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Thomas Adjei-Duodu, 2014.
Physical, chemical and functional properties of tiger nuts (*Cyperus esculentus*) selected from Ghana, Cameroon and UK market (Spain)

By

THOMAS ADJEI-DUODU

A thesis submitted to the University of Plymouth
in partial fulfilment for the degree of

DOCTOR OF PHILOSOPHY

School of Biological sciences,
Faculty of science and Environment

January, 2014
Abstract
The tiger nut (*Cyperus esculentus*) has attracted a lot of unsubstantiated health claims, yet there is a dearth of research investigation within Ghana specifically in the area of food product development. This study addresses the development of ‘functional bread and biscuit’ from tiger nuts obtained from UK market (Spain). The chemical constituents; carbohydrate, lipid, protein, dietary minerals and antioxidants, the functional properties of three varieties of tiger nuts obtained from Ghana (black and brown), Cameroon (yellow) and UK market (Spain) (brown) were investigated using standard analytical methods as well as the blood glucose response of healthy adults who consumed the developed bread. Tiger nuts were found to be good sources of carbohydrate (51-82g/100g) and lipids (21-37g/100g). The dietary fibre components ranged between 18, 1, 19 and 19g/100g for IDF, SDFP, HMWDF, and ITDF respectively, while the available carbohydrate as sugars were 45, 0.5 and 5g/100g for glucose, maltose and xylose respectively. Minerals that were found to be inherent to the crop were; potassium, phosphorus, magnesium, copper, iron, zinc and selenium and do not depend on place of origin. The following ratios for Sodium/Potassium 0.04, Calcium/Phosphorus 0.13 and Calcium/Magnesium 0.28 were obtained. Tiger nuts had TPC ≥ 134GAE per g, DPPH and stability index of 0.9-8.7mmol/litre and 3- 4 respectively. In conclusion the tiger nuts originating from different geographical locations were good sources of health giving minerals and had diversity of physical properties and chemical constituents which could inform future research in the functional food industry. Tiger nut could be added to the Ghana food basket and the product developed from it could be a potential functional food because of its effect on glucose response and phytochemical contents. It could again be used to replace artificial antioxidants (BHA or BHT) which are used in the food industry to inhibit lipid and protein oxidation especially the black variety.
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DEDICATION

To the Almighty God for preserving and seeing me through this work, to the memory of my beloved Parents (David Kofi Duodu and Sara Akua Yinka) and mother in law (Margaret Akosua Anima)
## FREQUENTLY USED ABBREVIATIONS

The abbreviations and symbols most used in the thesis are listed below.

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<tr>
<td>µM</td>
<td>Micrometre</td>
</tr>
<tr>
<td>AACC</td>
<td>American Association of Cereal Chemists</td>
</tr>
<tr>
<td>AAI</td>
<td>Antioxidant Activity Index</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AOAC</td>
<td>Association of Official Chemists</td>
</tr>
<tr>
<td>BC</td>
<td>Before Christ</td>
</tr>
<tr>
<td>BHA</td>
<td>Butylated Hydroxyanisol</td>
</tr>
<tr>
<td>BHT</td>
<td>Butylated Hydroxytoluene</td>
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<tr>
<td>CHO</td>
<td>Crude carbohydrate</td>
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<tr>
<td>db</td>
<td>dry basis</td>
</tr>
<tr>
<td>DF</td>
<td>Dietary fibre</td>
</tr>
<tr>
<td>Dg</td>
<td>Geometric mean diameter</td>
</tr>
<tr>
<td>DP</td>
<td>Degree of polymerisation</td>
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<td>DPPH</td>
<td>1,1-Diphenyl-2-picrylhydrazyl</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agricultural Organisation</td>
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<tr>
<td>FCR</td>
<td>Folin-Ciocateu’s Reagent</td>
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<tr>
<td>FF</td>
<td>Functional Food</td>
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<tr>
<td>FOSHU</td>
<td>Foods for Specific Health Use</td>
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<tr>
<td>GAE</td>
<td>Gallic Acid Equivalent</td>
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<td>GI</td>
<td>Glycaemic Index</td>
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<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
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<td>HMWDF</td>
<td>High molecular weight dietary fibre</td>
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<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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<td>IUAC</td>
<td>Incremental Area Under the blood glucose response curve</td>
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<td>kcal</td>
<td>Kilocalorie</td>
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<td>Millimetre</td>
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<tr>
<td>mmol</td>
<td>Millimole</td>
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<tr>
<td>NDO</td>
<td>Non digestible oligosaccharides</td>
</tr>
<tr>
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<td>Recommended daily allowance</td>
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<tr>
<td>RS</td>
<td>Resistant starch</td>
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<td>Soluble dietary fibre soluble in 78% alcohol</td>
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<td>Whole Tiger nut Bread/biscuits</td>
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ACKNOWLEDGEMENTS

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AUTHOR’S DECLARATION

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the graduate committee.

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Poster presented at a conference

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Signed: ........................................... Date: ..............................
CHAPTER 1

GENERAL INTRODUCTION

1.1. Rationale and research framework

Whilst food provides us with energy and nutrients for growth and the maintenance of body functions, it is becoming clear that, in the context of the lifestyle of people in developed and even in some developing countries, it can offer much more. For example O’Toole and Claesson (2010), carried out a review on gut flora and stated that, most western countries are experiencing a significant increase in the proportion of their elderly citizens (>65 years), as a result of improved sanitation, hygiene, healthcare and diet. Their views have been supported by the US Census Bureau who according to Kinsella and He (2009) has estimated that by 2040, the proportion of older people will double from 7 to 14 percent of the total world population. The report has also indicated that within 10 years, for the first time in human history there will be more people aged 65 and older than children under 5 in the world (Kinsella and He, 2009). Significantly, the proportion of elderly citizens in the developing countries is also increasing all as a result of good nutrition.

Food companies are increasingly recognising the need to connect with public health messages. They are responding to mounting pressure to reduce some ingredients in the foods such as sugar, excessive calories and fat contents (Tapsell et al., 2005). Apart from capacity to contribute to and control many metabolic, physiological and psychological functions of the body, it has effects
beyond what is traditionally accepted as nutrition. Food can play an important role in reducing the risk of disease and, equally importantly for a healthy population can help to optimise and enhance normal functions and thereby improve quality of life (Guittard, 2006).

1.2. **Rationale of the study**

There is a dearth of research investigation within Ghana specifically concerning unsubstantiated health claims and the possibility of using tiger nut’s tubers to sustain the food security situation. It has been with us for millennia. It is among one of the first domesticated crops cultivated by the ancient Egyptians and found in paintings in the tomb of Rekhmire, in the tomb was an inscription detailing how to make small loaves of a mixture of tiger nuts and honey (Kaufman, 2006).

In Ghana, it is one crop that has attracted unsubstantiated health claims, and elsewhere in South Africa, porridge prepared from tiger nut extracted milk is given to females to alleviate menstrual pain, yet it is the least utilised and studied crop among the nation’s agriculture and food scientists. Again, in spite of the importance attached to this crop, its usage in Ghana is limited to chewing it raw, which always leaves a whole chunk of fibrous bran with large particles in the mouth which makes its eating unappealing. The coarse chaff generated becomes difficult to swallow and therefore have to be discarded. The discarded chaff to the environs put a lot of pressure on the already poor waste management system in the country and serves as fodder for rodents which have been found to be carriers of food borne diseases.
It is public knowledge, which is supported by traditional herbalists and alcoholic beverage manufacturers that a product that contains tiger nuts as one of its ingredients may be used to cure a wide range of diseases. For example one herbal preparation containing tiger nuts as one of its ingredients can claim to cure malaria, diarrhoea, nausea, skin infections, sexually transmissible diseases (vaginal thrush, gonorrhoea, and syphilis) and sexual impotency.

A very common feature that runs through almost every herbal product when one of its ingredients is tiger nuts is the suggestion that it has aphrodisiac properties, and is identified as a local alternative to Viagra.

The best extent that the food and drug regulating body Food and Drugs Authority (FDA) has done is to ban television and radio stations from airing these claims (Anonymous, 2006).

According to studies carried out by (Salem et al., 2005), tiger nuts exhibit anti-inflammatory properties and immunostimulatory effects in immuno-competent hosts (Apolipoprotein deficient mice). It has however been shown that arginine, which is the predominant amino acid in tiger nuts, substantially reduces blood pressure and peripheral vascular resistance in healthy adults and in patients with vascular disease (Bosch et al., 2005, Moore, 2004, Cordian et al., 2005). However, none of the authors have indicated the quantity of tiger nuts that can be used to achieve an effective dose on the body.

The bran which is firmly attached to the seedling makes it difficult to remove as it is done during the milling of cereals such as wheat, again chewing and swallowing the bagasse is unappealing and as a result discarded. One of the most popular uses of the crop in Spain is the use of the extracted milk from the
nut in the production of beverages; the waste generated has little use and could become an environmental issue. Additionally, there are opportunities for adding value to agricultural products (bagasse) for example in the development of ‘functional foods’. With the high amount of fibre, antioxidants and micro-elements in tiger nuts as reported by Linssen et al. (1989) and Parker et al. (2000), the bagasse may be useful in the development of functional foods; prebiotic bread and biscuit. Again, the fibres have the potential to provide non-nutritive bulk to low-fibre fabricated foods for example the bread and biscuits. Additionally, they provided a high concentration of solid matter to an aqueous, food system without significantly altering the viscosity of the system. In a review carried out by Shalini and Gupta (2010), they reported that Shah and Masoodi (1994) conducted studies on the utilisation of wastes from apple processing plants. They reported that in large scale apple processing plants about 25% apple pomace and 1.5% apples are discarded as processing wastes. Such industrially discarded apples were converted to homogenous pulp after autoclaving. The pulp when preserved with potassium metabisulphite (KMS) @ 1.0 g/kg of pulp stored well for over one year at 20°C. Beverages prepared from pulp were highly acceptable. With this success story from apple pomace or bagasse, it is hoped that this could be introduced in Ghana where these materials are dried and burnt.

In recent years, consumers have become sophisticated about the nutritional and therapeutic aspects of the food they eat. As a result of this, according to Adel (2001), have turned their attention to plants instead of dietary supplements to obtain their dietary requirements for a healthy existence because there is increasing evidence that the consumption of whole foods due to the effects of their phytonutrients is better than the consumption of nutrients taken as dietary
supplements. Marinangeli and Jones (2010), have stated that bioactive isolated from nuts, legumes, cereals, grains, fruits and vegetables have been shown to be useful in reducing lipid and cholesterol levels, increasing bone mineral density and antioxidant status as well possessing anticancer properties.

Bread is one of the most staple foods consumed around the globe or by humanity. Traditionally, wheat has been the most commonly used ingredient in bread making, being a highly effective grain owing to many of its components including gluten forming proteins that allows the bread to stretch and rise, giving a unique and desirable texture, making it a difficult material to replace (Meuser, 1996). Apart from it being a good source of calories and other nutrients, it is considered nutritionally poor, as the cereal proteins are deficient in essential amino acids such as lysine and threonine. Grain legumes or nuts contribute significantly towards protein, mineral and B-complex vitamin needs of people in developing countries. Therefore, supplementation of wheat flour with inexpensive staples, such as tiger nut bagasse will helps in improving the nutritional quality of wheat products. As far back as 1971, (Pomeranz and Shellenberger) postulated that in countries where wheat is not grown, cereals, nuts, roots and tubers such as maize (corn), rye, barley, oats, sorghum, millet, yam, cassava, potatoes and peanuts have been utilised in bread-making to reduce the proportion of wheat flour being used if economic condition necessitate the reduction of wheat imports. Afolabi et al. (2001) agreed that the importation of wheat into developing countries is not economically viable, which is why locally grown tubers, legumes and cereals like corn, rice, millet or sorghum are sought as a supplement. The above researchers have reported that supplementing foods with a native crop can increase yield from the crop and creates a potential to penetrate western
markets thus increasing revenue from exportation of the crop or value-added to the crop.

Milling of grain to white flour, ready-made squeezed juices and many heat treated canned vegetables and fruits have clearly cut down the supply of some important phytochemical compounds like dietary fibre, minerals and antioxidants. These are removed during processing and the bagasse that contains these compounds is used as animal feed. For the past decades there has been a paradigm shift from this practice and now instead of giving them to animals we are rather using them as our fore fathers or distant ancestors did.

Although there are a wide range of DF sources; oats bran, wheat bran, mango, guava, apple pomace bagasse, vegetables and fruits that have been identified, yet numerous research work are on-going to explore DF in some underutilised crops which have been used several years as food or herbal medicine. Tiger nut is one of such crops that have been used since the days of the Pharaohs in ancient Egypt and has attracted a lot of health claims, in spite of this only few researchers have worked on tiger nut dietary fibre either on the waste generated after milk extraction or the whole nut.

A focus on post-harvest activities, differentiated value added products and increasing links with niche or speciality markets would appear to be a strategy open to smallholders (Lundy et al., 2000). Lundy et al. (2000) have indicated that the impact of non-traditional crops on the livelihoods of rural producers in Mexico resulted in improved market links and product diversification which increased incomes by 58% while value adding activities accounted for a 350% increase in farmer income, thereby changing the farm environment (Ramirez, 2000).
A potential economic benefit of this study could be that Ghanaian tiger nuts growers stand to gain by exporting high premium products (both raw and processed) to other countries for profiting in hard currencies. It would be expected that once the export market of tiger nuts increases, more people (especially the youth) who have migrated from rural areas to urban centres may go back to do tiger nuts farming. The Food and Drug Authority; government regulatory body and the world as a whole can use the knowledge to execute their activities more confidently and scientifically. This work will again help growers, processors and retailers to have a range of specific quality and safety parameters that relate directly to the needs of functional/health food users. And another advantage would be that food services in delivering ‘functional meals’ would be improved, and the possibility of their frequent consumption increased.

Literature reviewed so far has no evidence to indicate that tiger nuts have been proved scientifically to have medicinal value, however the fibre, antioxidants, the minerals, monounsaturated fatty acids and amino acids (arginine) contents have shown why this nut was one of the earliest domesticated and cherished crop in Old Egypt (Zohary and Hoff, 2000). From time immemorial, tiger nut cultivation in Ghana has predominantly been practised on a subsistence scale by the poor rural women with the application of rudimentary technology resulting in low productivity and unattractive to the youth. However, with an improved and ready to be used equipment the youth and the men would be attracted to this area of crop production.

