department of Nutrition and Food Science, University of Ghana.

**Narayana, K and Narasimga, R. N. M. S.** (1982). Functional properties of raw and heat processed winged bean flour. *Journal of Food Science* 47, 1534-1538

**Navarro, J.L. Schwartz, M., Gasque, F., Alberola, J., Perez, R. and Lafuente B.** (1984). Influencia de la e'oca de recoleccion de la chufa (Cyperus esculentus L.) sobre las caracteristicas analiticas y sensoriales de la horchata. *Revista de Agroquimica y Tecnologia de los Alimentos* 24, 199-203

**Ndjouenkeu, R. and Djomdi, Ejoh, R.** (2007). Soaking behavior and milk extraction performance of tiger nut (Cyperus esculentus) tubers. *Journal of Food Engineering* 78, 546-550

**Nelson, A. L., Steinberg, M. P., and Wei, L. S.** (1976). Illinois process for preparation of soymilk. *Journal of Food Science* 41, 57-61

**Nelson, A.I., Wei, I.S and Weingartner, K.E.** (1987). Home and Village preparation of soy products. In soybean for the Tropics. Edited by Singh S.R. Rachie, K.O. Dashiell, K.E. John Wiley and Sons ltd.

**Nout M.J.R.** (1993). Processed weaning foods for tropical climates. *International Journal of Food Science and Technology* 43,213-221

**Nyarko, D.N., Tagoe, D.N.A. and Aniweh, Y.** (2011). Assessment of microbiological safety of tiger nuts (Cyperus Esculentus L.) in the Cape Coast Metropolis of Ghana. *Archieves of Applied Science Research* 3 (6), 257-262

**NVTI** (1985) Ghana cookery' book. Ghana Publishing Corporation. Accra.Ghana. P27

**Odoemelam, S.A.** (2003). Chemical composition and functional properties of conophor nut (Tetracarpidium conophorum) flour. *International Journal of Food Science and technology* 38, 729-734

**Ofori E.** (1994) Production and Marketing of *Cyperus Esculentus* at Aduamoa in the Kwahu South District of Ghana. Dissertation submitted to School of Agric, U.C.C.

**Ofori-Anti A.O.** (2000) Tiger nuts (Cyperus Esculentus): production processing and product development. Dissertation submitted to the Department of Nutrition and Food Science, University of Ghana.

**Oh k, Hu, F.B., Manson J.E., Stampfer M.J., and Willett W.C. (2005).** Dietery fat intake and risk of coronary heart diseases in women: 20years follow up of the nurses' health study. *American Journal of Epidemiology* 161:672-9

**Okezie, B.O. and Bello, A.B. (1988).** Physico-chemical and functional prperties of winged bean flourand the isolate compound with soy isolate. *Journal of Food Science*, 43,450-454

**Oladele, A.K. and Aina, J.O.** (2007). Chemical composition and functional properties of flour produced from two varieties of tiger nut (Cyperus esculentus) *African Journal of Biotechnology* 6 (21), 2473-2476,

**Olayiwola, I.O., Abubakar, H.N., Adebayo, G.B. and Oladipo, F.O.** (2009). Study of sweet potato (Ipomea batatas Lam) foods for indigenous

consumption through chemical and anti-nutritive analysis in Kwara State, Nigeria. *Pakistan Journal of Nutrition* 8 (12): 1894-7.

**Opare, J.** (2005). Marketing of Tigerimt in the Central and Brong AhafoRegions of Ghana. Dissertation presented to the School of Agriculture. University of Cape Coast, Cape Coast, Ghana.

**Ouhrabková, J., Gabrovsk á, D., Rysov á, J., Vavreinová, S., Paulíčková, I., Němečková, I., Roubal, P. and Pechačová, M.** (2010). Utilization of vegetable raw materials as alternatives to cow's milk. *Agronomy Research* 8 (Special Issue II), 327-331

**Pan, Z. and Tangratanavalee, W.** (2003) Characteristics of soybeans as affected by soaking conditions. *LWT- Food Science and Technology* 36 (1), 143-151

**Park Y.W., Ju´arez M., Ramosc, M. and Haenlein G.F.W.** (2007). Physico-chemical characteristics of goat and sheep milk. *Small Ruminant Research* 68, 88–113

**Pascual B, Maroto JV, Lopez-Galarza S, Sanbautista A, and Alagarda J**, (2000). Chufa (Cyperus esculentus L. var. sativus Boeck.): An unconventional crop. Studies related to applications and cultivation. *Economy Botany* 54, 439-448

**Pattee, H.E. and Singleton, J.A.** (1980). Peanut quality: its relationship to volatile compounds. A review in quality of selected fruits and vegetable of north America (Eds.)R Teranishi and H Barrea-Benitez American chemical society Washington, D.C. pp 147-161

**Pennington, J.A.T. and Church, H.N.** (1985) *Bowes and Church's food values of portions commonly used*. Philadelphia: J. B. Lippincott, Co.

**Priepke, P.E., Wei, L.S., Nelson, A.I., and Steinberg, M.P.** (1980). **Suspension Stability of Illinois Soybean Beverage.** *Journal of Food Science*, 45: 242-245

**Quasem, J. M. Mazahreh, A. S. and Abu-Alruz, K.** (2009). Development of Vegetable Based Milk from Decorticated Sesame (Sesamum Indicum) *American Journal of Applied Sciences* 6 (5), 888-896

**Rackis, J.J, Sessa, D.J, and Honig, D.H.** (1979). Flavor problems of vegetable food proteins. *Journal of the American Oil Chemists Society* 56, 262-271

**Ramli, N., Zamzarina, N. and Zawawi, A.** (2006) Sensory evaluation and physical characteristics of chocolate using goat's milk. *International Journal of Dairy Science* 1, (2), 146-154

**Reid, W.S., Hergert, G.B., and Fagan, W.E.** (1972). Development of a proto-type mechanical harvester for chufa (Cypersu esculentus L. car. Sativus Beek). *Canadian Agricultural Engineering* 14(2), 89-95

**Rhee, K.C., Cater, M.C. and Matil, K.F.** (1972). Simultaneous recovery of protein and oil from raw peanuts in an aqueous system *Journal of Food Science* 39, 90

**Riaz, M.N.** (1999). Soybeans as functional foods. *Cereal Foods World* 44(2), 88-92

**Richards, D. J., Ivanova, L. K., Smallman, D. J. and Zheng, B.** (2005). Assessment of waste degradation using acid digestible fibre analysis.

International Workshop 'Hydro-Physico-Mechanics of Landfills. LIRIGM, Grenoble 1 University, France, pp 1-5, 21-22.

**Robertson, G.L.** (1993). Food Packaging: Principles and Practice. New York: Marcel Dekker Inc.

**Rosengarten, Jnr. F.** (1984).The book of edible nuts Walker Publication Inc East 2nd street, Niveda, NY 11501 pp 331

**Rubert, J., Sabastià, N., Soriano, J. M., Soler, C. and Mañes J.** (2011). One-year monitoring of aflatoxins and ochratoxin A in tiger-nuts and their beverages. *Food Chemistry* 127, 822-826

**Rubico, S.M, Resurreccion, A.V.A, Frank, J.F, and Beuchat, L.R.** (1987). Suspension stability, texture, and color of high temperature treated peanut beverage. *Journal of Food Science* 52(6), 1676-1679

**Russell, K. & Delahunty, C.** (2004). The effect of viscosity and volume on pleasantness and satiating power of rice milk. *Food Quality and Preference* 15, 743-750.

**Saguy, I. and Karel, M.** (1987). Index of deterioration and simulation of quality loses. In Objective Methods in Food Quality Assessment, ed. J. G. Kapsalis, Boca Baton, Fla. CRC Press. pp 233-260

**Sampedro, F., Geveke, D.J., Fan, X., Rodrigo, D. and Zhang, Q.H**. (2009). Shelf life study of an Orange juice –milk based beverage after PEF and thermasl processing, *Journal of Food Science* 74, 107-112,

**Sánchez-Zapata, E.,Fuentas-Zaragosa, E., Fernandez-Lopez, L., Sendra, E. Sayas- Babera, E. Navarro, C.** (2009) Preparation of dietary fiber powder

from tiger nuts (Cyperus esculentus) milk ("horchata") by-product and its physicochemical properties. *Journal of Agricultural and food Chemistry* 57, 7719-7725

**Sánchez-Zapata, E., Muñoz, C.M., Fuentes, E., Fernández-López, J., Sendra, E., Sayas, E., Navarro, C. and Pérez-Alvarez, J.A.** (2010). Effect of tiger nut fiber on quality characteristics of pork burger. *Meat Science* 85. 70-76

**Sanful, R. E.** (2009) Production and sensory evaluation of tiger nut beverages *Pakistan Journal of Nutrition* 8 (5), 688-690

**Sanni, A.I., Onilude, and Adeleke, E.O.** (1999). Preparation and characteristics of lactic acid fermented cowpea milk. *Z Lebensm Unters Forsch* 208, 225-229

**Schwartz, M., Costell, E. and Gasque, F.** (1984). Efecto de los tratamientos de estabilizacie la horchata de chufa (Cyperus esculentus L.) sobre su color y su viscosidad. Revista de Agroquca y Tecnologde Alimentos, 24, 271 -277.

**Sendra, E., Fayos, P., Y., Fernandez-Lopez., J., Sayas-Barbera, E., & Perez-Alvarez., J.A.** (2008). Incorporation of citrus fibres in fermented milk containing probiotic bacteria. *Food Microbiology* 25, 13-21

**Shi T.G. and Ren, L. (**1993). Soyfood Technology (Chinese). China's Light Industry Publisher, Beijing, China.

**Shirai, K., Gutierrez-Duran, M., Marshall, V. M. E., Revah-Moiseev, S., and Garcia-Garibay, M.** (1992). Production of a Yoghurt-like product from plant foodstuffs and whey. Sensory evaluation and physical attributes. *Journal of the Science of Food and Agriculture* 59(2), 205-210

**Shukla, V.K.S.** (1994). Milk fat in sugar and chocolate Confectionery. In Moran, D.P.J. and K.K.Rajah, Fats in Food Products: London: Blackie Academic and Professional London, pp.256

**Sopade, P.A. and Obekpa, J.A.** (1990). Modeling Water Absorption in Cowpeas, Soyabean and Peanut at Three Temperatures Using Peleg's Equation. *Journal of Food Science*, 55, 1084– 1807.

**Sørenson, L.B. and Astrup, A. (2011)** Eating dark choclate milk chocolate: a randomized crossover study of the effects on appetite and energy intake. *Nutrition and diabetes* 1, e 21;doi:10.1038

**Statistics, Research and Information Directorate** (SRID). 2011. Agriculture in Ghana. Facts and figures. Ministry of Food and Agriculture. Accra. Ghana.

**Takrama, J.F. Aculey, P.C., Owusu, E., Botchway, F., Frimpong, K., Pawar, G., Bediako, S., and Asante, F.** (2007). Physical and biochemical assessment of Ghana's cocoa for quality assurance. Proceedings of the 15 th International Cocoa Research Conference, San Jose, 54 American Journal of Food and Nutrition Costa Rica, 9-14 October, Cocoa Producers Alliance. 1179-1185,

**Tamime, A. Y., and O'Connor, T. P.** (1995). Kishk-a dried fermented milk/ cereal mixture. *International Diary Journal* 5(2), 109-118

**ter Borg, S., Schippers, P.** (1992). Distribution of varieties of Cyperus esculentus and the possible migration in Europe, 1: 417-425, Dijon, France, ANPP

**Tetteh, J.P. and Achearnpong. A.** (1999). Effects of manure on the growth and yield tiger nut (Cyperus esculentus L.). Proceedings: Soil Science Society, Ghana. Vol 16, (1999)

**Tetteh J.P., & Ofori E, A.** (1998). Baseline survey of tiger nuts (Cyperus esculentus) production in the Kwahu South District of Ghana. *Ghana* **Journal of Agricultural Science 31, 211-216**

**Tigernuts Traders S.L.** (1997-2010) Profile on Tiger nut. http://www.tigernut.com/pdf/tigernut profil.pdf (Accessed October 2010).