In spite of the importance attached to tiger nuts in Ghana, its usage in the past was only limited to eat them raw or unprocessed as snacks. It is only recently that the nuts have found its way in the alcoholic and non-alcoholic industries; however,
due to the high content of starch, it leaves a chalky mouth feeling which is not appealing to consumers. With this study, it is envisaged that a desirable quality and safety products that will be acceptable to consumers could be produced. Physical properties of the nuts will give an indication of its effective processing characteristics; design a universal equipment for harvesting, grading, transporting, storage, marketing, milling and efficient extraction of tiger nuts milk leading to possible value added components.

The results obtained from the physical properties of the nuts would help agricultural engineers to develop machines for harvesting, sorting and grading, cleaning and milling into different fractions. The products analysis (fibre, antioxidants, oil, trace minerals, starch and flour) carried out on the nuts could be used as product ingredient or formulated products in the food and pharmaceutical industrials.

1.2. Scope of the study

1.2.1. Aims

The aim for this study was to investigate and compare the physical, chemical and functional properties of tiger nuts selected from three countries (Ghana, UK market (Spain) and Cameroon). Understanding the physical and chemical properties of the nuts may be useful for designing appropriate equipment for sorting, separation, storage and extraction of the milk and as dietary information or be used as active ingredient in formulation of ‘functional foods’. To provide quantitative information about the content of various nutrients in the tiger nuts’ tubers of Cameroon, Ghana and UK market which could be used by nutritionists,
medical personal, pharmaceutical companies and public health workers to improve the diets of populations in this part of the world.

1.2.2. Objectives of the study

The main objectives of this present work are;

1. To critically analyse the differences between selected tiger nuts species obtained from Ghana (two types), Cameroon and UK market in terms of the physical, chemical, functional and value-adding properties.
2. To contribute to the knowledge base necessary for development of “functional/health food”, labelling purposes and claims labelled to tiger nuts.
3. To characterise and develop added value ingredients for confectionery/ bakery industry from the whole nuts and the waste generated (bagasse) after the extraction of tiger nuts’ milk.
4. To investigate the medicinal properties (dietary fibre, mineral and antioxidant) properties as claimed by Ghanaian herbalists.
5. To evaluate the glycaemic index of tiger nut products.
CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

Azizpour et al. (2009), posited that increasing cost of health care, the steady increase in life expectancy and the desire of the elderly to improve quality of their lives are gradually becoming the driving forces for research and development in the area of functional foods. Although the functional foods concept was introduced by Hippocrates (430 BC) when he posited that “Let thy food be thy medicine and medicine be thy food”, it is only recently that the body of evidence have started to support the hypothesis that diet may play an important role modulation of important physiological functions in the body.

2.2. Aim

This literature review aims to provide an evidence-based rationale to support choice of topic, exploration of the relevant literature and to identify gaps on research work carried out on tiger nuts.

2.3. Search strategy

A search strategy was conducted using the following key words Food, ‘tiger nuts (Cyperus esculentus’), physco-chemical properties of vegetable and nuts, ‘functional food’ (prebiotics, antioxidants, macrominerals, microelements), food product development, bread making, biscuit making, stability of fats, particle technology, dietary fibre, glycaemic index, value-adding (bagasse)
etc. to extract relevant literature from the University’s Electronic Library (Primo), British Library, Electronic-journals; Food Chemistry, Lebensmitteltechnologie, Food engineering, Food and nutrition research, Dairy, Food Science, Technology and Nutrition, American Journal of Nutrition, European Journal of nutrition, Food Chemistry and Agricultural, Bioresource Technology etc. The databases used included Web of Science, Medline, ScienceDirect, Elsevier, Taylor and Francis online, SwetsWise, Cochrane, CINAHL, Google scholar and Google etc. Information on tiger nuts is limited so no limit was put on the search year.

2.4. Definition of Foods

Man has found that a wide range of plant varieties, animals, and some microbial sources can be consumed as food. Rahman and Labuza (1999), have defined foods as materials, in a raw, processed, or formulated form, which are consumed orally by humans or animals for growth, health, and satisfaction or pleasure. Food and Agriculture Organisation (FAO) and World Health Organisation (WHO) (2001) in a consultative meeting held in Geneva defined food as any substance, whether processed, semi-processed or raw, which is intended for human consumption, and includes drink, chewing gum and any substance which has been used in the manufacture, preparation or treatment of “food” but does not include cosmetics or tobacco or substances used only as drugs. Unlike drugs, there are generally no limitations on the amount of foods that may be consumed.

Originally, food raw materials were selected on the basis of materials that could be consumed without any harmful effects, and it was subsequently found that processing of foods by heat could improve the texture, and in some cases inactivate harmful components within the food. Chemically, foods components
are classified into macronutrients, which are present as bulk components: proteins, fats and carbohydrates, and micronutrients, which are present at lower level, are the minerals and organic compounds (Kearney and Gleissler, 2011). Minerals are present in the form of salts and organic substances are the vitamins, emulsifiers, acids, antioxidants, pigments or flavours (Lutz and Przytulski, 2001). Energy is mainly derived from the macronutrients: proteins, fats, carbohydrates and alcohol.

2.5. Chemical Characteristics of macronutrients

Food analysis is the discipline dealing with the development, application and study of analytical procedures for characterizing the properties of foods and their constituents. These analytical procedures are used to provide information about a wide variety of different characteristics of foods, including their composition, structure, physicochemical properties and sensory attributes. This information is critical to our rational understanding of the factors that determine the properties of foods, as well as to our ability to economically produce foods that are consistently safe, nutritious and desirable and for consumers to make informed choices about their diet (Nielsen, 1998, Owusu-Apenten, 2005).

Food Analysis plays an important role in assessment and maintenance of food quality and safety, both in industry as well as for enforcement authorities at national and international level. In the past, food analysis was concerned with food adulteration only, but nowadays there is an increasing tendency to examine food from a more positive and broader view point. Processed foods are produced within the limits of prescribed manufacturing formulations, set also to comply with legal and/or other requirements. Proximate analysis refers to the analysis of
foodstuffs to establish their composition. It provides information related to the content of moisture, protein, carbohydrate, lipid, fibre, ash and total minerals (Owusu-Apenten, 2005). This is done by analysis of the product at different stages of processing starting at the farm level. The regulatory requirements for the analysis of food additives and contaminants at very low level have necessitated the development of instrumental techniques suitable for rapid assessment. In case of proximate analysis, the methods may vary for different categories of food products. Hence, the results obtained for a particular food constituent depends on the procedure adopted. However, as long as the same standard procedure is applied to the same food each time, the results are usually reproducible, and thus provide an adequate basis for interpretation.

In the food industry, various food components and parameters are analysed in both raw and processed products. Knowledge of the chemical composition of food is important for the health, well-being and safety of the consumers and manufacturers in understanding the importance of various nutritional constituents so that the level of essential nutrients may be maintained or improved during and after processing. The knowledge of the principles of different food analysis techniques is useful for the selection of an appropriate technique for analysing a particular food (Nielsen, 1998).

2.5.1. Carbohydrates

Carbohydrates are chemical compounds that are manufactured by green plants during photosynthesis, a complex process in which sugars, starches and non-starch polysaccharides (hemicellulose, cellulose, pectins, β-glucans, pentosans, heteroxylans, and xyloglucan) are formed in the plant from the combination of
carbon dioxide from the air and water from the soil. They are the key components of the cell walls of various grains and cover a great variety of biological functions and chemical structures (Kumar et al., 2012). Sunlight and the green plant pigment chlorophyll are necessary for the conversion. Through this process, the sun’s energy is transformed into food energy in the form of carbohydrates. It is important in foods as a major source of energy (4.0kcal/g) in the human diet, as imparters of crucial physical properties, and as modifiers of human physiological processes. Carbohydrates provide to foods many attributes, such as bulk, viscosity, stability to emulsions and foams, water-binding capacity, browning, aromas, flavours and a whole range of desirable textures (from crispiness to smooth, soft gels. Carbohydrates also provide satiety (Kearney and Gleissler, 2011).

The chemical structure of each carbohydrate determines whether it is sugar or starch. Sugars include monosaccharides and disaccharides and the starches are the polysaccharides. Carbohydrate may be determined by the following methods: by difference, energy, refractometry, polarimetry, colourimetry, titrations, enzymatic and high performance liquid chromatography (Gordon et al., 2011).

The main sources of carbohydrates in the diet are foods of plant origin: cereals, vegetables, legumes and fruit. A distinction is now made between the slowly absorbed polysaccharides (starch) contained in natural vegetables, often associated with indigestible polymers (dietary fibre), and the rapidly absorbed simple carbohydrates (sugars) contained in milk (lactose), fruit (fructose) and table sugar (sucrose) (Salvadóa et al., 2006).

Polysaccharides often have beneficial effects on the profile and glucose metabolism. Simple carbohydrates induce hypertriglycerideridemia and
hyperinsulinism, and favour the development of obesity and diabetes in predisposed people (Parks and Hellerstein, 2000).

Parks and Hellerstein (2000), reiterated that carbohydrate-rich diets (≥60% Energy) that contain simple sugars are associated with increased triglyceride levels, reduced HDL cholesterol and a poor metabolic control of diabetes. However, when substituting polysaccharides for fat in the diet, both the lipid profile and glucose control are improved, and these effects may be ascribed in part to dietary fibre.

2.5.1.1. Dietary Fibre

Mudgil and Barak (2013) assert that the concept of ‘dietary fibre’ first came into existence in 1953 after Hipsley observed rare incidents of pregnancy toxaemia in those people who consumed high fibre foods and included cellulose, hemicelluloses and lignin. However the origins of interest in dietary fibre stems from the writings of Mr Denis Burkitt and Dr Hugh Trowell whilst working in East Africa, they were intrigued by the difference patterns in the prevalence of non-infective diseases in Africa as compared to Western Europe. They were also fascinated in the emergence of “Western” disease patterns within the communities where they were working and the different incidence rates of many non-communicable diseases in different countries. Based on their observation, they posited in 1975, that ‘diets rich in foods containing plant cell walls are protective against a range of diseases which show a high prevalence in Western societies such as obesity, type 2 diabetes, coronary heart disease, gall stones, diverticular diseases and large bowel cancer (Brownlee, 2011, Ktenioudaki and Gallagher, 2012).
Dietary fibres are generally considered as ‘roughage’ material which is indigestible in the human small intestine. It comprises mainly the portions of food that are not broken down by the secretions of the human digestive tract. Types of dietary fibre may also be categorized according to their sources, solubility, fermentability and physiological effects. Dietary fibres usually include non-starch polysaccharides, oligosaccharides, lignin and associated plant substances (Champ et al., 2003, Hispley, 1953).

Dietary fibre has been defined by the American Association of Cereal Chemists (AACC) as “the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine” (American Association of Cereal Chemists (AACC), 2001).

From the Table 2-1, in addition to the cellular components (non-starch polysaccharides) within cereals, the portion of starch and starch products which resist digestion in the small intestine has been described as resistant starch (RS) and it is now regarded as a component of dietary fibre (American Association of Cereal Chemists (AACC), 2010). Starch may become resistant to digestion due to several reasons, as it may be physically inaccessible (RS1), compact granular structure (RS2), retrograded or crystalline non-granular (RS3), chemically modified or re-polymerized (RS4) or amylose-lipid complexed (RS5) starches.
Table: 2-1. Composition of dietary fibre (Adapted from (American Association of Cereal Chemists (AACC), 2001, Thebaudin et al., 1997)

<table>
<thead>
<tr>
<th>Non-Starch Polysaccharides</th>
<th>Analogous Carbohydrates</th>
<th>Non-glucidic components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>Indigestible dextrins</td>
<td>Lignin</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>Resistant maltodextrins</td>
<td></td>
</tr>
<tr>
<td>Arabinoxylans</td>
<td>Resistant potato dextrins</td>
<td></td>
</tr>
<tr>
<td>Arabinogalactans</td>
<td>Synthesised carbohydrate</td>
<td></td>
</tr>
<tr>
<td>Polyfructoses</td>
<td>compounds</td>
<td></td>
</tr>
<tr>
<td>Inulin</td>
<td>Polydextrose</td>
<td></td>
</tr>
<tr>
<td>Oligofructans</td>
<td>Methyl cellulose</td>
<td></td>
</tr>
<tr>
<td>Galactooligosaccharides</td>
<td>Resistant Starch</td>
<td></td>
</tr>
<tr>
<td>Gums</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucilages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pectins</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dietary fibre often has beneficial effects on the lipid profile and glucose metabolism. Simple carbohydrates induce hypertriglyceridemia and hyperinsulinism, and favour the development of obesity and diabetes in predisposed people (Parks and Hellerstein, 2000).

Dietary fibre has been defined by Codex Alimentarius Commission (CAC) (2006) as carbohydrate polymers with a degree of polymerization not lower than 3, which are neither digested nor absorbed in the small intestine. A degree of polymerisation not lower than 3 is intended to exclude mono- and disaccharides. It is not intended to reflect the average degree of polymerisation of a mixture.

Dietary fibre consists of one or more of: edible carbohydrate polymers naturally occurring in the food as consumed; carbohydrate polymers obtained from food raw material by physical, enzymatic, or chemical means; synthetic carbohydrate polymers. Dietary fibre generally has properties such as: decrease intestinal transit time and increase stool bulk; fermentable by colonic micro flora; reduce
blood total and/or LDL cholesterol levels; reduce post-prandial blood glucose and/or insulin levels.

Elleuch et al. (2011) have stated that there are two types of dietary fibre, defined by their physical behaviour in water: insoluble fibre, such as cellulose, lignin and some hemicelluloses, which are abundant in wholegrain cereals (wheat bran is particularly rich in this type of fibre), and soluble fibre, such as gums, mucilages and pectins, contained specially in fresh vegetables, legumes and fruit, and the beta-glucans, present in oats, barley and some yeasts. Different plant foods have different amounts and types of fibre. Whole wheat flour, bran and vegetables are, in general, good sources of cellulose, while bran and wholegrain cereals contain sizeable amounts of hemicellulose. Lignin is mainly found in ripe vegetables, wheat and some edible seeded fruits, such as strawberries. Oats, barley and legumes (from which ‘guar’ is derived) are rich in gum, while apples, citrus fruits, strawberries and carrots contain appreciable amounts of pectin.