**Torrell, J.M., Thornton M.K., and Morishita D.W.** (1993) Yellow nutsedege. Extention bulleting PNW 452

**Tunde-Akintunde, T.Y. and Souley A.** (2009) effect of processing methods on the quality of soymilk *Pakistan journal of nutrition* 8(8), 1156-1158

**Ukwuru, M.U. and Ogbodo A.C.** (2011). Effect of Processing Treatment on the Quality of Tiger nut Milk. *Pakistan Journal of Nutrition* 10, 1, 95-100

**Ukwuru, M.U. Ibeneme C.L. and Agbo G.I.** (2011). New product development from tiger nut (*Cyperus Esculentus*) and their sensory, proximate and microbiological evaluation *Pakistan Journal of Nutrition* 10(2), 101-105

**Umeri, S.C. and Enebeli, J.N.** (1996). Malt caramel from tubers of Cyperus esculentus. *Bioresource Technology* 57(2), 215-216.

**Umerie, S. C., Okafor, E. O. and Uka, A.S.** (1997). Evaluation of the tubers and oil of Cyperus esculentus. *Bioresource Technology* 61(2), 171-173

**Vegetarian Nutrition** (2008). www.vegetarian-nutrition.info, (Accessed October 2009)

**Voutsinas, L.P. & Nakai, S.** (1983). A simple turbidometric method for determining the fat binding capacity of proteins. *Journal of Agriculture Food & Chemistry* 31, 58-61

**Wang, H.J, Murphy, P.A.** (1994). Isoflavone composition of American and Japanese soybeans in Iowa; effect of variety, crop year and location. *Journal of Agriculture and Food Chemistry* 12: 1674-1677

**Wang, Z.H., Don J., Macura, D., Durance T.D., and Nakai S.** (1997). Solid phase extraction for GC analysis of beany flavor in soymilk *Food Research International* 30, 503-511

**Wang, H.** (1980). The importance of traditional fermented foods. *Biological science* 30,402-404

**Wang B, Xiong YL, and Wang C.** (2001). Physicochemical and sensory characteristics of flavored soymilk during refrigeration storage. *Journal of Food Quality* 24:513-526

**Wester P.O.** (1987) Magnesium. *American Journal of Clinical Nutrition* 45, 1305-12

**Wickramarathna G.L. and Arampath P.C.** (2003). Utilization of Okara in Bread Making, *Ceylon Journal of Science* (Bio. Sci), 31, 29-33

**Wills, J. B.** (1962). *Agriculture and land use in Ghana* (Wills, J. B., ed.) Oxford University Press, london. pp 378.

**Xiang, L., Xing, D., Lei, F., Wang, W., Xu, L., Nie, L., Du, L.** (2008). Effects of Season, Variety, and Processing Method on Ellagic Acid Content in Pomegranate Leaves *Tsinghua Science And Technology*, 13, 4, 460-465

**Ye, C., and Jiang, C.** (2011). Optimization of extraction process of crude polysaccharides from Plantago asiatica L. by response surface methodology. *Carbohydrate Polymers* 84, 495-502.

**Yeboah, S.O., Mitie, Y.C., Ngila, J.C., Wessjohann, L. and Schmidt, J.** (2011). Compositional and Structural Studies of the Oils from Two Edible Seeds: Tiger nut, Cyperus esculentum, and Asiato, Pachira insignis, from Ghana, *Food Research International*, doi: 10.1016/j.foodres.2011.06.036

**Zaruwa, M. Z., Umar I. A. and Chibuzo, E.C.** (2005). Formulation and evaluation of phytomilk from soybean (Glycine max_L) as an alternative to dairy milk. *ChemClass Journal* 2, 88-92

## APPENDICES

### Analytical methods

A1 Determmination of fibers

Preparation of Sample

A combination of enzymatic and gravimetric methods was used to prepare the sample. Blanks were also run using the procedure to ensure that any contributions to residue from reagents catered for.

1-gram of the sample was first weighed into beakers after which 50ml of pH 6.0 buffer was added to 0.1ml α-amylase and mixed well. The beaker then was then covered with aluminium foil and placed in a boiling water bath for 15 minutes at 95 ˚C. The beaker was shaken at every 5 minutes intervals. The Solutions was cooled to room temperature and 10ml of 0.275 NaOH added after which the pH was adjusted to 7.5.

A 0.1ml of 50mg/ml solution of just prepared protease in phosphate butter was then pipetted into the beaker and covered with aluminium foil and placed in a 60 ˚C water bath with continuous agitation for 30 minutes. The Solutions was then cooled to room temperature and 10ml of 0.325 M HCl added and the pH adjusted to between 4.0 and 4.6.

0.1ml of Amyloglucosidase was then added to the beaker and covered with aluminium foil and placed in a 60 ˚C water bath with continuous agitation for 30 minutes. The Solution was cooled to room temperature and the pH of the solution adjusted to 4.5 with HCl. The solution was filtered using a gentle suction through a dried and weighed crucible containing 0.5g of dry celite. Once all solution was

filtered, each beaker was washed and again filtered with 2x10ml of distilled water.

*Residue - Insoluble Fiber*

The beaker was washed with 2 x 10ml of 95% ethanol and 2 x 10ml of acetone. The residue was dried in an oven for 12 hours at 105˚C then cooled in a desiccator and weighed as (D1). It was then incinerated at 550˚C overnight. After cooling in a desiccator, residue was reweighed as (I1)

Insoluble fiber was finally determined as D1-I1/1

*Filtrate – Soluble Fiber*

To the determine the soluble fiber, the filtrate and washing liquid was adjusted to 100ml. 400ml of 60˚C 95% ethanol was added to the beaker and allowed to precipitate for one hour. The solution was again filtered through a dry and weighed crucible containing 0.5g of celite. The beaker was washed again with 2 x 10ml of 78% ethanol, 2 x 10ml of 95% ethanol and 2 x 10ml of acetone. The crucible was then dried for 12 hours in an oven at 105˚c. After cooling in a desiccator, the residue was weighed (D2). The residue was incinerated at 550˚C overnight cooled and residue reweighed as (I2). Soluble fiber was determined as D2-12/1

Appendix A2

Antioxidants

Total Antioxidant Capacity in the tiger nut milk, milk powder and fibre was determined using a modified method of the Ferric Reducing Antioxidant Power (FRAP assay) by Benzie and Strain (1999).

Preparation of sample: All samples were extracted in water in ice water bath for 15 min and centrifuged in 1.5 mL tubes at 12.402 × g for 2 min at 4°C. The concentration of antioxidants in the supernatant of the centrifuged samples was used for the determinations.

The FRAP reagent used was a combination of Acetate buffer 300 mM pH 3.6, 10mM ferric tripyridyltriazine in 40 mM HCl and FeCl3. 6H2O 20 mM in the ratio of 10:1:1 mixed together.

Preparations of samples for the standard curve: The method of Lado, *et al.*, (2004) was used for calibration. A 100ml stock solution of 278mg ferrous sulphate was dissolved in water and mixed well. Out of the solution 10ml was measured with a pipette and diluted to 100ml with water.

*Method for standard Calibration Curve and Tiger Nut Samples*

FRAP solution warmed at 37˚C, was used to prepare different concentrations as shown in Table A 2.1 to create a calibration curve. The spectrophotometer was set to 593nm.

Table A 2.1 Different concentrations used for the calibration curve

| Concentration (mM) | 1mM Standard Solution | Water |
|---|---|---|
| 0.025 | 0.25ml | 9.75ml |
| 0.05 | 0.5ml | 9.5ml |
| 0.10 | 1ml | 9ml |
| 0.50 | 5ml | 5ml |

| | | |
|---|---|---|
| 0.70 | 7ml | 3ml |
| 0.90 | 9ml | 1ml |

1. 300µl distilled water was added to 3.000ml to the FRAP reagent in a test tube. It was then mixed on a vortex mixer and transferred to a cuvette labelled 'blank'.
2. 18 test tubes were labelled and filled with the 3 standard solution concentrations (each one in triplicate).
3. 100µl of one ferrous sulphate solution was transfered into 3 test tubes.
4. 300µl of distilled water was transferred into each tube.
5. 3.000ml of working FRAP reagent was next added to each tube
6. Tubes were mixed on a vortex mixer and transferred into cuvettes
7. The Spectrophotometer was set to zero with the cuvette containing reagent 'blank'.
8. The absorbance of each of the cuvettes was measured.
9. Cuvettes were then placed into cuvette stand and for 5 minutes put in a water bath at 37˚C.
10. The absorbance of the cuvettes was again measured.

For the Tiger Nut Fibre samples steps 2 to 10 were completed however, instead of ferrous sulphate solution, the Tiger Nut Samples were used. For the tiger nut milk, milk powder and fibre three replicates were made

Appendix A3

Total Phenol Content

Total Phenol Content followed the method by Mojca-Skerget *et al.* (2005).

The chemicals used were Foilin-Ciocalteu Reagent (diluted 10 times with water), 75g/l solution of $Na_2CO_3$ and Gallic Acid Stock Solution prepared by mixing 0.50 g of dry Gallic acid in 10ml Ethanol and topped up to 100ml in a volumetric flask. 0.5ml of solution was first measured into a boiling tube before 2.5ml of diluted Folin-Ciocalteu reagent added and the tubes stored in a dark cupboard for 5 minutes. 2ml of 75g/l $Na_2CO_3$ solution was then transferred into the tubes and then incubated at 50 ˚C in a water bath for 5 minutes after which the tubes were removed and cooled to room temperature. Each sample was finally poured into 3 cuvettes each and the absorbance measured using a spectrophotometer at 760 nm.

**Appendix B: Survey Questionnaires**

Appendix B1 QUESTIONNAIRE FOR EVALUATION OF TIGER NUT MILK FROM DIFFERENT SITES

NAME……..……DATE:……

You have received three (6) different samples of Tiger nut milk. Please evaluate them from left to right in the order as indicated. Rinse your mouth with some of the water provided and wait for 1 minute before evaluating the next sample.

On the scale of 1 to 6 please rank these samples for overall acceptability. The most acceptable should be ranked 1st and the least 6th. Enter codes in the spaces provided.

Samples Order:

| | | | | | |
|---|---|---|---|---|---|
| 478 | 975 | 157 | 678 | 278 | 874 |
| ….. | …….. | ……. | …… | …….. | …….. |
| 1st | 2nd | 3rd | 4th | 5th | 6th |

COMMENTS……

……

……

……

Appendix B2

QUESTIONNAIRE FOR EVALUATION OF TIGER NUT MILK AFTER DIFFERENT PRE-TREATMENTS

NAME……..……DATE:……

You have received three (3) different samples of Tiger nut milk. Please evaluate them from left to right in the order as indicated. Rinse your mouth with some of the water provided and wait for 1 minute before evaluating the next sample.

On the scale of 1 to 9 (see below for key), rate the samples according to the attributes specified.

Samples Order: 482 424 539



1. Flavor:

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|

2. After-taste:

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|

3. Appearance:

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|

4. Mouth feel:

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|

5. Overall Acceptability:

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|

Comments……
……
……
……

KEY:

1 – Dislike extremely
2 – Dislike very much
3 – Dislike moderately
4 – Dislike slightly
5 – Neither like nor dislike
6 – Like slightly
7–Like moderately
8– Like very much
9 – Like extremely

Appendix B3

QUESTIONNAIRE FOR EVALUATION OF TIGER NUT MILK/DAIRY MILK CHOCOLATE BEVERAGE

NAME……..……DATE:……

You have received five (5) different samples of Tiger nut milk chocolate beverage. Please evaluate them from left to right in the order as indicated. Rinse your mouth with some of the water provided and wait for 1 minute before evaluating the next sample.

On the scale of 1 to 5 please rank these samples for overall acceptability. The most acceptable should be ranked 1st and the least 5th. Enter codes in the spaces provided.