**Health Benefits**

Trinidad et al. (2010), have indicated that dietary fibre has been implicated in the prevention of risks of non-communicable chronic diseases such as cancer, CVD and diabetes mellitus. It comes from the family of carbohydrates; non-starch polysaccharides (NSP) are not digested in the small intestine but may be fermented in the colon into short chain fatty acids (SCFA) such as acetate, propionate and butyrate. SCFA contribute 1·5–2·0 kcal/g dietary fibre (Roberfroid, 1997). They enhance water absorption in the colon, and thus prevent constipation. Propionate has been shown to inhibit the activity of the enzyme hydroxy-3-methylglutaryl-CoA reductase, the limiting enzyme for cholesterol
synthesis. Dietary fibre has the ability to bind with bile acids and prevents their reabsorption in the liver, and thus inhibit cholesterol synthesis (Williams et al., 1991). Butyrate enhances cell differentiation, thus preventing tumour formation in the colon (Anderson et al., 2010). Dietary fibre’s viscous and fibrous structure can control the release of glucose with time in the blood, thus helping in the proper control and management of diabetes mellitus and obesity (Pashankar and Loening-Bauke, 2005, Samuel et al., 2003).

**Glycaemic index**

The glycaemic index (GI) concept was introduced in the 1980s by Jenkins et al. (1983) and since then it has been used as a dietary tool to improve the glycaemic control of people living with diabetes. The management of blood glucose is relevant today more than ever, with an estimated 2.9 million people currently affected by type 2 diabetes in the UK as cited by Sleeth et al. (2013). However, the GI as a concept has dipped in and out of scientific fashion. By its nature, the GI is highly complex in that it hinges on a number of physico-chemical properties of carbohydrates, such as the chemical structure of the carbohydrate, the surrounding food matrix and the processing it undergoes (Wolever et al., 1991). The fact that GI measurements are confounded by other food components within the diet serves as an additional layer of complexity (Levitan et al., 2007). Consequently, the concept of the GI has been met, at times, with scepticism and some have found it challenging to embrace.

The GI has been defined by the FAO/WHO expert consultation on carbohydrates in human nutrition (Food and Agriculture Organisation (FAO) and World Health Organisation (WHO), 1998) as the incremental area under the blood glucose
response curve of a 50g carbohydrate portion of a test food expressed as a percent of the response to the same amount of carbohydrate from a standard food taken by the same subject. GI of food has been classified as 0-55 low, 56-69 as medium and >70 as high (Foster-Powell et al., 2002).

Liu (2006), asserted that low post prandial glucose concentration diets are with a reduced risk for the development of diabetes mellitus, obesity and cardiovascular disease. Foods that raise blood sugar and steadily give continuous energy are low Glycaemic index food while high Glycaemic index foods includes a sharp rise in blood glucose, which declines within a short time (Akinlua et al., 2013).

The normal tolerance plasma glucose as recommended by American Diabetes Association (2001) for non-diabetic people generally should not rise higher than 7.8 mmol/l in response to meals and typically returns to pre meal levels within two to three hours. The organisation has gone further to defined the baseline for postprandial hyperglycaemia as a plasma glucose level which is more than 7.8 mmol/l two hours after ingestion of food. The World Health Organization (WHO) (2006) defines normal glucose tolerance as <7.8 mmol/l two hours following ingestion of a 75-g glucose load in the context of an oral glucose tolerance test. A body’s homeostatic mechanism, when operating normally, restores the blood sugar level to a narrow range of about 4.4 to 6.1 mmol/L, as measured by a fasting blood glucose test. The mean normal blood glucose level in humans is about 5.5 mmol/L, however, this level fluctuates throughout the day (American Diabetes Association, 2000).

Table 2.4 shows the recommended target blood glucose levels for different groups of people.
Table 2-2: NICE recommended target blood glucose level ranges

<table>
<thead>
<tr>
<th>Target levels by Type</th>
<th>Before meals (pre-prandial)</th>
<th>2 hours after meals (post prandial)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non diabetic</td>
<td>4.0 to 5.9 mmol/L</td>
<td>under 7.8 mmol/L</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>4 to 7 mmol/L</td>
<td>under 8.5 mmol/L</td>
</tr>
<tr>
<td>Type 1 diabetes</td>
<td>4 to 7 mmol/L</td>
<td>under 9 mmol/L</td>
</tr>
<tr>
<td>Children with type 1 diabetes</td>
<td>4 to 8 mmol/L</td>
<td>under 10 mmol/L</td>
</tr>
</tbody>
</table>

Adapted from (Diabetes.co.uk, 2014)

2.5.2. Lipids

Lipids include not only ‘visible fats’ such as butter and margarine, cooking fats and oils and the fat on meat, but also the ‘invisible fats’ which occur in foods such as nuts, cheese, cake, biscuits and vegetable foods. They consist of a broad range of organic molecules that dissolve easily in organic solvents such as alcohol, chloroform, ether, or acetone etc. but are much less soluble in water.

The main classes of lipids found in food and in the body are triglycerides, phospholipids, and sterols.

Triglycerides are the largest category of lipids. In the body, fat cells store triglycerides in adipose tissue. In foods, we call triglycerides “fats and oils,” with fats usually being solid and oils being liquid at room temperature (Insel et al., 2014). Like carbohydrates, fats are compounds of carbon, hydrogen and oxygen only, but the proportion of oxygen is lower. Chemically, food fats consist mainly of mixtures of triglycerides. Each triglyceride is a combination of three fatty acids
with a unit of glycerol, and the differences between one fat or oil and another are largely the result of the different fatty acids in each (Lutz and Przytulski, 2001).

Dietary fats are converted to energy (9 kcal/g), but they are also sources of essential fatty acids. Fats make an important contribution to food characteristics such as texture and palatability. Fat content of foods are estimated by the following methods: gravimetric solvent extraction procedures, volumetric procedures and instrumental methods.

2.5.3. Proteins

All proteins are compounds of carbon, hydrogen and oxygen, but unlike carbohydrates and fats, they are nitrogen-containing macromolecules that occur in major foods. Most proteins also contain sulphur and some contain phosphorus. Dietary proteins provide energy (4 kcal/g), but they are also sources of amino acids which are essential for the synthesis of a wide variety of proteins with important functions, including carriers of vitamins, oxygen and carbon dioxide plus enzymes and structural proteins (Kearney and Gleissler, 2011). The presence of nitrogen in proteins is often used as the basis of the estimation of protein in foods. Some of the methods used to determine protein estimation include: The Kjeldahl, direct distillation, formol titration and spectroscopic (James et al., 1999).

2.5.4. Ash content in Foods

Ash refers to the inorganic residue remaining after either ignition at a temperature of around 500°C or complete oxidation of organic matter in a foodstuff. Ash content represents the total mineral content in foods. Determining the ash content
may be important for several reasons. It is a part of the proximate analysis for nutritional evaluation. Ashing is the first step in the preparation of a foods sample for specific elemental analysis (Harbers, 1998).

2.5.4.1. Minerals contents in food

Webb (2008), has indicated that there are at least 15 essential minerals recommended as daily mineral intake but for the purpose of this review 12 minerals namely, calcium, sodium, potassium, magnesium, phosphorus, copper cobalt, chromium, iron, manganese, selenium and zinc will be dealt with in this review.

Dietary minerals (also known as mineral nutrients) are inorganic substances, other than the four elements carbon, hydrogen, nitrogen, and oxygen present in all body tissues and fluids and their presence is necessary for the maintenance of certain physicochemical processes which are essential to life (Eruvbetine, 2003). They have played an important role in medicine for years, ever since humans have walked the planet. Many are essential in our diets in varying quantities, although people have only recently realised their significance. This could probably be attributed to our increased awareness of our personal and families’ health, and increased media involvement in our lives.

Minerals in the diet are essential for biological processes and play a vital role in metabolic functions, normal growth and development. Physiologically, the most important macro minerals are calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na) and potassium (K). The main roles of these elements can be described as maintenance of pH, osmotic pressure, nerve conductance, muscle
contraction, energy production and almost all other aspects of biological life (Institute of Medicine, 1997, Institute of Medicine, 2005). Consequently, health problems can be attributed to inadequate dietary intakes, which lead to a deficiency or an excess of these elements. The importance of optimal intakes of essential mineral elements to maintain peak health is therefore widely recognised. However at the other extreme, certain metals remain toxic in trace amounts, which can enter the body via a variety of routes and often cannot be excreted leading to metal toxicity. Until recently many people were unaware of the full extent of the risk of metal toxicity, for example in the use of lead piping in houses; the legacy of which continues to this very day.

Most minerals that enter into the dietary physiology of organisms consist of simple compounds of chemical elements. Larger aggregates of minerals need to be broken down for absorption. Plants absorb dissolved minerals in soils, which are subsequently picked up by the herbivores that eat them and so on, the minerals move up the food chain. Larger organisms may also consume soil (geophagia) and visit mineral licks to obtain limiting mineral nutrients they are unable to acquire through other components of their diet (Woywodt and Kiss, 2002, Abrahams, 2012).

Albert et al. (1980), affirm that bacteria play an essential role in the weathering of primary minerals that result in the release of nutrients for their own nutrition and for the nutrition of others in the ecological food chain. One mineral, cobalt, is available for use by animals only after having been processed into complicated molecules (e.g., vitamin B12), by bacteria. Scientists are only recently starting to
appreciate the magnitude and role that microorganisms have in the global cycling and formation of biological minerals.

The importance of minerals as food ingredients depends not only on their nutritional and physiological roles. They also contribute to food flavour, texture and activate or inhibit enzymes-catalysed and other reactions.

2.5.4.1.1. Calcium

Calcium is the most common mineral in the body (Webb, 2002). Calcium is an important factor in bone health, and a high intake is recommended particularly during pregnancy and infancy (Insel et al., 2011), calcium together with phosphorus, magnesium and potassium is important for growth and maintenance of bone, teeth and muscle (Insel et al., 2011) and bone metabolism (Ilich et al., (2003)); (New, 2003); (Bonjour et al., 2009)).

The majority of it is present in the bones and teeth, and a small percentage is found in the blood and soft tissues, e.g. in the heart and kidneys, where it is responsible for nerve impulses and muscle contractions (Rogers and Simon, 1999). A deficiency in Ca and magnesium has been associated with an increased risk of cardiovascular diseases such as hypertension (Heine et al., 2013).

Calcium is thought to decrease the symptoms of premenstrual syndrome (PMS), reduce the risk of preeclampsia, and may have a protective role against colon cancer (Kumar et al., 2010). In UK, the reference nutrient intake of calcium for adults is 900mg/day.
2.5.4.1.2. Chromium

The chromium content of the body varies depending on the region; the range is 6-12mg. The daily intake varies greatly between 5 to 200 micrograms (Belitz et al., 2004). Chromium (Cr$^{3+}$) is an essential trace element functioning in glucose and insulin metabolism. For instance, it activates the enzyme phosphoglucomutase and increases the activity of insulin. It is associated with age related chronic diseases including diabetes and CVD (Ravina A et al., 1999, Cefalu and Hu, 2004). Cr also acts in regulating corticosteroid metabolism (Cefalu and Hu, 2004). It is postulated to decrease losses in bone density and lean body mass and protects against the loss of cognitive function in the elderly (Anderson, 1998, Sharma and Chauhan, 2000). Cheng et al. (2004) have postulated that Cr acts both as an insulin potentiating factor and antioxidant and could, therefore, counteract the increased oxidative stress associated with insulin resistance in the elderly.

2.5.4.1.3. Cobalt

Cobalt is the active centre of coenzymes called cobalamins, the most common example of which is vitamin B$_{12}$ which is essential for the maturation of red blood cell and normal function of all cells. The dietary requirement for cobalt is expressed in terms of vitamin B$_{12}$. Approximately 2 to 3 µg of vitamin B$_{12}$ is needed daily (Anderson, 2000).
2.5.4.1.4. Copper

Copper is ranked as the third most abundant dietary trace metal after iron and zinc. The amount of copper in the body is 80–100mg. This essential trace element is required as a component of numerous oxido-reductase enzymes; cytochrome oxidase (takes part in energy production), dopamine monooxygenase (necessary for neurotransmission in the brain), superoxide dismutase (protects cells from the damage which free radicals may cause) and converts iron from the form in which it is ingested to one which may be absorbed (Belitz et al., 2004). Adult human dietary recommendation for copper (estimated safe and adequate dietary intake) was set at between 1.5 and 3.0 mg Cu/day (Milne, 1998). Food sources of copper include shellfish, liver, kidney, nuts, cocoa powder, chocolate, offal and wholegrain cereals (about a third of intake in the UK is from cereals) (Webb, 2008).

2.5.4.1.5. Iron

The WHO lists iron deficiency as the 6th leading cause of illness and disease in low income countries (Antoine et al., 2012). The iron content in the human body is 4-5grams (Belitz et al., 2004). Most of it is present in the haemoglobin (blood) and myoglobin (muscle tissue) pigments. Iron is also present in a number of enzymes (peroxidase, catalase, hydroxylases and flavine enzymes); hence it is an essential ingredient of the daily diet. The iron requirement depends on the age and sex of the individual; it is about 1.5-2.2mg/day. Iron supplied in the diet must be around 18mg/day in order to meet this daily requirement. The dietary iron is found in the following food, red meat, poultry and fish, liver, kidney, heart, dried
fruit, e.g. raisins, prunes, vegetables, Clams, oysters, wholegrain cereals, nuts and beans and bananas (Belitz et al., 2004).

2.5.4.1.6. Magnesium

Magnesium is found in a wide variety of foods and beverages. Beverages rich in magnesium are coffee, tea, and cocoa. Foods particularly high in magnesium are nuts, legumes, whole-grain cereals (oats and barely), spices, seafoods, and green leafy vegetables. Chlorophyll found in the green leafy vegetables contains the magnesium. Tap water also may represent a source of magnesium (hard water and mineral water). Food processing and preparation may substantially reduce the magnesium content of some foods. For example, the refining of whole wheat with removal of the germ and outer layers can reduce the magnesium content by 80% (Gropper et al., 2005, National Research Council (NRC), 1989).