Samples Order:

| | | | | |
|---|---|---|---|---|
| 321 | 711 | 478 | 916 | 612 |
| …….. | …….. | ……. | …… | …….. |
| 1st | 2nd | 3rd | 4th | 5th |

COMMENTS……

……

……

……

Appendix B4

QUESTIONNAIRE FOR TIGER NUT MILK /DAIRY MILK CHOCOLATE BEVERAGE

NAME……..……DATE:……

You have received three (3) different samples of Tiger nut/dairy milk chocolate drinks. Please evaluate them from left to right in the order as indicated. Rinse your mouth with some of the water provided and wait for 1 minute before evaluating the next sample.

On the scale of 1 to 9 (see below for key), rate the samples according to the attributes specified.

Samples Order: 482 612 113

1. Appearance:

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|

**2.** Mouth feel:

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|

3. Flavor:

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|

4. After-Taste:

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|

5. Overall Acceptability:

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|

Comments……
……
……
……

KEY:

1 – Dislike extremely
2 – Dislike very much
3 – Dislike moderately
4 – Dislike slightly
5 – Neither like nor dislike
6 – Like slightly
7 – Like moderately
8 – Like very much
9 – Like extremely

Appendix B5

QUESTIONNAIRE FOR EVALUATION OF TIGER NUT MILK CAKE CHOCOLATE SPREAD

NAME……..……DATE:……

You have received six (5) different samples of Tiger nut chocolate milk drink. Please evaluate them from left to right in the order as indicated. Rinse your mouth with some of the water provided and wait for 1 minute before evaluating the next sample.

On the scale of 1 to 5 please rank these samples for overall acceptability. The most acceptable should be ranked 1st and the least 5th. Enter codes in the spaces provided.

**Samples Order:**

| 321 | 711 | 478 | 916 | 612 |
|---|---|---|---|---|
| ……..… | ……. | ……. | ……. | …….. |
| 1st | 2nd | 3rd | 4th | 5th |

COMMENTS……

……

……

……

Appendix B6

QUESTIONNAIRE FOR TIGER NUT MILK CAKE CHOCOLATE

NAME……..……DATE:……

You have received four (4) different samples of Tiger nut cake chocolate. Please evaluate them from left to right in the order as indicated. Rinse your mouth with some of the water provided and wait for 1 minute before evaluating the next sample.

On the scale of 1 to 9 (see below for key), rate the samples according to the attributes specified.

Samples Order: 482 732, 113 612

1: Appearance:

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|

2: Mouth feel:

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|

3: Flavor**:**

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|

4: After-Taste**:**

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|

5: Taste**:**

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|

6: Overall Acceptability:

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|

Comments……

……

……

……

**KEY:**

1 – Dislike extremely

2 – Dislike very much

3 – Dislike moderately

4 – Dislike slightly

5 – Neither like nor dislike

6 – Like slightly

7 – Like moderately

8 – Like very much

9 – Like extremely

**Appendix C:**

Analysis of Variance Tables For proximate and mineral composition of Black tubers for both planting periods

Key: AMP BK, Ampanyi;BAW BK, Bawjiase; DAN BK, Danyameso; EBU BK, New Ebu;TAMP BLK, Tampiong; TAN BLK, Tanoso;BAW BLK m, Bawjiase minor season; DAN BLK m, Danyameso minor season

Appendix C1 Anova-Protein

Source DF SS MS F P
Factor 7 67.9018 9.7003 346.80 0.000
Error 16 0.4475 0.0280
Total 23 68.3493

S = 0.1672 R-Sq = 99.35% R-Sq(adj) = 99.06%

Individual 95% CIs For Mean Based on Pooled StDev

Level N Mean StDev ------+---------+---------+---------+---
AMP BK 3 4.0400 0.1900 (*)
BAW BK 3 2.2733 0.1286 (*-)
DAN BK 3 4.4667 0.1401 (-*)
EBU BK 3 3.3867 0.2754 (-*)
TAMP BLK 3 3.8200 0.1803 (*-)
TAN BLK 3 4.3400 0.1153 (*)
BAW BLK m 3 7.3433 0.0208 (*)
DAN BLK m 3 7.2867 0.1716 (-*)

Appendix C2 Anova-Fat

Source DF SS MS F P
Factor 7 557.893 79.699 498.68 0.000
Error 16 2.557 0.160
Total 23 560.450

S = 0.3998 R-Sq = 99.54% R-Sq(adj) = 99.34%

Individual 95% CIs For Mean Based on Pooled StDev

Level N Mean StDev --+---------+---------+---------+-------
AMP BK 3 26.277 0.560 (-*)
BAW BK 3 17.473 0.276 (-*)
DAN BK 3 15.537 0.100 (*)
EBU BK 3 16.597 0.662 (*-)
TAMP BLK 3 24.230 0.182 (-*)

TAN BLK 3 20.310 0.497 (*)
BAW BLK m 3 25.403 0.239 (-*)
DAN BLK m 3 29.540 0.322 (*)

Appendix C3 Anova-Fiber

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 7 | 43.956 | 6.279 | 25.50 | 0.000 |
| Error | 16 | 3.940 | 0.246 | | |
| Total | 23 | 47.896 | | | |

S = 0.4962 R-Sq = 91.77% R-Sq(adj) = 88.17%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | -----+---------+---------+---------+---- |
|---|---|---|---|---|
| AMP BK | 3 | 8.490 | 0.140 | (---*---) |
| BAW BK | 3 | 10.920 | 0.246 | (---*---) |
| DAN BK | 3 | 10.340 | 0.217 | (---*---) |
| EBU BK | 3 | 7.417 | 0.291 | (---*---) |
| TAMP BLK | 3 | 8.877 | 0.060 | (---*---) |
| TAN BLK | 3 | 10.380 | 0.850 | (---*---) |
| BAW BLK m | 3 | 10.407 | 0.941 | (---*---) |
| DAN BLK m | 3 | 11.840 | 0.383 | (---*---) |

Appendix C4 Anova-Ash

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 7 | 1.14440 | 0.16349 | 58.74 | 0.000 |
| Error | 16 | 0.04453 | 0.00278 | | |
| Total | 23 | 1.18893 | | | |

S = 0.05276 R-Sq = 96.25% R-Sq(adj) = 94.62%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ---+---------+---------+---------+------ |
|---|---|---|---|---|
| AMP BK | 3 | 1.2300 | 0.1212 | (-*--) |
| BAW BK | 3 | 1.5433 | 0.0115 | (--*-) |
| DAN BK | 3 | 1.9433 | 0.0404 | (--*-) |
| EBU BK | 3 | 1.6667 | 0.0252 | (--*-) |
| TAMP BLK | 3 | 1.3567 | 0.0404 | (-*--) |
| TAN BLK | 3 | 1.7700 | 0.0557 | (--*-) |
| BAW BLK m | 3 | 1.5200 | 0.0200 | (--*-) |
| DAN BLK m | 3 | 1.7567 | 0.0058 | (-*--) |

Appendix C5 Anova-Phosporus

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 7 | 989.423 | 141.346 | 586.60 | 0.000 |
| Error | 16 | 3.855 | 0.241 | | |
| Total | 23 | 993.278 | | | |

S = 0.4909 R-Sq = 99.61% R-Sq(adj) = 99.44%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ----+---------+---------+---------+----- |
|---|---|---|---|---|
| AMP BL M | 3 | 28.309 | 0.227 | (*) |
| BAW BL M | 3 | 37.537 | 0.293 | (*) |
| DAN BL M | 3 | 27.933 | 0.191 | (*) |
| EBU BL M | 3 | 47.741 | 0.089 | (*) |
| TANT BL M | 3 | 29.429 | 0.060 | (*) |
| TAN BL M | 3 | 35.420 | 0.060 | (*) |
| BAW BL m_1 | 3 | 36.255 | 0.933 | (*) |
| DAN BL m_1 | 3 | 28.395 | 0.931 | (*) |

Appendix C6 Anova-Calcium

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 7 | 1.78598 | 0.25514 | 29.67 | 0.000 |
| Error | 16 | 0.13760 | 0.00860 | | |
| Total | 23 | 1.92358 | | | |

S = 0.09274 R-Sq = 92.85% R-Sq(adj) = 89.72%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | --------+---------+---------+---------+- |
|---|---|---|---|---|
| AMP BK | 3 | 0.7667 | 0.0416 | (---*--) |
| BAW BK | 3 | 1.1267 | 0.0306 | (---*--) |
| DAN BK | 3 | 1.5800 | 0.0600 | (---*--) |
| EBU BK | 3 | 0.8267 | 0.0503 | (---*--) |
| TAMP BLK | 3 | 1.2267 | 0.0306 | (---*---) |
| TAN BLK | 3 | 1.1133 | 0.0503 | (---*---) |
| BAW BLK m | 3 | 1.2800 | 0.1400 | (---*--) |
| DAN BLK m | 3 | 1.5267 | 0.1922 | (---*---) |

Appendix C7 Anova-Magnesium

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 7 | 926.9 | 132.4 | 7.46 | 0.000 |
| Error | 16 | 284.0 | 17.7 | | |
| Total | 23 | 1210.9 | | | |

S = 4.213 R-Sq = 76.55% R-Sq(adj) = 66.29%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | +---------+---------+---------+--------- |
|---|---|---|---|---|
| AMP BK | 3 | 53.533 | 3.602 | (------*-----) |
| BAW BK | 3 | 66.167 | 2.608 | (------*-----) |
| DAN BK | 3 | 68.900 | 1.908 | (-----*------) |
| EBU BK | 3 | 58.100 | 1.513 | (------*-----) |
| TAMP BLK | 3 | 65.267 | 2.281 | (------*-----) |
| TAN BLK | 3 | 65.967 | 0.907 | (-----*------) |
| BAW BLK m | 3 | 74.667 | 2.274 | (-----*------) |
| DAN BLK m | 3 | 69.400 | 10.250 | (------*-----) |

Appendix C8 Anova-Potassium

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 7 | 760755 | 108679 | 113.84 | 0.000 |
| Error | 16 | 15275 | 955 | | |
| Total | 23 | 776030 | | | |

S = 30.90 R-Sq = 98.03% R-Sq(adj) = 97.17%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | --------+---------+---------+---------+- |
|---|---|---|---|---|
| AMP BK | 3 | 675.0 | 21.9 | (-*-) |
| BAW BK | 3 | 1089.1 | 22.7 | (*-) |
| DAN BK | 3 | 1165.9 | 43.9 | (-*-) |
| EBU BK | 3 | 887.7 | 32.1 | (-*-) |
| TAMP BLK | 3 | 877.9 | 18.7 | (-*-) |
| TAN BLK | 3 | 902.3 | 36.0 | (-*-) |
| BAW BLK m | 3 | 1003.5 | 4.4 | (-*-) |
| DAN BLK m | 3 | 1278.0 | 44.9 | (-*-) |

Appendix C9 Anova-Zinc

Source DF SS MS F P
Factor 7 26.73180 3.81883 719.53 0.000
Error 16 0.08492 0.00531
Total 23 26.81671

S = 0.07285 R-Sq = 99.68% R-Sq(adj) = 99.54%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | --------+---------+---------+---------+- |
|---|---|---|---|---|
| AMP BK | 3 | 3.6757 | 0.0733 | (*) |
| BAW BK | 3 | 4.7517 | 0.0645 | (* |
| DAN BK | 3 | 4.5103 | 0.0386 | (*) |
| EBU BK | 3 | 3.2877 | 0.0414 | (*) |
| TAMP BLK | 3 | 2.3080 | 0.0160 | (*) |
| TAN BLK | 3 | 6.0577 | 0.0722 | (* |
| BAW BLK m | 3 | 4.6380 | 0.0645 | (*) |
| DAN BLK m | 3 | 4.6347 | 0.1417 | (*) |

Appendix C10 Anova-Copper

Source DF SS MS F P
Factor 7 0.49432 0.07062 48.40 0.000
Error 16 0.02334 0.00146
Total 23 0.51766

S = 0.03820 R-Sq = 95.49% R-Sq(adj) = 93.52%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ---+---------+---------+---------+------ |
|---|---|---|---|---|
| AMP BK | 3 | 0.33167 | 0.02761 | (---*---) |
| BAW BK | 3 | 0.18333 | 0.01724 | (---*---) |
| DAN BK | 3 | 0.46233 | 0.05859 | (---*--) |
| EBU BK | 3 | 0.13633 | 0.02754 | (---*---) |
| TAMP BLK | 3 | 0.14467 | 0.01955 | (---*---) |
| TAN BLK | 3 | 0.48300 | 0.01967 | (---*---) |
| BAW BLK m | 3 | 0.19967 | 0.00153 | (---*---) |
| DAN BLK m | 3 | 0.47133 | 0.07516 | (---*---) |