2.5.4.1.7. Manganese

The body contains a total of 10-40 mg of manganese. This metal is essential for the normal structure of bone and between 2 and 5 mg is the RDA (recommended daily amount), is met by normal daily food intake (2-48 mg manganese/day). Manganese (Mn) is an essential nutrient, involved in the metabolism of amino acids, proteins, and lipids, but in excess, can be a potent neuro-toxicant. Occupational and environmental exposure to airborne manganese has been associated with neurobehavioral deficits in adults and children (Zoni et al., 2007). In exposed workers, neuro-behavioural deficits have been shown to correlate with manganese deposition in the brain observed by magnetic resonance imaging (Chang et al., 2009).
2.5.4.1.8. Phosphorus

Phosphorus is the second most abundant inorganic mineral only to calcium in the human body. Approximately 800g of phosphorus is found in the average adult human. Of this amount, approximately 85% is found in the bones and bones as its inorganic constituent a calcium phosphate salt called calcium hydroxyapatite. The remainder is found as part of essential intracellular components such as nucleic acid, coenzymes and phospholipids (Gropper et al., 2005). Little free phosphorus is found in the living body (Westheimer, 1987). Phosphorus is generally found in the body as part of the phosphate ion. The reference nutrient intake of the element is 3,200mg and the majority of this is absorbed in the gut either by active or passive transport. Phosphorus is widely distributed in foods. The best food sources of phosphorus include meat, poultry, fish, eggs, milk products. Nuts, legumes cereals, and grains also contain phosphorus; however, animal products are superior sources of available phosphorus compared with most plant foods, coffee, tea and soft drink are also good sources of this element. (Insel et al., 2006b, Gropper et al., 2005). It is not usually found free in nature. Dietary phosphorus occurs in both an inorganic form and an organic form. In its organic form, phosphorus is bound to a variety of compounds such as protein, sugars and lipids. The relative amounts of inorganic and organic phosphorus vary with the type of diet. For example, about one-third of the phosphorus in milk is in the form of inorganic phosphates. Meat contains phosphorus that is largely bound to organic compounds and thus requires hydrolysis for absorption to occur. Over 80% of the phosphorus in grains, such as wheat, rice and corn is found as phytate (also called phytic acid or myoinositol hexaphosphate). Phosphorus in
the form of phytate is also found in beans, legumes and nuts. The bioavailability of phosphorus from phytate is limited to about 50% (Gropper et al., 2005).

2.5.4.1.9. Potassium

Potassium, like sodium, is naturally widely distributed in foods. Legumes, nuts, dried fruits, banana, melons, avocados, and kiwi fruit are rich sources of this element. Major vegetable sources of potassium are potatoes and spinach (Strain and Cashman, 2009). Potassium, together with sodium, regulates muscle contraction and nerve impulse transmission, and a high potassium/sodium ratio may assist the excretion of excessive salt and water (Arthey and Ashurst, 2001).

2.5.4.1.10. Selenium

Selenium is a chemical element resembling sulphur. Selenium content in human, according to Belitz et al. (2004), is 10-15 mg, while the daily intake is 0.05-0.1mg. Most selenium found in nature is a part of protein. Foods rich in this element include sea-foods, organ meats; kidney and liver, other meats, and grains grown in soil abundant in selenium.

2.5.4.1.11. Sodium

Sodium is essential to humans, used for regulating blood pressure and blood volume and critical for muscle and nerve function. Excess dietary sodium can lead to hypertension, and may have more serious implications in people with congestive heart failure, cirrhosis or renal disease (Antoine et al., 2012). Whitney and Rolfes (2008), have reported that, excess serum sodium (hypernatremia) is
generally caused by inadequate hydration rather than excess sodium intake. Symptoms of excess sodium in the blood include vomiting, diarrhoea, excess sweating, mental status changes due to cerebral oedema, seizures and death (Brauer et al., 2009). In general, processed foods have the most sodium, whereas unprocessed foods such as fresh fruits, vegetables, milk and meats have the least. Whitney and Rolfes (2008), have reiterated that as much as 75 per cent of the sodium in people’s diet comes from added to foods by manufacturers; about 15 per cent comes salt added during cooking and at the table and only 10 per cent comes from the natural content in foods.

2.5.4.1.12. Zinc

According to Samman (2007), zinc is available widely in the food supply chain but its bioavailability from different foods is highly variable. Zinc in animal products, crustacean and a mollusc is more readily absorbed than from plant foods. Rich sources of zinc include oysters, red meat, liver, cheese cereal grains, legumes and nuts. The zinc content of refined cereals, legumes and nuts is lower than their unrefined forms because the bran is noted to contain most of the phytate.

The total zinc content in adult human’s tissue is between 1.2 and 4 grams of zinc. The daily requirement of 5-10mg is provided by a normal diet (6-22mg zinc per day) (Belitz et al., 2004, Samman, 2007).

The functions of zinc fall into three categories: catalytic, structural and regulatory. In its structural role, zinc helps fold proteins into functional shapes. As a regulator, zinc helps control many diverse functions, including gene expression, cell death, and nerve transmission (Insel et al., 2006b). The highest concentrations of this
element are found in the choroid of the eye and the prostate gland (semen has 100 times the concentration of plasma), but most of the body zinc is in the bones and muscles. Zinc, according to Samman (2007), is the most common catalytic metal ion in the cytoplasm of mammalian cells. Some examples of zinc metalloenzymes are; alcohol dehydrogenase, lactate dehydrogenase, malate dehydrogenase, glutamate dehydrogenase, carboxypeptidases A and B, carbonic anhydrase, superoxide dismutase and alkaline phosphatase. Zinc helps to provide structural integrity or helps to activate these enzymes. Zinc is essential for the synthesis and action of insulin. It also helps to stabilise the proinsulin and insulin hexamers by forming complexes with them.

2.5.5. Importance of pH in Foods

The natural pH in foods is significant to determine the type of organisms that can grow and survive during food storage. Natural pH in foods can be lowered by adding acidulants (Acetic, Citric, Ascorbic and Lactic acids). It is useful in many fermented foods (dairy, meat and alcoholic beverages). The antimicrobial effect results are mostly due to the decrease of pH below the microbial growth range through production of lactic or acetic acids.

An acid and alkaline product would taste sour and bitter respectively. According to McClendon and Sharp (1919) pH in foods are all on the acids side of neutrality no matter what the condition of freshness, mode of storage, stage in the preparation (cooking) or dilution with water. Potatoes, sweet potato juice, carrot juice like all root and tuber crops have a pH range of 5.80 to 9.44
2.5.6. Energy content in foods

The energy evaluation was done by multiplying the protein, fat and carbohydrate by the factors 4, 9 and 4 respectively. The energy content of foods depends on the components of food that provide energy (protein, fat, carbohydrate, alcohol, polyols, organic acids and novel compounds). For the purpose of calculation, the quantity of each individual component is converted to food energy using a generally accepted factor that expresses the amount of available energy per unit of weight. Next, the food energies of all components are added together to represent the nutritional energy value of the food for humans. The energy conversion factors and the models currently used assume that each component of a food has an energy factor that is fixed and that does not vary according to the proportions of other components in the food or diet (Food and Agriculture Organisation (FAO), 2011).

New lifestyles of modern countries have contributed to the appearance of chronic diseases such as obesity or cardiovascular diseases, which are mainly due to bad eating habits. Solutions can be found in providing the consumers with functional foods with health capability. With the supplementation of food products during the first step with for example vitamins, minerals ($\text{Ca}^{2+}$, $\text{Mg}^{2+}$) or essential fatty acids to improve their properties, another strategy has grown up in the last decade with the development of low-fat products. These lipids, originally present in the food product (and remove), are responsible for its texture. The solution has been in the discovery of the polysaccharides, so called fat replacers, with specific physiochemical behaviour, which also possess healthy properties as dietary fibre and probiotics (Warrand, 2006).
The primary role of food has moved from being a source of energy and body-forming substance to the more subtle action of biologically active food components on human health (Adams, 1999, Grajek et al., 2005).

Adams (1999), affirms that food is now seen as more than just a collection of nutrients, but consists of an enormous collection of different molecules which is classified into two major groups; Nutrients and “Functional Foods” (Healthy Foods or Nutricines) see Figure 2-1.
For example Warrand (2006) has indicated that, in recent years, accumulating evidence has pointed out clearly to a role of certain dietary components (bioactive ingredients) in the prevention of cardiovascular disease, some kinds of cancer, osteoporosis, inflammatory conditions, obesity and modulation of the immune system. Bioactivity refers to the application of nutraceuticals or bioactive ingredients in food such as probiotics, probiotics, bioactive carbohydrates (fibre), bioactive peptides and sterols. Functional foods are endowed with specific physiological benefits that discriminate them from traditional foods.

In the industrialised world, there has been an explosion of consumer interest in the active role of foods in the well-being and life prolongation as well as in the prevention of initiation, promotion and development of cancer, cardiovascular diseases and osteoporosis.
2.5.7. Phytochemicals

The term phytochemicals according to Johnson and Williamson (2003) refer to biologically-active, non-nutritive secondary metabolites, which are closely related to plant pigments, flavour and natural toxicity to pests. Brightly coloured fruits and vegetables tend to have the highest levels of phytochemicals and nutrients. These natural bioactive compounds work together with vitamins, minerals, and fibre to promote good health, but also have protective or disease-preventive properties. Although phytochemicals are not considered nutritious, they have valuable antioxidant properties. Johnson and Williamson (2003), have classified phytochemicals into three main groups:

- Phenolic compounds (including flavonoids and phytoestrogens);
- Glucosinolates;
- Carotenoids

2.5.7.1. Antioxidants

Antioxidants are substances that are able to prevent or inhibit oxidation processes in human body, plants and food products (Babbar et al., 2011, Gramzaa et al., 2006). Kaur et al. (2008) affirmed that the body’s enzymes as well as too much exposure to environmental pollution, ultraviolet light, cigarette smoke, excessive intakes of iron and food additive are capable of converting oxygen to hydroxyl radicals. These generated free radicals are highly reactive and can cause damage to the body’s cell proteins, DNA and fats. These damages according to Daker et al. (2008), can contribute to numerous degenerative diseases including coronary heart, inflammatory disease, cancer,
diabetes mellitus and reperfusion injury. Free radical by definition is 'any species capable of independent existence (hence the term free) that contains one or more unpaired electrons' (Gutteridge and Halliwell, 1996).

Consumption of fruits, vegetables and nuts are well patronised natural products because they are believed to be rich in phenolic compounds. Shahnawaz et al. (2010), report that phenolic compounds are potent antioxidants in plants extracts and therefore contribute to fruit quality and nutritional value by modifying colour, taste, aroma, and flavour, and also by providing beneficial health effects. These compounds also play a role in plant defensive mechanisms by counteracting reactive oxygen species (ROS), thus minimizing molecular damage due to microorganisms, insects, and herbivores. These are large groups of secondary metabolites that have common features and properties similar to hydroxyl-substitute benzene ring (Megias et al., 2009).

Lipids and lipid-based foods deteriorate during processing (heat treatment), storage of raw materials and subsequent storage of finished products is a leading cause of shelf-life failure and rejection on the part of consumers in fat containing foods, generating nutritional value losses, off-flavours, aromas, taste, texture and changes in colour. Lipid oxidation is initiated by exposure to the enzyme lypoxygenase, increased levels of unsaturated fats, polyunsaturated fatty acids, oxygen, heat, ionising radiation, light, meat/heme pigments, metal ions and metalloprotein catalysts (Daker et al., 2008, Tepe et al., 2005, Fernández-López et al., 2007). Oxidation affects many foods, such as omega-rich products, fried foods, meats, dairy products and nuts. On the positive side it is essential to many living organisms for the production of energy necessary for biological processes (Babbar et al., 2011).
Oxygen-centred free radicals, also known as reactive oxygen species (ROS), including superoxide, hydrogen peroxide, hydroxyl (OH·), peroxyl (ROO·) and alkoxyl (RO·), are produced in vivo during oxidation (Bloknina et al., 2003). The deterioration of lipid as described above is referred to as autoxidation. Lipid oxidation Autoxidation of lipids (RH) according to (Ostrowska-Ligeza et al., 2010) proceeds through a radical mechanism involving three steps: (i) initiation or formation of initial free radicals (RH → R• + H•), (ii) propagation of the free radicals and formation of primary oxidation products such as hydroperoxide (R• + O2 → ROO•, ROO• + RH → ROOH + R•) and (iii) termination and formation of non-radical products of recombination, or disproportionation of radicals and peroxide decomposition to inert products that are commonly known as secondary oxidation products (R• + R• → R•, R• + ROO• → ROOR, ROO• + ROO• → O2 + ROOR). In highly oxidised fats and oils, aldehydes, ketones, furanones, lactones, hydrocarbons, organic acids and polymeric compounds are present. They have an unpleasant odour and taste and are toxic (Gonzaga and Pasquini, 2006, Kanner and Rosenthal, 1992).

Aruoma (1998) and Burns et al. (2001) have reiterated that ROS play an important role in the degenerative or pathological processes of various diseases in humans, such as aging. In the Food industry, the rate of the reactions stated above may be reduced by freezing, refrigeration, packaging under inert gas in the absence of oxygen and vacuum packaging (Daker et al., 2008). In the event that these methods are neither economically feasible nor practical from the nutritional and technological point of view, it is highly desirable to control these undesirable reactions with the addition of antioxidants.
Synthetic antioxidants like BHA, BHT and TBHQ have been used in foods for many years; and while they can be effective, they often carry a negative consumer image. Although efficient in preventing autoxidation, only a few synthetic compounds are currently approved for use in the food industry because of their potential toxicity and carcinogenicity. BHA and BHT are both anti carcinogenic and carcinogenic (Lölliger, 1991, Botterweck et al., 2000, Grun et al., 2006, Johnston et al., 2005). Thus, to satisfy consumers’ preference and meet regulatory authorities for natural food additives over synthetic ones, there is increasing importance in searching for natural antioxidants from herbs, fruits, vegetables, nuts and spices as a less harmful alternative to synthetic antioxidants.

For example plant extracts oils (i.e. rosemary) have been successfully used to reduce lipid oxidation in meat products, burgers and sausages (Kuri et al., 2005) and crispy bacon (Viuda-Martos et al., 2010a).

2.5.8. Functional Foods

In recent years new food products with increased health benefits and potential to reduce risk of diseases have been developed and marketed, as a result of advances in food technology and nutritional sciences. The dietary concerns of both today’s ageing population and people with fast paced lifestyles have moved from foods that prevent nutritional deficiency and associated diseases to foods that offer longer-term prevention of chronic diseases. Countries are currently faced with health challenges arising from changing population demographics (e.g. an ageing population) and increases in lifestyle-related diseases. Consumers are becoming more aware of the relationships between diet and disease (Sun-Waterhouse, 2011).
The commonly accepted definition for “functional foods” refers to foods or their ingredients that provide an additional physiological benefit beyond their contribution to basic nutrition (Day et al., 2009). From this point of view, whole foods, such as fruits and vegetables, represent the simplest form of functional foods, since they are rich in fibre and bioactive phytochemicals (Day et al., 2009).