Appendix C11 Anova-Manganese

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 7 | 1.819030 | 0.259861 | 451.02 | 0.000 |
| Error | 16 | 0.009219 | 0.000576 | | |
| Total | 23 | 1.828249 | | | |

S = 0.02400 R-Sq = 99.50% R-Sq(adj) = 99.28%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | -+---------+---------+---------+-------- |
|---|---|---|---|---|
| AMP BK | 3 | 0.01833 | 0.00306 | (*) |
| BAW BK | 3 | 0.10000 | 0.00624 | (*) |
| DAN BK | 3 | 0.14233 | 0.00850 | (*) |
| EBU BK | 3 | 0.18400 | 0.00656 | (*-) |
| TAMP BLK | 3 | 0.01167 | 0.00153 | (*-) |
| TAN BLK | 3 | 0.92200 | 0.04828 | (*) |
| BAW BLK m | 3 | 0.13500 | 0.04331 | (*-) |
| DAN BLK m | 3 | 0.18967 | 0.01537 | (-*) |

Appendix C12 Anova-Sodium

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 7 | 5726.410 | 818.059 | 12679.17 | 0.000 |
| Error | 16 | 1.032 | 0.065 | | |
| Total | 23 | 5727.442 | | | |

S = 0.2540 R-Sq = 99.98% R-Sq(adj) = 99.97%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | -------+---------+---------+---------+-- |
|---|---|---|---|---|
| AMP BK | 3 | 55.030 | 0.147 | * |
| BAW BK | 3 | 77.825 | 0.168 | * |
| DAN BK | 3 | 92.407 | 0.627 | * |
| EBU BK | 3 | 92.062 | 0.158 | (* |
| TAMP BLK | 3 | 52.344 | 0.094 | (* |
| TAN BLK | 3 | 52.119 | 0.055 | *) |
| BAW BLK m | 3 | 68.215 | 0.136 | * |
| DAN BLK m | 3 | 70.063 | 0.132 | *) |

Appendix C13 Anova-Iron

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 7 | 0.1983425 | 0.0283346 | 1646.57 | 0.000 |
| Error | 16 | 0.0002753 | 0.0000172 | | |
| Total | 23 | 0.1986178 | | | |

S = 0.004148 R-Sq = 99.86% R-Sq(adj) = 99.80%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ----------+---------+---------+---------+-- |
|---|---|---|---|---|
| AMP BK | 3 | 0.32133 | 0.00208 | *) |
| BAW BK | 3 | 0.27233 | 0.00306 | (*) |
| DAN BK | 3 | 0.46467 | 0.00777 | (*) |
| EBU BK | 3 | 0.34100 | 0.00200 | (* |
| TAMP BLK | 3 | 0.54733 | 0.00321 | *) |
| TAN BLK | 3 | 0.51700 | 0.00100 | (* |
| BAW BLK m | 3 | 0.40467 | 0.00666 | (* |
| DAN BLK m | 3 | 0.44700 | 0.00200 | (*) |

Appendix D:

Analysis of Variance Tables For proximate composition of Brown tubers for both planting periods

Key: AMP BR, Ampanyi;BAW BR, Bawjiase; DAN BR, Danyameso; EBU BR, New Ebu;KWAHU, Kwahu;TWI PR, Twifo Praso; BAW BLK m, Bawjiase minor season; DAN BLK m, Danyameso minor season

Appendix D1 Anova-Protein

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 7 | 61.5196 | 8.7885 | 100.64 | 0.000 |
| Error | 16 | 1.3973 | 0.0873 | | |
| Total | 23 | 62.9169 | | | |

S = 0.2955 R-Sq = 97.78% R-Sq(adj) = 96.81%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ---+---------+---------+---------+------ |
|---|---|---|---|---|
| AMP BR | 3 | 2.9767 | 0.4304 | (--*-) |
| BAW BR | 3 | 3.9067 | 0.3024 | (-*-) |
| DAN BR | 3 | 5.6900 | 0.1664 | (-*-) |
| EBU BR | 3 | 3.8733 | 0.3062 | (--*-) |
| KWAHU | 3 | 6.7600 | 0.2343 | (-*-) |
| TWI PR | 3 | 5.6633 | 0.4798 | (--*-) |
| BAW BR m | 3 | 7.5100 | 0.0265 | (-*-) |
| DAN BR m | 3 | 7.3100 | 0.1212 | (--*-) |

Appendix D2 Anova-Fat

Source DF SS MS F P
Factor 7 615.3138 87.9020 949.27 0.000
Error 16 1.4816 0.0926
Total 23 616.7954

S = 0.3043 R-Sq = 99.76% R-Sq(adj) = 99.65%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ---------+---------+---------+---------+ |
|---|---|---|---|---|
| AMP BR | 3 | 22.260 | 0.201 | (*) |
| BAW BR | 3 | 12.873 | 0.346 | (*) |
| DAN BR | 3 | 16.547 | 0.258 | (*) |
| EBU BR | 3 | 12.873 | 0.346 | (*) |
| KWAHU | 3 | 19.137 | 0.420 | (*) |
| TWI PR | 3 | 17.657 | 0.150 | (*) |
| BAW BR m | 3 | 25.280 | 0.275 | (*) |
| DAN BR m | 3 | 27.527 | 0.346 | (*) |

Appendix D3 Anova-Fiber

Source DF SS MS F P
Factor 7 89.845 12.835 92.31 0.000
Error 16 2.225 0.139
Total 23 92.070

S = 0.3729 R-Sq = 97.58% R-Sq(adj) = 96.53%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | -----+---------+---------+---------+---- |
|---|---|---|---|---|
| AMP BR | 3 | 8.590 | 0.312 | (-*-) |
| BAW BR | 3 | 8.530 | 0.272 | (--*-) |
| DAN BR | 3 | 8.987 | 0.108 | (-*-) |
| EBU BR | 3 | 10.293 | 0.219 | (-*--) |
| KWAHU | 3 | 7.527 | 0.220 | (--*-) |
| TWI PR | 3 | 11.623 | 0.734 | (-*-) |
| BAW BR m | 3 | 7.857 | 0.397 | (-*--) |
| DAN BR m | 3 | 13.537 | 0.369 | (--*-) |

Appendix D4 Anova-Ash

Source DF SS MS F P
Factor 7 0.96413 0.13773 40.61 0.000
Error 16 0.05427 0.00339
Total 23 1.01840

S = 0.05824 R-Sq = 94.67% R-Sq(adj) = 92.34%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ----+---------+---------+---------+----- |
|---|---|---|---|---|
| AMP BR | 3 | 1.4567 | 0.0321 | (---*--) |
| BAW BR | 3 | 1.5567 | 0.0321 | (---*--) |
| DAN BR | 3 | 1.5633 | 0.0513 | (--*---) |
| EBU BR | 3 | 1.6400 | 0.1229 | (---*---) |
| KWAHU | 3 | 1.5467 | 0.0379 | (--*---) |
| TWI PR | 3 | 1.2267 | 0.0709 | (--*---) |
| BAW BR m | 3 | 1.4367 | 0.0252 | (---*--) |
| DAN BR m | 3 | 0.9967 | 0.0153 | (---*--) |

Appendix D5 Anova-Phosporus

Source DF SS MS F P
Factor 7 1749.738 249.963 1380.46 0.000
Error 16 2.897 0.181
Total 23 1752.635

S = 0.4255 R-Sq = 99.83% R-Sq(adj) = 99.76%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ---------+---------+---------+---------+ |
|---|---|---|---|---|
| AMP BR | 3 | 36.600 | 0.163 | (*) |
| BAW BR | 3 | 47.837 | 0.195 | (*) |
| DAN BR | 3 | 25.353 | 0.090 | (*) |
| EBU BR | 3 | 47.685 | 0.163 | *) |
| KWAWU | 3 | 37.135 | 0.091 | (*) |
| T PRASO BR | 3 | 39.831 | 0.075 | *) |
| DAN BR m | 3 | 25.865 | 0.225 | (*) |
| BAW BR m | 3 | 46.988 | 1.134 | (*) |

Appendix D6 Anova-Potassium

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 7 | 999552 | 142793 | 169.84 | 0.000 |
| Error | 16 | 13452 | 841 | | |
| Total | 23 | 1013004 | | | |

S = 29.00 R-Sq = 98.67% R-Sq(adj) = 98.09%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | --+---------+---------+---------+------- |
|---|---|---|---|---|
| AMP BR | 3 | 831.6 | 29.9 | (-*) |
| BAW BR | 3 | 1252.3 | 22.1 | (-*) |
| DAN BR | 3 | 975.8 | 22.0 | (-*-) |
| EBU BR | 3 | 859.3 | 21.3 | (-*-) |
| KWAHU | 3 | 936.4 | 26.0 | (-*-) |
| TWI PR | 3 | 805.2 | 26.4 | (-*-) |
| BAW BR m | 3 | 1017.6 | 34.8 | (-*-) |
| DAN BR m | 3 | 1424.1 | 42.7 | (-*-) |

Appendix D7 Anova-Calcium

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 7 | 2.08892 | 0.29842 | 92.29 | 0.000 |
| Error | 16 | 0.05173 | 0.00323 | | |
| Total | 23 | 2.14065 | | | |

S = 0.05686 R-Sq = 97.58% R-Sq(adj) = 96.53%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ------+---------+---------+---------+--- |
|---|---|---|---|---|
| AMP BR | 3 | 0.7933 | 0.0416 | (-*--) |
| BAW BR | 3 | 1.2667 | 0.0757 | (-*--) |
| DAN BR | 3 | 1.5600 | 0.0400 | (-*-) |
| EBU BR | 3 | 1.0133 | 0.0611 | (--*-) |
| KWAHU | 3 | 1.1333 | 0.0416 | (--*-) |
| TWI PR | 3 | 1.6800 | 0.0346 | (-*-) |
| BAW BR m | 3 | 1.6133 | 0.0416 | (--*-) |
| DAN BR m | 3 | 1.4400 | 0.0917 | (-*-) |

Appendix D8 Anova-Magnesium

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 7 | 848.07 | 121.15 | 18.27 | 0.000 |
| Error | 16 | 106.08 | 6.63 | | |
| Total | 23 | 954.15 | | | |

S = 2.575 R-Sq = 88.88% R-Sq(adj) = 84.02%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ------+---------+---------+---------+--- |
|---|---|---|---|---|
| AMP BR | 3 | 55.067 | 2.996 | (----*---) |
| BAW BR | 3 | 69.500 | 2.921 | (---*----) |
| DAN BR | 3 | 67.833 | 4.484 | (----*---) |
| EBU BR | 3 | 58.667 | 1.604 | (----*---) |
| KWAHU | 3 | 58.767 | 1.007 | (----*---) |
| TWI PR | 3 | 64.033 | 1.405 | (---*----) |
| BAW BR m | 3 | 65.533 | 2.899 | (----*---) |
| DAN BR m | 3 | 74.000 | 1.212 | (----*---) |

Appendix D9 Anova-Zinc

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 7 | 15.46390 | 2.20913 | 260.64 | 0.000 |
| Error | 16 | 0.13561 | 0.00848 | | |
| Total | 23 | 15.59951 | | | |

S = 0.09206 R-Sq = 99.13% R-Sq(adj) = 98.75%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ----+---------+---------+---------+----- |
|---|---|---|---|---|
| AMP BR | 3 | 3.4890 | 0.0830 | (-*) |
| BAW BR | 3 | 4.1053 | 0.1022 | (-*) |
| DAN BR | 3 | 5.5337 | 0.0589 | (-*-) |
| EBU BR | 3 | 3.8447 | 0.0564 | (-*-) |
| KWAHU | 3 | 3.3570 | 0.0756 | (-*-) |
| TWI PR | 3 | 3.8433 | 0.0701 | (-*-) |
| BAW BR m | 3 | 5.5840 | 0.1658 | (-*) |
| DAN BR m | 3 | 4.2397 | 0.0756 | (-*) |