In the development and formulation of new products, functional foods play an important role for the food industry. Some functional products are intended for people with specific health problems such as cardiovascular disease, hypertension, diabetes, morbid obesity and gluten intolerance (Doporto et al., 2011).

It is a new or novel food, developed to have specific health benefits, in addition to their usual functions. Examples are spreads with added phytosterols, to lower serum low-density lipoprotein cholesterol and the risk of coronary heart disease, and the development of starchy products with resistant starch and lower glycaemic indices, to help control blood glucose levels. These foods may help to improve or restore nutritional status in many people. However, much more should be known about suitable biomarkers to test their efficacy, variability in human response to specific food products, safety, consumer understanding, and how their health messages must be formulated, labelled, and communicated (Voster, 2009).

Functional foods represent a value adding opportunity for both the food manufacturing and food service sectors. Advances in bio-technology, micro-encapsulation, ingredients synthesis/extraction/purification, non-thermal processing and predictive modelling support the design of functional foods. Rodgers (2004) has suggested that food service operators need to increase their
technological and marketing sophistication: knowledge of the available range of functional ingredients, their physiological benefits and effects on product quality; legislative requirements; marketing trends and consumer communication techniques.

Perez-Lopez and Carbonell-Barrachina (2005), posited that, the diet prevailing in industrialised countries is characterised by an excess of energy-dense foods, rich in fats and sugar but with a deficiency of complex carbohydrates, that constitute the major portion of dietary fibre. And an epidemiology research has demonstrated a relationship between this type of diet and the emergence of a range of chronic diseases, including colon cancer, obesity, cardiovascular diseases and several other disorders (Kaefferstein and Clugston, 1995). Therefore, an increased in the level of dietary fibre in the daily diet has been recommended by Johnson and Southgate (1994). For these reasons, there is a growing interest in the consumption of all foods that can supply fibre to daily food intake.

2.5.8.1. Value adding (Bagasse)

In recent years, valorisation of agricultural bagasse has been receiving more attention; in view of this researchers are evaluating the conversion of bagasse into food ingredients and other value-added materials (Makris et al., 2007). The residual substances (bagasse) that remain after isolating the main component of the total sub-product are abundant, and represent an inexpensive material that has been under-valued until now, being only used as a combustible or fertilizer. These residuals substances have found its way in the jam industry (Grigelmo-Miguel and Martín-Belloso, 2000). The generated bagasse during food
processing may still contain many valuable substances, such as, pigments, sugars, organic acids, flavours and bioactive compounds with antioxidant and antimicrobial activities, as well as being valuable sources of dietary fibre (Fernández-López et al., 2007). Dietary fibres obtained from bagasse may also contain appreciable amounts of colorants, antioxidants or other substances with positive health effects, such as those that may protect against cardiovascular disease, provide improvements in gastrointestinal health; provide improvements in glucose tolerance and the insulin response, reduce the risk of developing some cancers and influence lipid digestion, hence contributing to some degree of weight management (Ayala-Zavala et al., 2011, Kuri et al., 2005, Viuda-Martos et al., 2010b). As mentioned above, fibre-rich bagasse may be incorporated into food products as inexpensive, non-caloric bulking agents for the partial replacement of flour, fat or sugar, as enhancers of water and oil retention and to improve emulsion or oxidative stabilities (Elleuch et al., 2011).

Rodriguez et al. (2006b) opined that market for dietary fibre is highly competitive and new fibres with healthy properties that satisfy the growing consumer requests that are demanded every day; there are a great variety of agronomic sub-products that are available. Insoluble fibre-rich fractions (FRFs), including insoluble dietary fibre, alcohol-insoluble solid, and water-insoluble solid, were isolated from the peel of Citrus sinensis L. Cv. Liucheng. They found that these three FRFs could effectively adsorb glucose, retard glucose diffusion, and inhibit the activity of α-amylase to different extents. They proposed that the mechanisms might create a concerted benefit in decreasing the rate of glucose absorption and eventually lower the concentration of postprandial serum glucose. The potential
hypoglycaemic effects of these FRFs suggested that they could be incorporated as low-calorie bulk ingredients in high-fibre foods to reduce calorie level.

There are many fruits, for example orange, apple, peach, pawpaw, pineapple, and olive, which are used for the extraction of their juices. They all contain a by-product from which can be recovered different high-added value compounds; among these, it is remarkable the fibre fraction has great potential in the preparation of functional foods. There are also several vegetables, such as pepper, artichoke, onion and asparagus that originate a waste during processing (Rodriguez et al., 2006a).

Among many other bioactive compounds, significant amounts of pectins and polyphenols can be recovered from apple by-products (Carle et al., 2001).

Other waste products are those coming from kiwi that contain about 25% fibre referred to dry matter (Martin-Cabrejas et al., 1995) and from the pineapple shell that has a high percentage of insoluble fibre (70% total fibre), which is mainly composed of neutral sugars, such as xylose and glucose, and presents a great antioxidant capacity (Salvi and Rajput, 1995). Olives that are largely destined for the production of olive oil also leave a by-product that is rich in different bioactive components, including phenolic compounds and fibre (Benitez et al., 1997).

A widely accepted definition of functional food is ‘a food which has a beneficial effect on one or more target functions of the body, above and beyond the usual effects of food, such as improving the state of health and well-being or reducing the risk of disease’ (Dimer and Gibson, 1998, Plaami and Dekker, 2001). This term was introduced in Japan in mid 1980s (Diplock et al., 1999) and this type of foods is known on the Japanese market as ‘Foods for Specified Health Use’
(FOSHU). FOSHU are required to provide evidence that the final food product is expected to exert a health or physiological effect. FOSHU products should be in the form of ordinary foods, (not as pills or capsules) and are assumed to be consumed as part of an ordinary diet and not as very occasional items linked to specific symptoms. The functional foods comprise: (i) convectional foods containing naturally occurring bioactive substances (e.g., dietary fibre), (ii) foods enriched with bioactive substances (e.g., probiotics, antioxidants), and (iii) synthesised food ingredients introduced to traditional foods (e.g., prebiotics) (Grajek et al., 2005).

Most products currently approved contain either oligosaccharides or lactic acid bacteria for promoting intestinal health.

In a study carried out by Kuri et al. (2002), it was revealed that the effect of inulin (oligosaccharide) on the rheological and textural characteristics of yog-ice cream desserts in relation to their sensory attributes, increases the viscosity and hardness of the ice cream. In addition, the mouth-feel and meltdown characteristics of the ice cream improved with the increase amount of inulin into the product.

Sanchez-Zapata et al. (2010), have successfully added tiger nut fibre (15%) to a fresh meat product (burger) and to a cooked meat product (type bologna). They asserted that addition of this product ingredient to burgers and bolognas improves its nutritional value (decreases its fat content and increases total dietary fibre content) without relevant modifications in their physiochemical characteristics.
Advances in food science and technology are now providing the food industries with increasingly sophisticated methods to control and alter the physical structure and the chemical realisation of the market potential for functional foods based on the principles of value-adding for health benefits.

These current changing concepts in nutrition are of particular importance to the consumers, food industries and academia in view of some significant trends in our present society.

Some of the reasons why functional foods are patronised are;

1. Increasing cost of health care and off days from work.
2. Continuing increase in life expectancy.
3. Increasing in the number of elderly people.
4. People’s desire for an improve quality of life.

Fruits, vegetables, nuts and common beverages as well as several herbs and plants with diverse pharmacological properties have been shown to be rich sources of micro-chemicals (‘functional foods’) with the potential to prevent human cancers (Waladkhani and Clemens, 2001). Food Chemists and natural product scientists have identified hundreds of phytochemicals that are being evaluated for the prevention of cancer. These include the presence in plant foods of such potentially anticarcinogenic substances as carotenoids, chlorophyll, flavonoids, indoles, isothiocyanates, polyphenolic compounds, protease inhibitors, sulphides and terpenes (Waladkhani and Clemens, 2001).
Table 2: Dietary sources of phytochemical compounds as cited by (Waladkhani and Clemens, 2001)

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Food sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotenoid</td>
<td>Apricot, Peach, Orange, Nectarine, Broccoli,</td>
</tr>
<tr>
<td></td>
<td>Cabbage, Spinach, Pea, Pumpkin, Carrot, Tomato,</td>
</tr>
<tr>
<td></td>
<td>Green tea, Black tea, Citrus fruits, Rice</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Onions, Broccoli, Cherry, Wheat, Corn, Tomatoes,</td>
</tr>
<tr>
<td></td>
<td>Spinach, Cabbage, Apples, Olives, Red wine, Soy</td>
</tr>
<tr>
<td></td>
<td>products, Soybean, Oats, Corn</td>
</tr>
<tr>
<td>Protease Inhibitors</td>
<td>Wheat, Peanuts, Rice</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>Corn, Potato, Grapes, Strawberry, walnut,</td>
</tr>
<tr>
<td></td>
<td>Raspberry</td>
</tr>
<tr>
<td>Sulphide</td>
<td>Cabbage, Paprika, Pomegranate</td>
</tr>
<tr>
<td>Terpenes</td>
<td>Cabbage, Chives, Allium, Onion, Garlic</td>
</tr>
<tr>
<td></td>
<td>Grape fruits, Lemon, Mint, Cherry, Lime, orange,</td>
</tr>
<tr>
<td></td>
<td>lavender and celery seed</td>
</tr>
</tbody>
</table>

The wide range of food products available to today’s consumer offers a wide variety of complex food components as summarised in figure 1. These have the potential to improve the health and well-being of individuals and maybe to reduce the risk from, or delay the development of major diseases such as cardiovascular diseases (CVD), cancer, osteoporosis, gastrointestinal disorders and dental caries (Diplock et al., 1999). Currently, Japan and United States of American (USA) have made a lot of progress in this new area.

In USA, ‘reduction of diseases risk’ claims have been allowed since 1993 on certain foods. These contain components where the Food and Drugs Administration (FDA) has accepted there is objective evidence for a correlation between nutrients or foods in the diet and certain on the basis of ‘the totality of
publicly available scientific evidence, and where there is substantial agreement amongst qualified experts that the claims were supported by the evidence’.

The new EU regulation on nutrition and health claims (No. 1924/2006), according to Grunert et al. (2011) has set down two general requirements with regard to consumer protection: that claims are not false, ambiguous or misleading to the consumer; and that claims shall be permitted only if the average consumer can be expected to understand the beneficial effects as expressed in the claim.

In Latin American scientific and regulatory communities, the functional foods concept has been associated with foods having health benefits beyond those of basic nutrition but is not defined officially in the emerging regulatory codes (Lajolo, 2002).

According to Stephen et al. (2002) health claims linking foods and food components to diseases are prohibited in Canada because of limitations of the Food and Drugs acts. Changes to the act to permit such claims would require a Bill to Parliament, lengthy and impractical solution.

In Ghana, the Food and Drugs Agency, is the body that has been assigned to regulate food and drugs in the country, and there is neither legislation nor scientifically constituted experts working on the area of ‘Functional foods’. The Board has banned any form of advertisement both in the print and electronic media from making any unsubstantiated claims on food and herbal medicine. The Agency is facing a lot of opposition from the food and herbal medicine industries, the advertising companies and the media as a result of the ban directives. These establishments feel that their livelihood or existence is going to be affected by being asked by the Establishment to prove their numerous unsubstantiated
claims scientifically before their products could be registered or allowed to sell to the general public.

Up to now, the approaches used for functional food science, both in Japan and to a lesser extent in the USA, to match these new concepts in nutrition have mostly been ‘product or food components-driven’, this approach is likely to be much influenced by local, traditional or cultural characteristics. In view of this a more scientific based, ‘Functional driven’ approach should be preferred, because the functions and their modulation are universal.

Functional Foods, defined above, can contain probiotics or prebiotics, but for the purpose of this work only the prebiotics term has been reviewed.

2.5.8.2. Prebiotic

Prebiotic is defined as non-digestible or low-digestible food ingredients that benefit the host organism by selectively stimulating the growth or activity of one or a limited number of beneficial bacteria in the colon (Dimer and Gibson, 1998, Manning and Gibson, 2004). This role is played by fermentable carbohydrates, which are not digested or poorly digested in the small intestine and stimulate, preferentially, the growth of bifidobacteria and some Gram-positive bacteria, belonging to the probiotics bacteria administered to humans. Complex carbohydrates pass through the small intestine to the lower gut where they become available for colonic bacteria present but are not utilised by the majority of the bacteria present in the colon. Lactulose, galactooligosaccharides, fructooligosaccharides, inulin and its hydrolysates, maltooligosaccharides, and resistant starch are probiotics commonly used in human nutrition. The main end
products of carbohydrate metabolism are short chain fatty acids, namely acetate, butyrate and propionate, which are further used by the host organism as an energy source.

In practice, the most common oligosaccharides are found in food raw materials as listed in Table 2.1

Oligosaccharides comprise glycosides that contain between three and ten moieties. The degree of oligosaccharide polymerisation is of importance. Usually, food-grade oligosaccharides are mixture of saccharides with a different degree of polymerisation (Crittenden and Playne, 1996). For example, the major fraction in inulin has a degree of polymerisation of about 14 as reviewed by Grajek et al. (2005).

Prebiotic oligosaccharides can be produced in three different ways: by extraction from plant materials, microbiological synthesis or enzymatic synthesis, and enzymatic hydrolysis of polysaccharides (Crittenden and Playne, 1996).

The majority of prebiotics oligosaccharides are produced on the industrial scale and are widely available on the market (Grajek et al., 2005).

In practice, combined mixtures of prebiotics and probiotics are often used because their synergic effects are conferred onto food products. For this reason, such mixtures are called synbiotics.

The selection of suitable sources to provide new dietary fibre products with high antioxidant capacity derived from natural associated sources as indicated by Adams (1999) this could be an appropriate tool with which to achieve a better antioxidant status along with the recommended higher dietary fibre intake.
2.6.  Particle size Reduction

2.6.1. Definition of milling

Milling is a general trade name, which normally means reduction of food grains into various and products like meal, flour, split products etc. Milling includes pearling, dehusking, grinding or size reduction, mixing, polishing etc. The meaning of the term milling vary with the crop for example milling of wheat refers to a grinding operation to produce flour, whereas in rice industry, milling refers to overall operation in a rice mill from cleaning of paddy to rice gradation. Milling also refers to extraction of juice and oil. Dehulling involves removal of the fibrous seed coat that tightly envelops the cotyledons. It is the major primary process, and improves the cooking quality, digestibility and appearance of the product (Huyghebaert and Dewettinck, 1999).

Different types of mills are used to prepare grain, with adjustments in operating conditions used to control the particle size distribution of the milled grain (Wondra et al., 1995).

Indeed milling technique may have a greater impact on whole wheat bread quality than the quality of wheat used for producing the flour or the formulation of the bread itself as reiterated by Kihlberg et al. (2004).

As stated by Kent and Evers (1994), the two predominant techniques for grinding whole grain flours are stone and roller mills. Whole grain flours could also notionally be produced with an impact or hammer mill but this is rarely used.
2.6.1.1. Stone milling

Stone mills are the oldest attrition mills used for making whole grain flours, which simultaneously use compression, shear, and abrasion to grind wheat kernels between two stones and produce a theoretical extraction rate of 100% (Kihlberg et al., 2004). Modern stone mills are metal plates with composition stones attached (Posner and Hibbs, 2005).

Stone mills generate considerable heat due to friction. This can result in considerable damage to starch, protein, and unsaturated fatty acids in comparison with other milling techniques (Prabhasankar and Rao, 2001). Furthermore, in large, continuous milling operations, heat generated from stone milling can pose a fire risk. Interestingly, there appears to be a marketing advantage by using the term “stone ground” with consumers, as evidenced by the preponderance of whole wheat flour products making this claim in both retail and commercial markets (Posner and Hibbs, 2005). Thus, some mills will “crack” the grain using a stone mill with the plates situated far enough apart to not generate excessive heat. Additional capital costs may be required to equip existing mills with such a set up. The cracked wheat is then reduced to flour on a roller mill.

2.6.1.2. Roller milling

Roller-milling is reportedly more efficient as it requires less energy for particle size reduction than hammer-milling; however, hammer-mills are more widely used in feed manufacturing because they are easier to operate and maintain, although they produce a wider range of particle sizes (Amerah et al., 2007).

The process of roller milling involves separation of the endosperm from the bran and germ followed by gradual size reduction of endosperm (Ziegler and Greer,
In this process, wheat is passed through a series of corrugated and smooth rollers accompanied by sifting between stages. Producing flour that fulfils the requirement for being whole grain is achieved by blending bran and germ back with the endosperm flour in the naturally-occurring proportions. Feeding the bran and germ milling streams with the endosperm flour stream is most often achieved in a continuous process, rather than collecting all fractions in separate bins and recombining at the end of milling. In this case, production of whole wheat flour would not involve additional capital expense beyond what is required for regular roller milling. Sometimes whole wheat flour is made by physically separating flour millstreams and then recombining at the end of the milling process. This is usually done when the bran will undergo some post-milling such as ultra-fine grinding or heating. In these cases, capital costs would be required for the post-milling, plus equipment for recombining the fractions.

When producing whole wheat flour on roller mills, a number of conditions are different from those used for wheat flour (Kent and Evers, 1994). First, conditioning (tempering) is less important when milling whole wheat flour. While wheat flour relies on proper conditioning to facilitate endosperm and bran separation, this is not required for whole wheat milling. Thus, in theory no conditioning should be required, although many mills will add 1-2% moisture to soften the grain and improve efficiency in terms of the energy required to produce the flour. Efficiency can also be improved by tightening the roll gap and using more open scalp covers to increase the break release, as well as changing some of the smooth rolls to corrugate during reduction. The purifier air valves should also be adjusted so that the bran and germ are not rejected but are returned to the reduction system (Kent and Evers, 1994). There are several noteworthy
advantages of making whole grain flour from roller mills as opposed to stone mills. First, the amount of grinding and reduction at each roll can be adjusted to accommodate variations in raw materials, which makes roller milling both economical and flexible (Posner and Hibbs, 2005). Second, the use of selective corrugations and differential speeds subjects the endosperm fraction to minimal shear and compressive forces during the grinding and reduction, which allows less heat to build on reduction rolls and results in less destruction to chemical components in the flour (Prabhasankar and Rao, 2001). A third advantage of making whole grain flours from roller mills is that wheat bran and germ can be separated from the endosperm fraction and subjected to further processing such as heating or fine grinding to affect the storage or functional properties of the flour (Posner and Hibbs, 2005).

Larger granules tend to swell more on cooking and hence granule size is an important factors affecting the starch functionality (Joshi et al., 2013a). However, from a nutritional standpoint, smaller particles could help in the release of vitamins and other components from the outer cells of the kernel (Kahlon et al., 1986). Thus, a moderate particle size (mean bran particle size of about 400-500μm) may be the most desirable in whole wheat flour for bread production.

Particle size of the bran fraction in whole wheat flour has an influence on functional properties of the flour. In general, large wheat bran particles (mean article size of more than about 500μm) lead to higher water absorption (Anderson and Eastwood, 1987, De Kock et al., 1999, Galliard and Gallagher, 1988, Mongeau and Brassard, 1982, Robertson and Eastwood, 1981, Zhang and Moore, 1999) and loaf volume (De Kock et al., 1999, Galliard and Gallagher, 1988, Zhang and
Moore, 1999) compared with finer bran particle sizes (mean particle size less than about 500μm). However, if bran particles are too coarse (>600μm), bread possesses a rough crust appearance and gritty texture (Zhang and Moore, 1999). Small particles have a greater negative impact on bread quality because chemical components in the bran can interact more readily with gluten and inhibit development (Noort et al., 2010). However, from a nutritional standpoint, smaller particles could help in the release of vitamins and other components from the outer cells of the kernel (Kahlon et al., 1986). Thus, a moderate particle size (mean bran particle size of about 400-500μm) may be the most desirable in whole wheat flour for bread production.

Products that do not require gluten development may have different particle size requirements compared with those that do. In an evaluation of 69 soft wheat cultivars for whole wheat cookie baking quality, cookie spread was influenced by whole wheat flour particle size. Small particles produced large cookies (more spread), while larger bran particles produced smaller cookies (less spread) (Gaines and Donelson, 1985). Furthermore, bran particle size influences cake quality. For instance, cakes produced with up to 36% (flour weight) wheat bran of different particle sizes (50, 80, 250μm) showed the greatest increase in firmness, chewiness, and yellowness when coarser particle sizes were used. In a sensory evaluation, these changes were not favoured by consumers; the best sensory acceptability was reached when finer particle sizes were used (Gomez et al., 2010).

From the review undertaken on Particle Technology, the author did not come across any documentation on precise categorisation of granule size, however, starch granules have been arbitrarily classified as large (>25μm), medium (10–
25μm), small (5–10μm), and very small (<5μm) as reported by Lindeboom et al. (2004). Manek et al. (2012) have reported that tiger nuts (*Cyperus esculentus*) starch granule size range between 2 to 17μm and consists of small- to medium-sized granules (percent volume basis) with a mean particle size of 8.25μm, and they have reiterated that their findings is fairly consistent with the previously reported range of 3–12μm (Umerie et al., 1997). Typical particle size distributions for commercially used starches include maize starch (2–32μm), potato starch (10–100μm), rice starch (2–20μm), tapioca starch (5–35μm), and wheat starch (2–45μm) (Rowe et al., 2003)

2.6.2. Particles in food systems

Particle size reduction according to Amerah et al. (2007) and Wang and Jeronimidis (2008) is a process which results in disruption of the outer seed coat to expose the endosperm, and consequent fracture of the endosperm. Van der Meeren et al. (2004), have indicated that accurate particle size measures are essential to the control of quality in many products: powders and granules e.g. pigments, cement, pharmaceutical ingredients; suspensions, intravenous emulsions and slurries e.g. vaccines, milk, mining muds; aerosols and sprays e.g. asthma inhalers and crop protection sprays.

Particles occur in powders, emulsions, and suspensions. In each of these cases, the particle size has a pronounced influence on the macroscopic behaviour of the product. In powders, the particle size distribution is of utmost important for their dustiness, as well as for their transport properties (Fellows, 1997). Van der Meeren et al. (2004) reiterated that large particles of high density are generally free flowing and can be easily discharged from a storage reservoir through an
opening in the bottom. They further went on to state that free-flowing products are more susceptible to segregation during pneumatic transport. However, smaller particles, on the other hand, are much more cohesive and may give rise to arching in discharging reservoirs.

As far as emulsions and suspensions are concerned, the particle size distribution largely affects creaming or sedimentation phenomena. Besides, the particle size distribution also affects the sensorial properties of some food products, such as chocolate, ice cream, mustard and ketchup: Ziegler et al. (2001) indicate that large particles may give rise to the oral perception of sandiness, grittiness, or chalkiness. Reduction of particle size can also facilitate handling and improve mixing of ingredients, but risks unwanted effects from very small particles due to dust and gastric ulcer-inducing properties in e.g. pigs (Ayles et al., 1999). Finally, the particle size influences the rheological properties of food products such as tomatoes concentrate and molten milk chocolate (Ouden, 1997, Ziegler et al., 2001).

The particle size of a dietary fibre has a role in colonic function by affecting transit time, fermentation, and faecal bulking (Guillon and Champ, 2000). Tosh and Yada (2010) has cited from Auffret et al. (1994) that increased initial rate of water absorption with decreased particle size of dietary fibre. However, the initial particle size of a fibre may also change after ingestion because of chewing, stomach grinding, and degradation by intestinal bacteria. Analysis of particle size distribution is typically carried out by dry sieving through a series of sieves with decreasing mesh size, as described originally by Parrott and Thrall (1978) in an investigation of physical properties among fibre sources, but for a more reliable data on tiger nut particle size Laser diffraction particle size analysers was used.
Laser diffraction particle size analysers provide indirect size measurements of spherically equivalent particles, based on the principle that particles of a given size diffract light through a given angle that increases logarithmically with decreasing size (Beuselinck et al., 1998).

2.7. Edible Nuts

Edible nuts are dry, one seeded fruit or seed of a great number of plants, which do not dehisce at maturity, and are usually enclosed by a rigid outer casing or shell. Botanically a nut is defined as a hard and indehiscent (one-seed fruit); of the ‘nut’ utilised by various peoples, only a few, such as the acorn, chestnut and the hazelnut meet the botanical definition (Heiser, 1981). Most nuts grow on large shrubs or trees and are known as tree nuts. Tree nuts include almonds (*Prunus amygdalus*), hazelnuts (*Corylus avellana*), Brazil nuts (*Bertholletia excelsa*), pecans (*Carya illinoensis*) and coconuts (*Cocos nucifera*). Although not covered strictly by the botanical definition, walnuts (*juglans regia*) or peanuts (*Arachis hypogaea*) are usually considered to be nuts (ICMSF, 2005). From this definition, tiger nut is treated as a nut in this work.

Nuts and seeds have constituted a part of mankind’s diet since pre-agricultural times (Eaton and Konner, 1985), providing a complex food rich in macronutrients (lipids and protein) and micronutrients and fibre, as well as other bioactive phytonutrients yet to be discovered. In addition, nuts and seeds are widely used for their oils as well as in butters and cookies, and they are consumed directly as food due to their characteristic flavours (Kim et al., 2000). Nuts of various kinds have long served as a highly concentrated source of nutrition. From the archaeological record, it is known that nuts of various wild plants were frequent
source of food in prehistoric times (Heiser, 1981). Nuts and seeds are lucrative and have anti-aging effects due to their abundance of in vitamins B and E (Venkatachalam and Sathe, 2006). Among the fatty acids commonly found in nuts and seeds, 70-80% is essential fatty acids, which are components of the plasma membrane and contain a lipoprotein found in brain cells known as lecithin. Therefore, nuts and seeds are excellent nutrients for growing children and good snacks for adults (Pattee and Young, 1982).

The chemical compositions of some selected nuts have been presented in Table 2.2

### 2.7.1. Nuts composition

Blomhoff et al. (2006), posited that nuts are highly nutritious and of prime importance for people in several regions in Asia and Africa. Nuts are good sources of lipids (e.g. pecan 70 %, macadamia nut 66 %, Brazil nut 65 %, walnut 60 %, almonds 55% and peanut butter 55 %). In addition, nuts are widely used for their oils as well as in butters and cookies, and they are consumed directly as food due to their characteristic flavours. They are lucrative and have anti-aging effects due to their abundance of in vitamins B and E (Kim et al., 2000, Venkatachalam and Sathe, 2006).

Despite their high fat content, they are not harmful because they contain a low proportion (4–16%) of saturated fatty acids. Nearly one half of the fat content of nuts consists of unsaturated fatty acids, which include both mono- (oleic acid) and poly- (linoleic and α-linolenic acid) unsaturated fatty acids (MUFA and PUFA respectively). The fatty fraction of nuts contains also plant sterols with antioxidant (Vivancos and Moreno, 2005) and cholesterol-lowering effects (Segura et
al., 2006). Nuts are also rich sources of other bioactive macronutrients, such as protein (25% of energy) and dietary fibre, which ranges from 4 to 11 g/100 g and in standard servings provide 5–10% of daily fibre requirements. The pH of all nuts and oilseeds is near neutral, in theory rendering them susceptible to growth of all kinds of microorganisms during development and before natural drying at maturity. In practice, shells provide a highly effective barrier to the entry of bacteria during nut growth (ICMSF, 2005).