Appendix D10 Anova-Copper

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 7 | 0.764854 | 0.109265 | 355.33 | 0.000 |
| Error | 16 | 0.004920 | 0.000307 | | |
| Total | 23 | 0.769774 | | | |

S = 0.01754 R-Sq = 99.36% R-Sq(adj) = 99.08%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ---------+---------+---------+---------+ |
|---|---|---|---|---|
| AMP BR | 3 | 0.12433 | 0.00862 | (*-) |
| BAW BR | 3 | 0.28533 | 0.01986 | (*) |
| DAN BR | 3 | 0.49033 | 0.03653 | (-*) |
| EBU BR | 3 | 0.10900 | 0.01114 | (*-) |
| KWAHU | 3 | 0.11333 | 0.00833 | (-*) |
| TWI PR | 3 | 0.03500 | 0.00361 | (*-) |
| BAW BR m | 3 | 0.55433 | 0.01940 | (*) |
| DAN BR m | 3 | 0.30267 | 0.00862 | (*-) |

Appendix D11 Anova-Manganese

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 7 | 1.867495 | 0.266785 | 399.88 | 0.000 |
| Error | 16 | 0.010675 | 0.000667 | | |
| Total | 23 | 1.878170 | | | |

S = 0.02583 R-Sq = 99.43% R-Sq(adj) = 99.18%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | +---------+---------+---------+--------- |
|---|---|---|---|---|
| AMP BR | 3 | 0.19833 | 0.00451 | (-*) |
| BAW BR | 3 | 0.74433 | 0.01457 | (*-) |
| DAN BR | 3 | 0.14700 | 0.01229 | (*-) |
| EBU BR | 3 | 0.06867 | 0.00351 | (*-) |
| KWAHU | 3 | 0.03867 | 0.00208 | (-*-) |
| TWI PR | 3 | 0.10133 | 0.00586 | (-*-) |
| BAW BR m | 3 | 0.16533 | 0.01845 | (*-) |
| DAN BR m | 3 | 0.76433 | 0.06755 | (*-) |

Appendix D12 Anova-Sodium

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 7 | 9170.422 | 1310.060 | 18440.74 | 0.000 |
| Error | 16 | 1.137 | 0.071 | | |
| Total | 23 | 9171.558 | | | |

S = 0.2665 R-Sq = 99.99% R-Sq(adj) = 99.98%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | --------+---------+---------+---------+- |
|---|---|---|---|---|
| AMP BR | 3 | 76.316 | 0.141 | * |
| BAW BR | 3 | 107.580 | 0.470 | * |
| DAN BR | 3 | 93.798 | 0.223 | (* |
| EBU BR | 3 | 64.212 | 0.017 | * |
| KWAHU | 3 | 53.856 | 0.105 | * |
| TWI PR | 3 | 48.451 | 0.119 | *) |
| BAW BR m | 3 | 87.024 | 0.222 | * |
| DAN BR m | 3 | 59.575 | 0.451 | (* |

Appendix D13 Anova-Iron

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 7 | 0.2091433 | 0.0298776 | 525.71 | 0.000 |
| Error | 16 | 0.0009093 | 0.0000568 | | |
| Total | 23 | 0.2100526 | | | |

S = 0.007539 R-Sq = 99.57% R-Sq(adj) = 99.38%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | --+---------+---------+---------+------- |
|---|---|---|---|---|
| AMP BR | 3 | 0.29333 | 0.00058 | (*) |
| BAW BR | 3 | 0.31100 | 0.00361 | (*-) |
| DAN BR | 3 | 0.53667 | 0.00777 | (-*) |
| EBU BR | 3 | 0.27700 | 0.00755 | (-*) |
| KWAHU | 3 | 0.49567 | 0.01320 | (-*) |
| TWI PR | 3 | 0.38600 | 0.00954 | (*) |
| BAW BR m | 3 | 0.47967 | 0.00153 | (-*) |
| DAN BR m | 3 | 0.36367 | 0.00751 | (*) |

Analysis of Variance Tables For proximate and mineral composition of Black and Brown tubers for both planting periods

Appendix E1 T-test, Protein

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK | 24 | 4.62 | 1.72 | 0.35 |
| BROWN | 24 | 5.46 | 1.65 | 0.34 |

Difference = mu (BLACK) - mu (BROWN)
Estimate for difference: -0.841667
95% CI for difference: (-1.823841, 0.140508)
T-Test of difference = 0 (vs not =): T-Value = -1.73 P-Value = 0.091 DF = 45

Appendix E2 T-test, Fat

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_1 | 24 | 21.92 | 4.94 | 1.0 |
| BROWN_1 | 24 | 19.27 | 5.18 | 1.1 |

Difference = mu (BLACK_1) - mu (BROWN_1)
Estimate for difference: 2.65167
95% CI for difference: (-0.28968, 5.59301)
T-Test of difference = 0 (vs not =): T-Value = 1.82 P-Value = 0.076 DF = 45

Appendix E3 T-test, Fiber

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_2 | 24 | 9.83 | 1.44 | 0.29 |
| BROWN_2 | 24 | 9.62 | 2.00 | 0.41 |

Difference = mu (BLACK_2) - mu (BROWN_2)
Estimate for difference: 0.215833
95% CI for difference: (-0.801104, 1.232770)
T-Test of difference = 0 (vs not =): T-Value = 0.43 P-Value = 0.670 DF = 41

Appendix E4 T-test, Ash

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_3 | 24 | 1.598 | 0.227 | 0.046 |
| BROWN_3 | 24 | 1.428 | 0.210 | 0.043 |

Difference = mu (BLACK_3) - mu (BROWN_3)
Estimate for difference: 0.170417
95% CI for difference: (0.043053, 0.297780)
T-Test of difference = 0 (vs not =): T-Value = 2.69 P-Value = 0.010 DF = 45

Appendix E5 T-test, Phosphorus

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK | 24 | 33.88 | 6.57 | 1.3 |
| BROWN | 24 | 38.41 | 8.73 | 1.8 |

Difference = mu (BLACK) - mu (BROWN)
Estimate for difference: -4.53429
95% CI for difference: (-9.03533, -0.03325)

T-Test of difference = 0 (vs not =): T-Value = -2.03  P-Value = 0.048  DF = 42

Appendix E6 T-test, Potassium

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_1 | 24 | 985 | 184 | 37 |
| BROWN_1 | 24 | 1013 | 210 | 43 |

Difference = mu (BLACK_1) - mu (BROWN_1)
Estimate for difference:  -27.8782
95% CI for difference:  (-142.5410, 86.7847)
T-Test of difference = 0 (vs not =): T-Value = -0.49  P-Value = 0.627  DF = 45

Appendix E7 T-test, Calcium

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_2 | 24 | 1.181 | 0.289 | 0.059 |
| BROWN_2 | 24 | 1.313 | 0.305 | 0.062 |

Difference = mu (BLACK_2) - mu (BROWN_2)
Estimate for difference:  -0.131667
95% CI for difference:  (-0.304490, 0.041156)
T-Test of difference = 0 (vs not =): T-Value = -1.53  P-Value = 0.132  DF = 45

Appendix E8 T-test, Magnusium

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_3 | 24 | 65.25 | 7.26 | 1.5 |
| BROWN_3 | 24 | 64.18 | 6.44 | 1.3 |

Difference = mu (BLACK_3) - mu (BROWN_3)
Estimate for difference:  1.07500
95% CI for difference:  (-2.91381, 5.06381)
T-Test of difference = 0 (vs not =): T-Value = 0.54  P-Value = 0.590  DF = 45

Appendix E9 T-test, Zinc

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_4 | 24 | 4.23 | 1.08 | 0.22 |
| BLACK_4 | 24 | 4.23 | 1.08 | 0.22 |

Difference = mu (BLACK_4) - mu (BLACK_4)
Estimate for difference:  0.000000
95% CI for difference:  (-0.627436, 0.627436)
T-Test of difference = 0 (vs not =): T-Value = 0.00  P-Value = 1.000  DF = 46

Appendix E10 T-test Copper

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_5 | 24 | 0.302 | 0.150 | 0.031 |
| BROWN_5 | 24 | 0.252 | 0.183 | 0.037 |

Difference = mu (BLACK_5) - mu (BROWN_5)
Estimate for difference:  0.049750
95% CI for difference:  (-0.047580, 0.147080)
T-Test of difference = 0 (vs not =): T-Value = 1.03  P-Value = 0.309  DF = 44

Appendix E11 T-test, Zinc

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_4 | 24 | 4.23 | 1.08 | 0.22 |
| BROWN_4 | 24 | 4.250 | 0.824 | 0.17 |

Difference = mu (BLACK_4) - mu (BROWN_4)
Estimate for difference: -0.016625
95% CI for difference: (-0.576041, 0.542791)
T-Test of difference = 0 (vs not =): T-Value = -0.06 P-Value = 0.952 DF = 42

Appendix E12 T-Test for Black, Brown Sodium For All Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_7 | 24 | 70.0 | 15.8 | 3.2 |
| BROWN_7 | 24 | 73.9 | 20.0 | 4.1 |

Difference = mu (BLACK_7) - mu (BROWN_7)
Estimate for difference: -3.84337
95% CI for difference: (-14.32066, 6.63391)
T-Test of difference = 0 (vs not =): T-Value = -0.74 P-Value = 0.463 DF = 43

Appendix E13 T-Test for Black, Brown Iron For All Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_8 | 24 | 0.4144 | 0.0929 | 0.019 |
| BROWN_8 | 24 | 0.3929 | 0.0956 | 0.020 |

Difference = mu (BLACK_8) - mu (BROWN_8)
Estimate for difference: 0.021542
95% CI for difference: (-0.033261, 0.076344)
T-Test of difference = 0 (vs not =): T-Value = 0.79 P-Value = 0.433 DF = 45

Appendix E14 T-Test for Black, Brown Carbohydrate For All Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| CARB BLK | 8 | 62.05 | 7.05 | 2.5 |
| CARB BRN | 8 | 64.29 | 7.30 | 2.6 |

Difference = mu (CARB BLK) - mu (CARB BRN)
Estimate for difference: -2.23500
95% CI for difference: (-9.98522, 5.51522)
T-Test of difference = 0 (vs not =): T-Value = -0.62 P-Value = 0.544 DF = 13

Appendix E15 T-Test for Black, Brown Energy Value For All Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| EV BLK | 8 | 470.8 | 23.6 | 8.4 |
| EV BRN | 8 | 458.5 | 24.2 | 8.5 |

Difference = mu (EV BLK) - mu (EV BRN)
Estimate for difference: 12.2375
95% CI for difference: (-13.5910, 38.0660)
T-Test of difference = 0 (vs not =): T-Value = 1.02 P-Value = 0.325 DF = 13

Appendix E16 T-Test for Black, Brown Protein For Major Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK | 18 | 3.721 | 0.773 | 0.18 |
| BROWN | 18 | 4.81 | 1.38 | 0.33 |

Difference = mu (BLACK) - mu (BROWN)
Estimate for difference: -1.09056
95% CI for difference: (-1.85758, -0.32354)
T-Test of difference = 0 (vs not =): T-Value = -2.92 P-Value = 0.007 DF = 26

Appendix E17 T-Test for Black, Brown Fat For Major Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_1 | 18 | 20.07 | 4.12 | 0.97 |
| BROWN_1 | 18 | 16.89 | 3.44 | 0.81 |

Difference = mu (BLACK_1) - mu (BROWN_1)
Estimate for difference: 3.17944
95% CI for difference: (0.60283, 5.75606)
T-Test of difference = 0 (vs not =): T-Value = 2.51 P-Value = 0.017 DF = 32

Appendix E18 T-Test For Black, Brown Fiber For Major Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_2 | 18 | 9.40 | 1.32 | 0.31 |
| BROWN_2 | 18 | 9.26 | 1.41 | 0.33 |

Difference = mu (BLACK_2) - mu (BROWN_2)
Estimate for difference: 0.145556
95% CI for difference: (-0.779224, 1.070335)
T-Test of difference = 0 (vs not =): T-Value = 0.32 P-Value = 0.751 DF = 33