<table>
<thead>
<tr>
<th>Micronutrients (mg)</th>
<th>Almonds</th>
<th>Walnuts</th>
<th>Pistachio</th>
<th>Hazelnuts</th>
<th>RDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>728</td>
<td>523</td>
<td>680</td>
<td>1042</td>
<td>2000</td>
</tr>
<tr>
<td>Calcium</td>
<td>248</td>
<td>61</td>
<td>114</td>
<td>110</td>
<td>800</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>474</td>
<td>513</td>
<td>290</td>
<td>485</td>
<td>700</td>
</tr>
<tr>
<td>Magnesium</td>
<td>275</td>
<td>201</td>
<td>163</td>
<td>120</td>
<td>375</td>
</tr>
<tr>
<td>Iron</td>
<td>4.3</td>
<td>3.12</td>
<td>4.7</td>
<td>4.2</td>
<td>14</td>
</tr>
<tr>
<td>Copper</td>
<td>1.11</td>
<td>1.36</td>
<td>1.73</td>
<td>1.33</td>
<td>1</td>
</tr>
<tr>
<td>Manganese</td>
<td>2.54</td>
<td>3.9</td>
<td>6.18</td>
<td>1.28</td>
<td>2</td>
</tr>
<tr>
<td>Selenium</td>
<td>2.8</td>
<td>17</td>
<td>2.4</td>
<td>9.3</td>
<td>55</td>
</tr>
</tbody>
</table>

Adapted from Alexiadou and Katsilambros (2011)

2.7.1.1. Health Benefits of Nuts

Nuts and seeds contribute significantly to the nutrition of human populations in many parts of the world. Consumed together with other food items, in sauces for example, or alone as snacks, these edible plants can be dried and stored for convenient use during the cold or dry season in West Africa (Glew et al., 2006). They are complex foods containing cholesterol lowering mono- and polyunsaturated fatty acids, arginine (a precursor to the vasodilator nitric oxide), soluble fibre, and several antioxidant polyphenols (Sabate and Ang, 2009).
Postprandial vascular reactivity is characterized by decreased bioavailability of nitric oxide and increased expression of pro-inflammatory cytokines and cellular adhesion molecules (Ros, 2010). It is not surprising that the evidence supporting the cardio protective effects of diets high in nuts is robust as multiple mechanisms work together to reduce risk. Prospective data from the Physicians’ Health Study (Albert et al., 2002) indicated reduced risk of sudden cardiac death associated with nut consumption originally perceived as being unhealthy because of their high-fat content.

2.7.2. Tiger nuts

Tiger nuts (Cyperus esculentus L), which is commonly known as yellow nut-grass or chufa flatsedge belongs to the Cyperaceae family, it is a perennial herbs from slender, scaly rhizomes ending with hard, greyish, orange to dark tubers that grows to heights between 24 and 55 cm (Swift, 1989). Other types of tiger nuts exist. For example, there are two types of variety which have been identified in USA; the cultivated (yellow nutsedge) and the wild (purple nutsedge). The stems are three-sided and triangular in cross-section while the leaves are yellow to green in colour with a distinct ridge. Its fibrous roots originate from the tubers, rhizomes, and basal bulbs (Defelice, 2002) and (Parker et al., 2000).

It is the only known tuber or root plant as affirmed by Manek et al. (2012) that produces underground storage organs containing a significant amount of all three storage reserves: starch, sucrose and oil, as compared to other known plants belonging to roots and tubers that normally accumulate high amount of starch or sugars in their roots or tubers tissues.
Fresh or rehydrated tiger nuts tubers are about 10-30mm in length with an obtuse, oval or cone end of irregular form when dry and spherical or egg-shaped when swollen in water, which has a crispy texture when chewed. Like ginger and Chinese water chestnut, tiger nuts do not gelatinised during cooking; this may be due to cross-links between diferulates attached to the cell wall polymers involved in cell adhesion as suggested by Parker et al. (2000).

Zohary and Hoff (2000), consider tiger nut tuber as being rank among the oldest cultivated plants in ancient Egypt. The cultivation of Tiger nuts originates from ancient Egypt. According to Darby et al. (1977), the nuts have been found in archaeological sites. From Egypt, the Arabs merchants helped to spread its cultivation North and West of Africa, Sicily and Spain. In Ancient Egypt it was cultivated for its sweetmeat, ingredient in barley beer and oil rich tubers.

Tiger nut’s tubers either grow wild as weed or cultivated as a cash crop. Even though it is normally found in tropical and temperate regions, it has been reported growing well in cold regions like Alaska (Holm et al., 1977, De Vries, 1991). It is found throughout the USA as a common weed in agronomic and horticultural crops. The fast-growing and tuberous habit of yellow nut-grass allows it to out-compete cultivated crops in fields, ornamentals in flower beds, and native species in other. It is considered to be a nuisance plant in some areas like: eastern and southern Africa, North and Central America, Poland and Israel, in view of this, all attempts are being made to exterminate this plant (Wang et al., 2009, Santos, 2009, Johnson III and Mullinix Jr, 2007).
According to Pascual et al. (2000), the tubers are used to feed hogs and pasture in the field in some states like Florida, Georgia and Alabama despite it being labelled as noxious plants in other states in the United State of American.

In Ghana, cultivation of this crop is predominantly carried out by women to earn a living. There are two distinct types of tiger nuts (both cultivated) that are found in Ghana; the Kwahu (South east) type with yellowish brown nuts and the Fanti (South west) type with a mixture of dark brown and black nuts. Although all the two are edible yet preference is normally given to the yellowish brown type because of its flavour and storage quality (Tetteh and Ofori, 1998).

Defelice (2002), has reported that there are many other names apart from yellow nutsedge for both the weedy and cultivated types most of which refer to either the grass-like (sedge) appearance or the edible underground tubers. Some of the names often use depending on where it is found or cultivated are; chufa, tiger nuts, chufa flatsedge, coco, ground almond, edible galingale, earth-almond, Hab’el aziz (“the precious grain”) or “the grain of the precious, “funeral gift”, northern nutgrass, rush nut, Zulu nut, yellow nutgrass, rush nut and ‘atadwe’ (‘battery charger’) in Ghana.

Due to the fibrous nature of tiger nut tubers, they are soaked prior to processing (milling and extraction of milk) or eating it raw. Soaking, according to Chiang and Yeh (2002) is a slow process controlled by the diffusion of water into food which is essential step in wet milling.

Physical properties of biological materials such as tiger nuts have unique characteristics which set them apart from other engineering materials. The irregular shape and fibrous nature of the nuts complicate the analysis of their
behaviour. Physical properties of tiger nuts like other agricultural products; cereals, fruits and vegetables are needed for the design of processing equipment. According to Bart-Plange et al. (2012), the study of physical properties plays an important role in developing sensors to control machines and processes. It helps to detect quality differences during harvesting, handling and storage. Various types of cleaning, grading and separation equipment are designed on the basis of their physical properties (Teye and Abano, 2012). According to Esref and Halil (2007) the knowledge of physical properties constitutes an important and essential engineering data in the design of machines, storage structures, and processing. Physical properties affect the converting characteristics of solid materials either by air or water, cooling and heating of food products (Sahay & Singh, 1994; Teye & Abano, 2012).

It has been reported by the following researchers (Cantalejo, 1997, Linssen et al., 1989, Mokady and Dolev, 1970, Negbi, 1992) that the nuts contain these nutrients; carbohydrate, fats (monounsaturated free fatty acid is the predominant, it is reported that the fat can be compared to olive oil in terms of composition of free fatty acids), protein, vitamins, dietary fibre, dietary minerals and starch.
Table 2-5: Proximate composition of tiger nut tuber (g/100) compared to other tubers

<table>
<thead>
<tr>
<th>Product</th>
<th>Scientific name</th>
<th>Moisture</th>
<th>Ash</th>
<th>Lipid</th>
<th>Protein</th>
<th>Carbohydrate</th>
<th>Starch</th>
<th>Reducing sugar</th>
<th>Total sugar</th>
<th>Sucrose</th>
<th>Fibre</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tiger nut</td>
<td><em>Cyperus esculentus</em></td>
<td>7.7-32.2</td>
<td>1.6-2.1</td>
<td>20.9-36.1</td>
<td>3.3-6.7</td>
<td>31.3-62.9</td>
<td></td>
<td>31.3-62.9</td>
<td></td>
<td></td>
<td>19.2</td>
<td>As reported in the Thesis</td>
</tr>
<tr>
<td>Potato</td>
<td><em>Solanum tuberosum</em></td>
<td>78.75</td>
<td>0.95</td>
<td>0.16</td>
<td>1.97</td>
<td>18.17</td>
<td>13.82</td>
<td>0.28</td>
<td>0.47</td>
<td>0.31</td>
<td>0.68</td>
<td>Lorbardo et al (2011)</td>
</tr>
<tr>
<td>Yam</td>
<td><em>Dioscorea spp.</em></td>
<td>62.50</td>
<td>1.5</td>
<td>0.2</td>
<td>2.55</td>
<td>34.6</td>
<td>18.89</td>
<td></td>
<td></td>
<td></td>
<td>1.68</td>
<td>Murniece et al (2011)</td>
</tr>
<tr>
<td>Jerusalem artichoke</td>
<td><em>Helianthus tuberosus</em></td>
<td>80.0</td>
<td>1.07</td>
<td>0.21</td>
<td>1.41</td>
<td>14.82</td>
<td>Inulin</td>
<td>16.18</td>
<td>2.54</td>
<td>0.12</td>
<td>1.15</td>
<td>3.12</td>
</tr>
<tr>
<td>Yacon</td>
<td><em>Smallanthus sonchifolius</em></td>
<td>80.69</td>
<td>1.1</td>
<td>0.13</td>
<td>2.22</td>
<td>13.8</td>
<td>Inulin</td>
<td>6.09</td>
<td>9.9</td>
<td>3.15</td>
<td>1.75</td>
<td>Scher et al. (2009)</td>
</tr>
<tr>
<td>Sweet Potato</td>
<td><em>Ipomoea batatas</em></td>
<td>70.54</td>
<td>1.49</td>
<td>3.88</td>
<td>0.66</td>
<td>25.74</td>
<td>10.29</td>
<td>0.39</td>
<td>6.63</td>
<td>4.77</td>
<td>0.88</td>
<td>Lai et al. (2001)</td>
</tr>
<tr>
<td>Cassava</td>
<td><em>Manihot esculentus</em></td>
<td>60.0</td>
<td>4.97</td>
<td>0.3</td>
<td>1.4</td>
<td>38</td>
<td>29.4</td>
<td>5.0</td>
<td>7.98</td>
<td>1.7</td>
<td>1.18</td>
<td>Maieves et al. (2011)</td>
</tr>
</tbody>
</table>

(Adapted from Sanchez Zapata et al. 2012)
Table 2-6: Proximate composition of tiger nut tubers (g/100g) compared to nuts

<table>
<thead>
<tr>
<th>Product</th>
<th>Scientific name</th>
<th>Moisture</th>
<th>Ash</th>
<th>Lipid</th>
<th>Protein</th>
<th>Carbohydrate</th>
<th>Total sugar</th>
<th>Fibre</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tiger nut</td>
<td><em>Cyperus esculentus</em></td>
<td>7.7-32.2</td>
<td>1.6-2.1</td>
<td>20.9-36.6</td>
<td>3.3-6.7</td>
<td>50.5-82.1</td>
<td>31.2-62.9</td>
<td>19.2</td>
<td>As reported in the Thesis</td>
</tr>
<tr>
<td>Walnuts</td>
<td><em>Juglans regia</em></td>
<td>2.7</td>
<td>1.82</td>
<td>65.2</td>
<td>1.41</td>
<td>14.82</td>
<td>0.7</td>
<td>6.4</td>
<td>Ros (2010) and Freitas and Naves (2010)</td>
</tr>
<tr>
<td>Pistachios</td>
<td><em>Pistachia pinea</em></td>
<td>5.74</td>
<td>3.21</td>
<td>44.4</td>
<td>2.22</td>
<td>13.8</td>
<td>2.2</td>
<td>9.0</td>
<td>Ros (2010), Panahi and Khezri (2011) and Venkatachalam and Sathe (2006)</td>
</tr>
<tr>
<td>Pine nuts</td>
<td><em>Pinus pinea</em></td>
<td>1.47</td>
<td>2.5</td>
<td>68.4</td>
<td>0.66</td>
<td>25.74</td>
<td>1.0</td>
<td>3.7</td>
<td>Ros (2010)</td>
</tr>
<tr>
<td>Peanuts</td>
<td><em>Arachis hypogea</em></td>
<td>3.53</td>
<td>2.27</td>
<td>49.2</td>
<td>1.4</td>
<td>38</td>
<td>1.1</td>
<td>8.5</td>
<td>Freitas and Naves (2010) Ros (2010)</td>
</tr>
</tbody>
</table>

Adapted from Sanchez Zapata et al. 2012
Tiger nut is not a real nut as described under the nuts: despite its name, it is considered to be tuber but for the purpose of this work it is treated as nuts as indicated previously. Its chemical composition shares characteristics with tubers Table 2.3 and with nuts Table 2.4. The moisture content is lower than the moisture contents reported for true tubers such as potato, yam, Jerusalem artichoke, sweet potato and cassava as indicated by Lombardo et al. (2012). The ash content according to Coskuner et al. (2002) is within the range for both tubers and nuts. From Table 2.2, 2.3 and 2.4, it could be observed that tiger nut’s lipid, protein and carbohydrate contents are higher than those in other tubers. However, if tiger nuts is compared to real nuts, it is observed that the fibre content is within the usual range for nuts, but the moisture and carbohydrate contents are much higher and the lipid and protein contents are lower than in real nuts. In general tubers have a high contents of carbohydrate, their profile and relative contents decreases and the reducing sugar contents increases during storage (Coskuner et al., 2002).

According to Sanchez-Zapata et al. (2009), tiger nut oil have the following free fatty acids; 14:0 (0.2%), 18:0 (3.2%), 20:0 (0.4%), 16:1 n-7 (0.3%), 18:1 n-9 (72.6%), 18:2 n-6 (8.9%) and 18:3 n-3 (0.4%). In a research carried out by Dubois et al. (2007) on some vegetable oils, they asserted that tiger nut oil has a monounsaturated profile (>60%)
Table 2- 7: Tiger nut tubers chemical composition (g/100g) dry basis according to different authors.