Appendix E19 T-Test for Black, Brown Ash For Major Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_3 | 18 | 1.585 | 0.253 | 0.060 |
| BROWN_3 | 18 | 1.498 | 0.147 | 0.035 |

Difference = mu (BLACK_3) - mu (BROWN_3)
Estimate for difference: 0.086667
95% CI for difference: (-0.055086, 0.228419)
T-Test of difference = 0 (vs not =): T-Value = 1.25 P-Value = 0.220 DF = 27

Appendix E20 T-Test for Black, Brown Carbohydrate for Major Period

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| CARB BLK | 6 | 65.22 | 4.28 | 1.7 |
| CARB BRN | 6 | 67.60 | 4.06 | 1.7 |

Difference = mu (CARB BLK) - mu (CARB BRN)
Estimate for difference: -2.38500
95% CI for difference: (-7.83600, 3.06600)
T-Test of difference = 0 (vs not =): T-Value = -0.99 P-Value = 0.348 DF = 9

Appendix E21 T-Test for Black, Brown Energy Value for Major Period

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| EV BLK | 6 | 465.4 | 25.0 | 10 |
| EV BRN | 6 | 449.9 | 21.1 | 8.6 |

Difference = mu (EV BLK) - mu (EV BRN)
Estimate for difference: 15.5800
95% CI for difference: (-14.6602, 45.8202)
T-Test of difference = 0 (vs not =): T-Value = 1.17 P-Value = 0.274 DF = 9

Appendix E22 T-Test for Black, Brown Phosphorus for Major Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK | 18 | 34.40 | 7.19 | 1.7 |
| BROWN | 18 | 39.07 | 7.86 | 1.9 |

Difference = mu (BLACK) - mu (BROWN)
Estimate for difference: -4.67856
95% CI for difference: (-9.78418, 0.42707)
T-Test of difference = 0 (vs not =): T-Value = -1.86 P-Value = 0.071 DF = 33

Appendix E23 T-Test for Black, Brown Potassium for Major Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_1 | 18 | 933 | 165 | 39 |
| BROWN_1 | 18 | 943 | 156 | 37 |

Difference = mu (BLACK_1) - mu (BROWN_1)
Estimate for difference: -10.4764
95% CI for difference: (-119.4379, 98.4850)
T-Test of difference = 0 (vs not =): T-Value = -0.20 P-Value = 0.846 DF = 33

Appendix E24 T-Test for Black, Brown Calcium for Major Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_2 | 18 | 1.107 | 0.279 | 0.066 |
| BROWN_2 | 18 | 1.241 | 0.317 | 0.075 |

Difference = mu (BLACK_2) - mu (BROWN_2)
Estimate for difference: -0.134444
95% CI for difference: (-0.336949, 0.068060)
T-Test of difference = 0 (vs not =): T-Value = -1.35 P-Value = 0.186 DF = 33

Appendix E25 T-Test for Black, Brown Magnessium for Major Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_3 | 18 | 62.99 | 5.84 | 1.4 |
| BROWN_3 | 18 | 62.31 | 5.82 | 1.4 |

Difference = mu (BLACK_3) - mu (BROWN_3)
Estimate for difference: 0.677778

95% CI for difference: (-3.279030, 4.634585)
T-Test of difference = 0 (vs not =): T-Value = 0.35 P-Value = 0.730 DF = 33

Appendix E26 T-Test for Black, Brown Zinc for Major Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_4 | 18 | 4.10 | 1.22 | 0.29 |
| BROWN_4 | 18 | 4.029 | 0.740 | 0.17 |

Difference = mu (BLACK_4) - mu (BROWN_4)
Estimate for difference: 0.069667
95% CI for difference: (-0.622079, 0.761413)
T-Test of difference = 0 (vs not =): T-Value = 0.21 P-Value = 0.838 DF = 27

Appendix E27 T-Test for Black, Brown Copper for Major Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_5 | 18 | 0.290 | 0.151 | 0.036 |
| BROWN_5 | 18 | 0.193 | 0.158 | 0.037 |

Difference = mu (BLACK_5) - mu (BROWN_5)
Estimate for difference: 0.097333
95% CI for difference: (-0.007373, 0.202040)
T-Test of difference = 0 (vs not =): T-Value = 1.89 P-Value = 0.067 DF = 33

Appendix E28 T-Test for Black, Brown Maganese For Major Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_6 | 18 | 0.230 | 0.325 | 0.077 |
| BROWN_6 | 18 | 0.216 | 0.249 | 0.059 |

Difference = mu (BLACK_6) - mu (BROWN_6)
Estimate for difference: 0.013333
95% CI for difference: (-0.183547, 0.210214)
T-Test of difference = 0 (vs not =): T-Value = 0.14 P-Value = 0.891 DF = 31

Appendix E29 T-Test for Black, Brown Sodium for Major Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_7 | 18 | 70.3 | 18.3 | 4.3 |
| BROWN_7 | 18 | 74.0 | 21.7 | 5.1 |

Difference = mu (BLACK_7) - mu (BROWN_7)
Estimate for difference: -3.73772
95% CI for difference: (-17.37804, 9.90260)
T-Test of difference = 0 (vs not =): T-Value = -0.56 P-Value = 0.581 DF = 33

Appendix E30 T-Test for Black, Brown Iron for Major Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_8 | 18 | 0.411 | 0.107 | 0.025 |
| BROWN_8 | 18 | 0.383 | 0.104 | 0.024 |

Difference = mu (BLACK_8) - mu (BROWN_8)

Estimate for difference: 0.027333
95% CI for difference: (-0.044165, 0.098831)
T-Test of difference = 0 (vs not =): T-Value = 0.78 P-Value = 0.442 DF = 33

Appendix E31 T-Test for Black, Brown Protein for Minor Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK | 6 | 7.315 | 0.114 | 0.046 |
| BROWN | 6 | 7.410 | 0.135 | 0.055 |

Difference = mu (BLACK) - mu (BROWN)
Estimate for difference: -0.095000
95% CI for difference: (-0.257786, 0.067786)
T-Test of difference = 0 (vs not =): T-Value = -1.32 P-Value = 0.219 DF = 9

Appendix E32 T-Test for Black, Brown Fat for Minor Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_1 | 6 | 27.47 | 2.28 | 0.93 |
| BROWN_1 | 6 | 26.40 | 1.26 | 0.52 |

Difference = mu (BLACK_1) - mu (BROWN_1)
Estimate for difference: 1.06833
95% CI for difference: (-1.44718, 3.58385)
T-Test of difference = 0 (vs not =): T-Value = 1.00 P-Value = 0.349 DF = 7

Appendix E33 T-Test for Black, Brown Fiber for Minor Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_2 | 6 | 11.12 | 1.01 | 0.41 |
| BROWN_2 | 6 | 10.70 | 3.13 | 1.3 |

Difference = mu (BLACK_2) - mu (BROWN_2)
Estimate for difference: 0.426667
95% CI for difference: (-2.860125, 3.713458)
T-Test of difference = 0 (vs not =): T-Value = 0.32 P-Value = 0.762 DF = 6

Appendix E34 T-Test for Black, Brown Ash for Minor Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_3 | 6 | 1.638 | 0.130 | 0.053 |
| BROWN_3 | 6 | 1.217 | 0.242 | 0.099 |

Difference = mu (BLACK_3) - mu (BROWN_3)
Estimate for difference: 0.421667
95% CI for difference: (0.156584, 0.686750)
T-Test of difference = 0 (vs not =): T-Value = 3.76 P-Value = 0.007 DF = 7

Appendix E35 T-Test for Black, Brown Phosphorus For Minor Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|

BLACK 6 32.33 4.38 1.8
BROWN 6 36.4 11.6 4.7
Difference = mu (BLACK) - mu (BROWN)
Estimate for difference: -4.10150
95% CI for difference: (-16.48226, 8.27926)
T-Test of difference = 0 (vs not =): T-Value = -0.81 P-Value = 0.449 DF = 6

Appendix E36 T-Test For Black, Brown Potassium For Minor Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_1 | 6 | 1141 | 153 | 62 |
| BROWN_1 | 6 | 1221 | 225 | 92 |

Difference = mu (BLACK_1) - mu (BROWN_1)
Estimate for difference: -80.0833
95% CI for difference: (-336.5206, 176.3540)
T-Test of difference = 0 (vs not =): T-Value = -0.72 P-Value = 0.492 DF = 8

Appendix E37 T-Test For Black, Brown Calcium For Minor Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_2 | 6 | 1.403 | 0.202 | 0.083 |
| BROWN_2 | 6 | 1.527 | 0.114 | 0.047 |

Difference = mu (BLACK_2) - mu (BROWN_2)
Estimate for difference: -0.123333
95% CI for difference: (-0.347523, 0.100856)
T-Test of difference = 0 (vs not =): T-Value = -1.30 P-Value = 0.234 DF = 7

Appendix E38 T-Test for Black, Brown Magnesium For Minor Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_3 | 6 | 72.03 | 7.24 | 3.0 |
| BROWN_3 | 6 | 69.77 | 5.05 | 2.1 |

Difference = mu (BLACK_3) - mu (BROWN_3)
Estimate for difference: 2.26667
95% CI for difference: (-6.04100, 10.57433)
T-Test of difference = 0 (vs not =): T-Value = 0.63 P-Value = 0.547 DF = 8

Appendix E39 T-Test for Black, Brown Zinc For Minor Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_4 | 6 | 4.6363 | 0.0985 | 0.040 |
| BROWN_4 | 6 | 4.912 | 0.745 | 0.30 |

Difference = mu (BLACK_4) - mu (BROWN_4)
Estimate for difference: -0.275500
95% CI for difference: (-1.064432, 0.513432)

T-Test of difference = 0 (vs not =): T-Value = -0.90  P-Value = 0.411  DF = 5

Appendix E40 T-Test for Black, Brown Copper For Minor Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_5 | 6 | 0.336 | 0.156 | 0.064 |
| BROWN_5 | 6 | 0.429 | 0.138 | 0.057 |

Difference = mu (BLACK_5) - mu (BROWN_5)
Estimate for difference:  -0.093000
95% CI for difference:  (-0.285799, 0.099799)
T-Test of difference = 0 (vs not =): T-Value = -1.09  P-Value = 0.304  DF = 9



Appendix E41 T-Test for Black, Brown Manganese For Minor Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_6 | 6 | 0.1623 | 0.0417 | 0.017 |
| BROWN_6 | 6 | 0.465 | 0.331 | 0.14 |

Difference = mu (BLACK_6) - mu (BROWN_6)
Estimate for difference:  -0.302500
95% CI for difference:  (-0.652676, 0.047676)
T-Test of difference = 0 (vs not =): T-Value = -2.22  P-Value = 0.077  DF = 5

Appendix E42 T-Test for Black, Brown Sodium For Minor Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_7 | 6 | 69.14 | 1.02 | 0.42 |
| BROWN_7 | 6 | 73.3 | 15.0 | 6.1 |

Difference = mu (BLACK_7) - mu (BROWN_7)
Estimate for difference:  -4.16033
95% CI for difference:  (-19.97794, 11.65727)
T-Test of difference = 0 (vs not =): T-Value = -0.68  P-Value = 0.529  DF = 5

Appendix E43 T-Test for Black, Brown Iron For Minor Periods

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| BLACK_8 | 6 | 0.4258 | 0.0236 | 0.0096 |
| BROWN_8 | 6 | 0.4217 | 0.0637 | 0.026 |

Difference = mu (BLACK_8) - mu (BROWN_8)
Estimate for difference:  0.004167
95% CI for difference:  (-0.063712, 0.072045)
T-Test of difference = 0 (vs not =): T-Value = 0.15  P-Value = 0.886  DF = 6

Appendix E44 T-test For Black, Brown Carbohydrate for Minor Period

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| CARB BLK | 2 | 52.56 | 3.99 | 2.8 |

CARB BRN 2 54.35 5.17 3.7
Difference = mu (CARB BLK) - mu (CARB BRN)
Estimate for difference: -1.78500
95% CI for difference: (-60.44230, 56.87230)
T-Test of difference = 0 (vs not =): T-Value = -0.39 P-Value = 0.765 DF = 1

Appendix E45 T-Test for Black, Brown Evergy Value For Minor Period

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| EV BLK | 2 | 486.8 | 10.2 | 7.2 |
| EV BRN | 2 | 484.57 | 7.06 | 5.0 |

Difference = mu (EV BLK) - mu (EV BRN)
Estimate for difference: 2.21000
95% CI for difference: (-109.18657, 113.60657)
T-Test of difference = 0 (vs not =): T-Value = 0.25 P-Value = 0.843 DF = 1

Appendix F HIGH FIBER CHOCOLATE
Appendix F1 ANOVA-Total dietery fiber

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 4 | 0.628467 | 0.157117 | 222.33 | 0.000 |
| Error | 10 | 0.007067 | 0.000707 | | |
| Total | 14 | 0.635533 | | | |

S = 0.02658 R-Sq = 98.89% R-Sq(adj) = 98.44%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | --+---------+---------+---------+------- |
|---|---|---|---|---|
| 0 | 3 | 0.9700 | 0.0200 | (--*-) |
| 1 | 3 | 1.1500 | 0.0361 | (-*-) |
| 2 | 3 | 1.2733 | 0.0153 | (--*-) |
| 3 | 3 | 1.4500 | 0.0300 | (--*-) |
| 4 | 3 | 1.5400 | 0.0265 | (-*-) |

Appendix F2 ANOVA-Fat

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 4 | 25.961 | 6.490 | 57.36 | 0.000 |
| Error | 10 | 1.132 | 0.113 | | |
| Total | 14 | 27.092 | | | |

S = 0.3364 R-Sq = 95.82% R-Sq(adj) = 94.15%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ---+---------+---------+---------+------ |
|---|---|---|---|---|
| 0 | 3 | 32.430 | 0.409 | (--*---) |

1 3 33.570 0.135 (---*--)
2 3 34.603 0.382 (--*---)
3 3 35.330 0.427 (--*---)
4 3 36.183 0.227 (---*--)

Appendix F3 ANOVA-Ash

Source DF SS MS F P
Factor 4 0.051333 0.012833 53.47 0.000
Error 10 0.002400 0.000240
Total 14 0.053733

S = 0.01549 R-Sq = 95.53% R-Sq(adj) = 93.75%

Individual 95% CIs For Mean Based on Pooled StDev

Level N Mean StDev -+---------+---------+---------+--------
0 3 1.4567 0.0208 (---*--)
1 3 1.5533 0.0153 (--*--)
2 3 1.5800 0.0173 (--*---)
3 3 1.6000 0.0100 (---*--)
4 3 1.6267 0.0115 (--*--)

Appendix F4 ANOVA-Moisture

Source DF SS MS F P
Factor 4 0.028440 0.007110 8.89 0.003
Error 10 0.008000 0.000800
Total 14 0.036440

S = 0.02828 R-Sq = 78.05% R-Sq(adj) = 69.26%

Individual 95% CIs For Mean Based on Pooled StDev

Level N Mean StDev -------+---------+---------+---------+--
0 3 2.1500 0.0200 (------*------)
1 3 2.1500 0.0361 (------*------)
2 3 2.0800 0.0173 (------*------)
3 3 2.0600 0.0265 (------*------)
4 3 2.0500 0.0361 (------*------)

Appendix F5 ANOVA-Protein

Source DF SS MS F P
Factor 4 0.324493 0.081123 138.28 0.000
Error 10 0.005867 0.000587
Total 14 0.330360

S = 0.02422 R-Sq = 98.22% R-Sq(adj) = 97.51%

Individual 95% CIs For Mean Based on

Pooled StDev

| Level | N | Mean | StDev | ---+---------+---------+---------+------ |
|---|---|---|---|---|
| 0 | 3 | 10.0333 | 0.0153 | (-*-) |
| 1 | 3 | 10.1500 | 0.0300 | (-*-) |
| 2 | 3 | 10.2467 | 0.0208 | (-*-) |
| 3 | 3 | 10.3267 | 0.0208 | (-*--) |
| 4 | 3 | 10.4633 | 0.0306 | (--*-) |

ANOVA HIGH FIBER SPREAD

Appendix G1 ANOVA FOR Moisture

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 4 | 0.20247 | 0.05062 | 7.41 | 0.005 |
| Error | 10 | 0.06827 | 0.00683 | | |
| Total | 14 | 0.27073 | | | |

S = 0.08262 R-Sq = 74.78% R-Sq(adj) = 64.70%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ---------+---------+---------+---------+ |
|---|---|---|---|---|
| 0 | 3 | 1.5433 | 0.0231 | (------*------) |
| 1 | 3 | 1.3167 | 0.1721 | (------*------) |
| 2 | 3 | 1.6467 | 0.0513 | (------*------) |
| 3 | 3 | 1.5600 | 0.0265 | (------*------) |
| 4 | 3 | 1.4167 | 0.0252 | (------*-------) |

Appendix G2 ANOVA-Fat

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 4 | 19.95763 | 4.98941 | 540.37 | 0.000 |
| Error | 10 | 0.09233 | 0.00923 | | |
| Total | 14 | 20.04996 | | | |

S = 0.09609 R-Sq = 99.54% R-Sq(adj) = 99.36%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | --------+---------+---------+---------+- |
|---|---|---|---|---|
| 0 | 3 | 40.3700 | 0.0964 | (-*) |
| 1 | 3 | 41.5700 | 0.1353 | (-*) |
| 2 | 3 | 43.5400 | 0.0985 | (*-) |
| 3 | 3 | 42.8633 | 0.0764 | (-*) |
| 4 | 3 | 43.0367 | 0.0551 | (*-) |

Appendix G3 ANOVA-Protein

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 4 | 0.5760 | 0.1440 | 6.55 | 0.007 |

Error 10 0.2200 0.0220
Total 14 0.7960
S = 0.1483 R-Sq = 72.36% R-Sq(adj) = 61.31%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | --------+---------+---------+---------+- |
|---|---|---|---|---|
| 0 | 3 | 2.3333 | 0.1528 | (------*-------) |
| 1 | 3 | 2.5333 | 0.2517 | (------*-------) |
| 2 | 3 | 2.8000 | 0.1000 | (-------*-------) |
| 3 | 3 | 2.2333 | 0.0577 | (------*-------) |
| 4 | 3 | 2.4000 | 0.1000 | (-------*-------) |

Appendix G4 ANOVA-Ash

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 4 | 0.038333 | 0.009583 | 35.94 | 0.000 |
| Error | 10 | 0.002667 | 0.000267 | | |
| Total | 14 | 0.041000 | | | |

S = 0.01633 R-Sq = 93.50% R-Sq(adj) = 90.89%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ---+---------+---------+---------+------ |
|---|---|---|---|---|
| 0 | 3 | 1.4567 | 0.0208 | (---*----) |
| 1 | 3 | 1.5533 | 0.0153 | (----*---) |
| 2 | 3 | 1.5800 | 0.0173 | (---*---) |
| 3 | 3 | 1.4833 | 0.0153 | (----*---) |
| 4 | 3 | 1.5767 | 0.0115 | (---*----) |

Appendix G5 ANOVA-Total dietery fiber

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 4 | 86.94033 | 21.73508 | 10416.17 | 0.000 |
| Error | 10 | 0.02087 | 0.00209 | | |
| Total | 14 | 86.96120 | | | |

S = 0.04568 R-Sq = 99.98% R-Sq(adj) = 99.97%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ---+---------+---------+---------+------ |
|---|---|---|---|---|
| 0 | 3 | 3.5433 | 0.0231 | (* |
| 1 | 3 | 6.4167 | 0.0252 | * |
| 2 | 3 | 9.9467 | 0.0513 | (* |
| 3 | 3 | 6.6200 | 0.0600 | * |
| 4 | 3 | 9.8733 | 0.0551 | *) |

Appendix G6 ANOVA-Sucrose

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 4 | 170.8093 | 42.7023 | 4270.23 | 0.000 |
| Error | 10 | 0.1000 | 0.0100 | | |
| Total | 14 | 170.9093 | | | |

S = 0.1 R-Sq = 99.94% R-Sq(adj) = 99.92%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ---------+---------+---------+---------+ |
|---|---|---|---|---|
| 0 | 3 | 34.833 | 0.115 | *) |
| 1 | 3 | 30.100 | 0.100 | *) |
| 2 | 3 | 25.467 | 0.058 | (* |
| 3 | 3 | 29.333 | 0.115 | *) |
| 4 | 3 | 33.900 | 0.100 | (* |

Appendix G7 ANOVA-Reducing sugar

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 4 | 3.04933 | 0.76233 | 163.36 | 0.000 |
| Error | 10 | 0.04667 | 0.00467 | | |
| Total | 14 | 3.09600 | | | |

S = 0.06831 R-Sq = 98.49% R-Sq(adj) = 97.89%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | -+---------+---------+---------+-------- |
|---|---|---|---|---|
| 0 | 3 | 3.2333 | 0.0577 | (-*--) |
| 1 | 3 | 3.2667 | 0.0577 | (-*--) |
| 2 | 3 | 2.8000 | 0.1000 | (--*--) |
| 3 | 3 | 2.1667 | 0.0577 | (--*-) |
| 4 | 3 | 2.3333 | 0.0577 | (--*-) |

Appendix G8 ANOVA-Total sugar

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 4 | 139.0573 | 34.7643 | 5794.06 | 0.000 |
| Error | 10 | 0.0600 | 0.0060 | | |
| Total | 14 | 139.1173 | | | |

S = 0.07746 R-Sq = 99.96% R-Sq(adj) = 99.94%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | -------+---------+---------+---------+-- |
|---|---|---|---|---|

0 3 37.1000 0.1000 *)
1 3 33.4000 0.1000 (*
2 3 28.2667 0.0577 *
3 3 31.5667 0.0577 *)
4 3 35.2333 0.0577 *

MILK EXTRACTION

Appendix H1 Anova- Milk Solids For Black Tubers

Source DF SS MS F P
Factor 5 5.55860 1.11172 125.38 0.000
Error 12 0.10640 0.00887
Total 17 5.66500

S = 0.09416 R-Sq = 98.12% R-Sq(adj) = 97.34%

Individual 95% CIs For Mean Based on Pooled StDev

Level N Mean StDev -+---------+---------+---------+--------
TAM BK 3 16.2433 0.0723 (--*-)
AMP BK 3 15.9400 0.1253 (--*-)
EBU BK 3 15.7100 0.0700 (-*--)
BAW BK 3 15.5233 0.0058 (-*--)
DAN BK 3 15.0100 0.1389 (-*--)
TAN BK 3 14.5933 0.0896 (--*-)

Appendix H2 Anova- Expected Milk Solids For Black Tubers

Source DF SS MS F P
Factor 5 261.7811 52.3562 819.85 0.000
Error 12 0.7663 0.0639
Total 17 262.5475

S = 0.2527 R-Sq = 99.71% R-Sq(adj) = 99.59%

Individual 95% CIs For Mean Based on Pooled StDev

Level N Mean StDev ---+---------+---------+---------+------
TAM BK 3 46.400 0.225 (*
TAN BK 3 43.650 0.252 (*)
AMP BK 3 43.140 0.407 (*)
EBU BK 3 41.810 0.184 *)
BAW BK 3 40.163 0.146 (*)
DAN BK 3 34.147 0.218 (*

Appendix H3 Anova- Milk Extract For Black Tubers

Source DF SS MS F P
Factor 5 9050.785 1810.157 3819.84 0.000
Error 12 5.687 0.474
Total 17 9056.472

S = 0.6884 R-Sq = 99.94% R-Sq(adj) = 99.91%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ----------+---------+---------+---------+-- |
|---|---|---|---|---|
| TAN BK | 3 | 299.117 | 0.126 | (* |
| TAM BK | 3 | 285.667 | 0.208 | (* |
| AMP BK | 3 | 270.633 | 0.416 | *) |
| EBU BK | 3 | 266.133 | 0.351 | * |
| BAW BK | 3 | 258.717 | 0.978 | *) |
| DAN BK | 3 | 227.503 | 1.238 | (* |

Appendix H4 ANOVA- Milk Solids For Brown Tubers

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 5 | 23.9804 | 4.7961 | 464.89 | 0.000 |
| Error | 12 | 0.1238 | 0.0103 | | |
| Total | 17 | 24.1042 | | | |

S = 0.1016 R-Sq = 99.49% R-Sq(adj) = 99.27%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ----+---------+---------+---------+----- |
|---|---|---|---|---|
| TWIFO | 3 | 17.183 | 0.142 | (*) |
| KWAHU | 3 | 16.397 | 0.095 | (*) |
| EBU BR | 3 | 15.210 | 0.072 | (*) |
| BAW BR | 3 | 14.720 | 0.087 | (*) |
| AMP BR | 3 | 14.573 | 0.095 | (-*) |
| DAN BR | 3 | 13.760 | 0.105 | (-*) |

Appendix H5 ANOVA- Expected Milk Solids For Brown Tubers

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 5 | 299.588 | 59.918 | 462.01 | 0.000 |
| Error | 12 | 1.556 | 0.130 | | |
| Total | 17 | 301.144 | | | |

S = 0.3601 R-Sq = 99.48% R-Sq(adj) = 99.27%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ----+---------+---------+---------+----- |
|---|---|---|---|---|
| KWAHU | 3 | 45.713 | 0.220 | (-*) |
| TWIFO | 3 | 44.897 | 0.414 | (*-) |
| EBU BR | 3 | 39.967 | 0.332 | (*) |
| BAW BR | 3 | 39.037 | 0.303 | (-*) |
| DAN BR | 3 | 37.450 | 0.550 | (*) |
| AMP BR | 3 | 34.000 | 0.233 | (*) |

Appendix H6 ANOVA- Milk Extract For Brown Tubers

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|

Factor 5 3661.64 732.33 664.67 0.000
Error 12 13.22 1.10
Total 17 3674.86
S = 1.050 R-Sq = 99.64% R-Sq(adj) = 99.49%

Individual 95% CIs For Mean Based on Pooled StDev

Level N Mean StDev -----+---------+---------+---------+----
KWAHU 3 278.80 0.25 (*)
DAN BR 3 272.18 1.92 *)
BAW BR 3 265.22 1.25 (*)
EBU BR 3 262.77 1.08 (*)
TWIFO 3 261.28 0.26 (*)
AMP BR 3 233.30 0.23 (*



Appendix H7-calculation of expected milk extracted (EMS)

Expected milk solids = Milk solids (MS) x milk extracted
Expected milk solids (EMS) =
MS x (milk extracted/weight of tubers)
EMS = MS x ME

SENSORY EVALUATION OF TIGER NUT CHOCOLATE DRINK DEVELOPED WITH MIXTURE DESIGN

Appendix I1 ANOVA-Appearance

Source DF SS MS F P
Factor 14 80.25 5.73 1.32 0.213
Error 90 391.71 4.35
Total 104 471.96
S = 2.086 R-Sq = 17.00% R-Sq(adj) = 4.09%

Individual 95% CIs For Mean Based on Pooled StDev

Level N Mean StDev -----+---------+---------+---------+----
C1 7 5.714 2.215 (---------*---------)
C2 7 4.714 1.254 (---------*---------)
C3 7 6.571 2.760 (---------*---------)
C4 7 4.714 1.890 (---------*---------)
C5 7 6.286 1.704 (---------*---------)
C6 7 6.714 0.488 (---------*---------)
C7 7 5.429 2.225 (---------*---------)
C8 7 5.857 2.545 (---------*---------)
C9 7 4.000 2.449 (---------*---------)
C10 7 6.571 1.718 (---------*---------)
C11 7 4.857 2.968 (---------*---------)
C12 7 5.714 1.976 (---------*---------)
C13 7 7.000 1.633 (---------*---------)

C14 7 5.714 2.430 (---------*---------)
C15 7 6.857 1.676 (---------*---------)

Appendix I2 ANOVA-Mouth feel

Source DF SS MS F P
Factor 14 163.10 11.65 2.54 0.004
Error 90 412.29 4.58
Total 104 575.39

S = 2.140 R-Sq = 28.35% R-Sq(adj) = 17.20%

Individual 95% CIs For Mean Based on Pooled StDev

Level N Mean StDev ---+---------+---------+---------+------
C1 7 6.714 1.496 (-------*-------)
C2 7 4.429 2.440 (-------*-------)
C3 7 6.143 2.116 (-------*-------)
C4 7 3.857 2.116 (-------*-------)
C5 7 3.000 1.528 (-------*-------)
C6 7 7.000 1.915 (-------*-------)
C7 7 5.000 2.828 (-------*-------)
C8 7 6.000 2.309 (-------*-------)
C9 7 3.571 2.225 (-------*-------)
C10 7 6.857 1.574 (-------*-------)
C11 7 6.000 2.646 (-------*-------)
C12 7 6.429 1.813 (-------*-------)
C13 7 6.571 1.988 (-------*-------)
C14 7 6.000 2.000 (-------*-------)
C15 7 6.286 2.563 (-------*-------)

Appendix I3 ANOVA-Flavor

Source DF SS MS F P
Factor 14 183.94 13.14 3.39 0.000
Error 90 348.86 3.88
Total 104 532.80

S = 1.969 R-Sq = 34.52% R-Sq(adj) = 24.34%

Individual 95% CIs For Mean Based on Pooled StDev

Level N Mean StDev --+---------+---------+---------+-------
C1 7 6.571 2.699 (-------*------)
C2 7 5.000 2.380 (------*------)
C3 7 7.714 0.951 (-------*------)
C4 7 4.143 2.193 (-------*------)
C5 7 3.714 1.890 (-------*------)
C6 7 5.429 2.440 (------*-------)
C7 7 6.571 2.699 (-------*------)
C8 7 7.143 1.345 (-------*------)
C9 7 3.143 1.574 (-------*------)
C10 7 6.714 1.704 (-------*------)

C11 7 4.714 2.360 (-------*------)
C12 7 6.571 0.535 (-------*------)
C13 7 6.571 1.988 (-------*------)
C14 7 7.000 0.816 (------*------)
C15 7 6.000 2.236 (------*------)

Appendix I4 ANOVA-After-Taste

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 14 | 209.90 | 14.99 | 3.02 | 0.001 |
| Error | 90 | 446.86 | 4.97 | | |
| Total | 104 | 656.76 | | | |

S = 2.228 R-Sq = 31.96% R-Sq(adj) = 21.38%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ------+---------+---------+---------+--- |
|---|---|---|---|---|
| C1 | 7 | 6.857 | 2.035 | (-------*--------) |
| C2 | 7 | 4.000 | 1.414 | (-------*-------) |
| C3 | 7 | 6.429 | 1.813 | (-------*--------) |
| C4 | 7 | 3.714 | 2.498 | (--------*-------) |
| C5 | 7 | 2.571 | 0.976 | (--------*-------) |
| C6 | 7 | 5.429 | 2.149 | (-------*--------) |
| C7 | 7 | 6.714 | 2.752 | (--------*-------) |
| C8 | 7 | 6.714 | 2.289 | (--------*-------) |
| C9 | 7 | 2.857 | 1.864 | (-------*--------) |
| C10 | 7 | 6.714 | 2.215 | (--------*-------) |
| C11 | 7 | 5.143 | 2.545 | (--------*-------) |
| C12 | 7 | 6.143 | 1.864 | (--------*-------) |
| C13 | 7 | 6.571 | 2.225 | (--------*-------) |
| C14 | 7 | 5.000 | 2.708 | (-------*-------) |
| C15 | 7 | 5.857 | 3.132 | (-------*--------) |

Appendix I5 ANOVA-Over All Acceptability

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor | 14 | 173.33 | 12.38 | 2.47 | 0.005 |
| Error | 90 | 451.43 | 5.02 | | |
| Total | 104 | 624.76 | | | |

S = 2.240 R-Sq = 27.74% R-Sq(adj) = 16.50%

Individual 95% CIs For Mean Based on Pooled StDev

| Level | N | Mean | StDev | ----+---------+---------+---------+----- |
|---|---|---|---|---|
| C1 | 7 | 7.000 | 1.633 | (-------*-------) |
| C2 | 7 | 4.571 | 2.149 | (--------*-------) |
| C3 | 7 | 7.000 | 1.000 | (-------*-------) |
| C4 | 7 | 4.143 | 1.952 | (--------*-------) |
| C5 | 7 | 3.286 | 2.059 | (-------*--------) |
| C6 | 7 | 6.571 | 2.370 | (--------*-------) |

C7 7 5.143 2.340 (--------*-------)
C8 7 6.000 2.309 (-------*-------)
C9 7 2.857 2.116 (-------*--------)
C10 7 6.857 1.773 (-------*--------)
C11 7 6.000 3.317 (-------*-------)
C12 7 6.286 1.604 (-------*--------)
C13 7 6.000 2.646 (-------*-------)
C14 7 6.000 2.646 (-------*-------)
C15 7 6.571 2.699 (--------*-------)

Appendix J1
Calculation of shelf life
The influence of temperature on the reaction rate may be described by using the Arrhenius relationship as follows $k = ko\ exp^{EA/RT}$
Where;

- ko is the pre exponential factor,
- EA is the activation energy,
- R is the ideal gas constant and
- T is the tempereature

The sharp increase in total plate count between day 1 and end of week 1 of beverage stored at 28$^{o}$C compared to beverage stored at 18$^{o}$C means that the reaction rate constant is influenced to a large extent by a change in temperature. This suggests the possible use of the Arrhenius equation to estimate the shelf life. Figure J1 is the logarithmic plot of the total plate count against time.

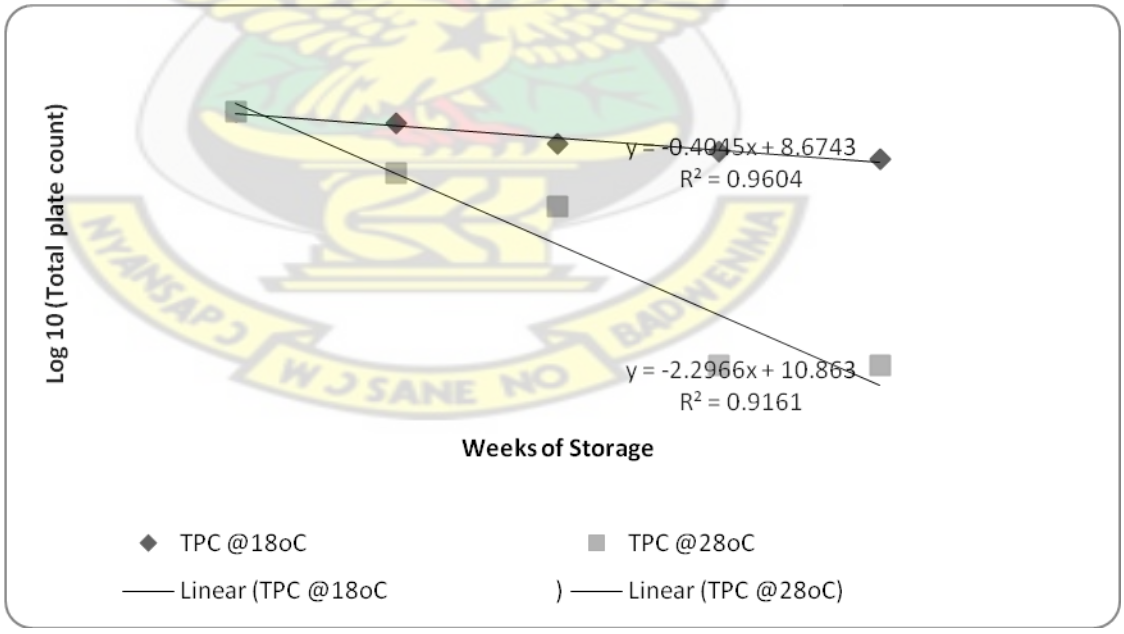



Figure J1: logarithmic plot of the total plate count against time

From the linear equation of the shelf life plot,

y = -0.4045x +8.6743 where

- y is the log 10 of the shelf life period and
- x is the storage temperature.

➢ At temperature of 18$^{o}$C log inverse of the shelf life was estimated at 24.55 weeks, approximately 6 months.