<table>
<thead>
<tr>
<th>Origin</th>
<th>Moisture</th>
<th>Carbohydrate</th>
<th>Fats</th>
<th>Fibre</th>
<th>Protein</th>
<th>Minerals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain 1983</td>
<td>7.1-9.7</td>
<td>31.8-38\textsuperscript{a}</td>
<td>23-28.3</td>
<td>9.8-11</td>
<td>8.2-9.2</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spain 1984</td>
<td>8</td>
<td>31.0\textsuperscript{a}</td>
<td>23.1</td>
<td>8.1</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR 2002</td>
<td>6.5-7.8</td>
<td>29.9\textsuperscript{a}</td>
<td>22.5-31.0</td>
<td>9.9-14.5</td>
<td>3.14-9.8</td>
<td>1.4-2.2</td>
</tr>
<tr>
<td></td>
<td>7.1</td>
<td>15.4\textsuperscript{b}</td>
<td>26.1</td>
<td>11.9</td>
<td>7.6</td>
<td>1.7</td>
</tr>
<tr>
<td>Nigeria 1989</td>
<td>5.8</td>
<td></td>
<td>25.7</td>
<td>5.5</td>
<td>7</td>
<td>1.9</td>
</tr>
<tr>
<td>Nigeria 1997</td>
<td>3.6</td>
<td>52.3</td>
<td>29.7</td>
<td>12.9</td>
<td>2.7</td>
<td>2.5</td>
</tr>
<tr>
<td>Turkey 2002</td>
<td>6.6-6.9</td>
<td>61.2-63.5</td>
<td>23.5-25.3</td>
<td>8.7</td>
<td>4.2-6.2</td>
<td>1.3-1.5</td>
</tr>
</tbody>
</table>

Adapted from (Toran and Rovira, 2002)

(a) Starch (b) Sucrose and other sugars

Manek et al. (2012), have found that tiger nuts (*Cyperus esculentus*) starch is a brilliant white, odourless powder with a warm bland taste and smooth texture. As indicated from the micrographs in Fig.2, tiger nuts (*Cyperus esculentus*) starch exhibits elliptical to spherical granules with a relatively smooth surface. Scanning electron microscopy indicates that tiger nuts (*Cyperus esculentus*) starch has uniform granular size, shape, and morphology. Size distribution of a potential excipient has been shown to affect various formulation characteristics like flowability, compactibility, water binding capacity, and drug release (Li et al., 2004).
2.7.2.1. Unsubstantiated health Claims

Tiger nuts have been used medically by the ancient Egyptians in these areas; mouth cleaning, enemata, ophthalmic, ointment, wound dressings, fumigation, to sweeten the smells of houses and clothes, together with myrr incense (Defelice, 2002). In modern Egypt, El-Shebini et al. (2010) have reported that daily consumption of raw tiger nut (30g) has been shown to contribute to effective weight loss and improvement of the metabolic disorders among obese diabetic patients.

In South Africa, the Zulus chew the roots for relief from indigestion; Zulu girls eat a porridge containing the mashed nuts to hasten menstruation. In West, North Africa and India, the nuts are considered as aphrodisiac spermatogens. Preventing inflammation of the respiratory passages has also been attributed to consumption of the nuts (Cantalejo, 1997).

In Ghana, the nuts are promoted by the herbalists and alcoholic beverage industries as, a digestive tonic, having a heating and drying effect on the digestive system, alleviating flatulence, increasing urine production, relieving menstrual pain, reducing cardiovascular diseases, improving intelligence, having aphrodisiac properties, and increasing breast milk output during lactation; especially when a processed food product contains tiger nuts as one of its ingredients.

There is really no scientific evidence to support the vast majority of health claims made on this nuts as far as Ghanaian community is concern.

Ejoh et al. (2006), have proposed that weaning food formulae can be developed from the flour of the nuts as a result of its high nutritional contents. In Spain a “hot cake” drink called horchata de chufa has been developed from it, which is currently being promoted as “health Food.” (Don Simon, horchata de Chufa de Valencia, 2006).
2.8 **New Product development (Bread and Biscuit)**

### 2.8.1. Bread

The word bread covers a considerable variety of products, with variations in recipe, processing, weight, shape, degree of baking, etc. but they share the same four basic ingredients (Brown, 1993). Mondal and Datta (2008), affirm that bread is a staple food which is consumed daily and its history dates back to the 9th century. Bread-making is a complex process that involves many physicochemical (i.e. texture, volume, colour) and organoleptic characteristics (e.g. volatiles) and structural transformations which lead to the production of an aerated baked product from basic ingredients such as wheat flour (flour type), water, yeast and salt. According to Ktenioudaki et al. (2011), the bread-making process is generally divided into three main steps: mixing, fermentation (proving), and baking (cooking time and temperature). During all these stages, deformations of different magnitudes take place and rheology is involved in every step of the process. During mixing, extreme deformations take place above the point of rupture as a result of forces exerted on the dough from the mixer, whereas during proving and baking the deformations are much smaller and are due to the difference in pressure between the gas cells and the atmosphere (Bushuk, 1985, Dall'Asta et al., 2013).

### 2.8.2. Biscuits

The word biscuit comes from the Latin *panis biscoctus*. This stands for “twice-baked bread.” A biscuit covers a wide range of flour baked products, commonly a biscuit is an unleavened cake or bread which is crisp and dry and in a flat, thin and small shape. Biscuits have evolved over many years to many different baking methods such as pastries, tarts and short bread. Biscuits are made from a number of different
ingredients. Flour is the most important ingredient. Changing the type of flour can change the biscuits properties such as its texture and crispness. Wholemeal wheat flour is used in the ‘digestive’ or ‘wheat-meal’ type of biscuits (Stradley, 2004).

Biscuits belong to the group of food products that are very popular in daily diet of almost all profiles of consumers (Popov-Raljić et al., 2013), having not only the nutritive purpose but influencing also on emotional status of consumers with the effects even on the positive mood enhancement (Turner et al., 2010). They are ideal for their nutritive value, palatability, compactness, convenience and low moisture content than cakes and bread, safer from microbiological spoilage and have long shelf-life, which results in their availability almost everywhere at any time (Sharif et al., 2003, Galdeano and Grossmann, 2006, Akubor, 2003, Kulkarni, 1997). Therefore, the alteration of composition of cookies directed to enhancement their nutritive and/or functional properties or to enabling of their consumption to the groups of consumers with special needs and demands has been the subject of interest of many researchers.

The process of biscuit making varies between recipes. However the common biscuit method is to first mix the ingredients into dough. The dough is then kneaded and rolled out to an even thickness and then cut into an even size and baked in an oven. Since the early 19th Century biscuit making has become mechanised due to the technology available. Biscuits are baked both commercially and in the home.

The role of the biscuits in the diet has also changed throughout the years. In the early 19th Century biscuits were only eaten by the upper class and were an expensive novelty. At this time biscuits were a minor role in the popular diet. It was not until the 1960s when the same quality of biscuits was eaten in the home. Nowadays, emphasis is on healthy biscuits with low glycaemic index, more protein and will
increase the dietary fibre intake, high resistant starch, decrease in calorie and carbohydrates of baked goods. These uses now popularly include health foods, slimming aids or digestive aids. They are now accompaniments to hot drinks, snacks or substitutes for bread (Stradley, 2004).

2.9. Literature review: Emergent Themes

The main themes that emerged from the reviewed literature are; conflicting information on the chemical composition especially the mineral contents of the tiger nut’s tubers, limited information on the physical properties of the tubers, the unsubstantiated health claims across the globe, possibility of using the tubers to achieve sustainable food security programmes in African (the tubers thrive at any soil, arable, rocky, or water logged), possibility of using the bagasse as an ingredient in preparation of prebiotic food products, developing new food products from the tubers for diabetic patients. Two schools of thought again emerged; one group of researchers are of the view that this crop is nuisance to cash crops (pepper, tomatoes, onions etc.) so it should be exterminated, whereas the other group of which most of the health claims are based, believe that it is a millennium crop and much attention ought to be given to it. In view of all what have been written and said about tiger nuts, it is wealth delving into it to ascertain the veracities of conflicting and unsubstantiated health claims on it.
CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1. Introduction

In this chapter, the materials and methods used in the analyses of the physical properties, the proximate compositions, characterisation of the nuts for product formulation and as product ingredients are based on the methods described in (Association of Official Analytical Chemists (AOAC), 2003, Association of Official Analytical Chemists (AOAC), 2005, Association of Official Analytical Chemists (AOAC), 2012) and manual developed for students who work in the Food and Nutrition Laboratory.

3.2. Physical characteristics

- Hydration properties of tiger nut at room temperature (19-21ºC)

Dried fresh tiger nuts were obtained from three countries; Ghana, (two different types, black and brown), Cameroon and Spain (UK market). Samples from Ghana and Cameroon were procured from local markets in Accra and Bamenda respectively, whilst the other nuts were purchased from UK market (Ludlow Nut Company, Ludlow, Shropshire). All samples were transported in polyethylene bags and stored in a fridge at a temperature of 4°C.

Tiger nuts from Spain (UK market) were use in this study because of sample availability at the time of performing this experiment. This was done to determine how long the nuts should be soaked for the purpose of easily milling and without quality change.
This experiment was conducted using the method proposed by Djombi et al. (2007) with slight modifications. Eight sets of 40 randomly selected nuts were counted and weighed (Sartorius, Model: L 620P, S/No: 8040467, Sartorius Ag. Goettingen, Germany). The nuts were put in labelled plastic cups (8) containing 100mls of distilled water and stored at room temperature. During this experiment, the water was changed every 24 hours. The nuts were superficially dried by blotting with tissue paper four to five times before they were weighed using electronic balance (Sartorius, model) (Turhan et al. (2002), Kashaninejad and Kashiri (2007) and Solomon (2007)). After weighing, the nuts were put back into the plastic cups and soaked again with fresh 100mls distilled water. This procedure was repeated 7 consecutive days. Each test was repeated three times to determine the mean values and the standard deviation. The weight uptake was calculated as follows:

\[ WU(\%) = \frac{W - W_0}{W_0} \times 100 \]

Where \( WU \) is weight uptake, \( W \) is weight of wet tiger nut at any time (g) and \( W_0 \) is initial weight of tiger nut (g).

- **Unit mass**

Five sets, each containing 20 tubers were randomly selected from a polythene bags which were used to store the bulk of samples from each country. Each set was weighed using an electronic balance with accuracy to 0.001g. Each set was soaked with distilled water for 24 hours at room temperature. The soaked samples were weighed and the value obtained were divided by 20 to get the unit mass of the tubers. Biological material test device used in the physical properties study and description of the two axes and two perpendicular dimensions of tiger nuts.
Figure 3-1: Biological material test device used in the physical properties study and description of the two axes and two perpendicular dimensions of tiger nuts. X-axis: the longitudinal axis through the two nodes A and B (Length (L)); Y-axis: the transverse axis (width, W1 and W2) at right angles to the X-axis.

- **Dimensions**

To determine the dimensions of the nuts, three groups of samples from each country consisting of 100 nuts were randomly selected. From each group 20 nuts were again selected randomly and soaked for three days before the length (L) and width (W1 and W2) were measured using a micrometer with accuracy to 0.01mm. The lengths were measured by holding nuts in an upright position between the two nodes (A and B). The diameter was measured by taking two readings from the broadest points near the two nodes and the average taking as the width. The smallest reading was divided by the biggest to get the thickness factor which was then multiplied by the averaged diameter to get the thickness. (See Figure: 3-1).

Geometric mean diameter ($D_g$), sphericity ($\Phi$), surface area (S) and Volume (V) were calculated by using the following equations:

Formulas quoted from (Baryeh, 2001)

\[
D_g = (LWT)^{\frac{1}{3}} \quad (1)
\]

\[
\Phi = \frac{D_g}{L} \quad (2)
\]
3.3. Flour preparation and characterisation

- Bagasse Flour Production

The flours prepared from samples selected from Cameroon (yellow), Ghana (black), Ghana (brown) and Spain (UK market (brown)) were used in the particle size distribution and the chemical characteristic experiments. For health and safety and traceability purposes of tiger nut, it was decided that, tiger nuts (*Cyperus esculentus*) purchased from UK market should be used in the product development. It is believed that the UK, company imported the nuts from Spain. In Spain, the tiger nut industry is well developed as compared to other tiger nut producing countries. For example the cultivation, harvesting, cleaning, drying, packaging, storage and marketing are all standardised as compared to nuts obtained from Ghana and Cameroon.

3.4. Whole nut flour Sample Preparation

All the samples used for the chemical analyses were soaked in water for three days at room temperature (19 ± 21 °C) and the steeping water changed every 24 hours. At the end of this period the nuts were milled using Robot Coupe, model: R 402 V.V (France). The milled nuts were dried in a convectional drying oven at temperature of 40 and 50°C for 24 hours. The two separated dried nuts were ground to fine powder.
• **Soaking and Extraction**

This method is based on the work done on the hydration properties of the nuts. Weighed amounts of the nuts were screened to remove stones and other physical hazards. The sorted nuts were then washed under tap water, nuts that floated to the surface of the water during the cleaning and soaking periods were discarded because they were considered to be not viable, whilst the sank nuts were considered to be healthy. The cleaned nuts were soaked in drawn tap at room temperature (19-21˚C) for 72 hours. The soaked water was changed every 24 hours with fresh tap water. This was done to prevent fermentation of the nuts.

At the end of the three days soaking period, the soaked nuts were washed with fresh water, drained and weighed to determine the amount of water to be used in the grinding. Weighed nuts to tap water ratio of 1:3 (w/v) were used. Due to the fibrous, hard texture nature of the cell wall and irregular shapes of the nuts, a Food processor (Robot Coupe model: R402 V. V France) was first used to reduce the nuts size before grinding and extracting the juice from the slurry. This was repeated twice for the first and second extracts (bagasse and milk) using a centrifuge juice extractor, (Warring juice extractor, manufactured at Toppington, Connecticut, USA, Model 11JE6).

• **Extracted Tiger nut milk**

The cold extracted milk (pooled from first and second extractions) was put in a cooking pan and heated to 85-90˚C for 15 minutes. At the end of this period the hot pan containing the milk was fast cooled in a sink that was loaded with ice cubes and water. The cooled milk was then put into plastic containers and stored in a deep freezer until they were ready to be used.
The extracted milk was heated and fast cooled not only for food safety reason but also to gelatinised the starch which gave chalky sensation in the mouth when the unheated extracted juice was used to formulate yoghurt and ice cream products in the initial studies of the programme. Again this chalky sensation in the mouth when *Chufa horchata* drink produced in Valencia, Spain was first tasted. This problem has since been resolved after the extracted milk was heated and fast cooled before usage.

3.5. Preparation of bagasse generated after the extraction of milk

The waste (solid fraction) were weighed, spread on trays and dried in an oven (Zanussi Combiwave; model number Fcvm/62E) which was set at temperatures of 40 and 50°C for 18 hours. The dried material was then cooled and milled in a coffee machine; Russell Hobbs grinding machine (model number 9702, manufactured in UK) to particle size between 1-200µm. The powder was vacuumed pack and stored at a temperature of (4-7°C) until ready to be used for particle size experiment and food product developments (biscuits and bread).

3.6. Particle Size

Particle size analysis was carried out using a Malvern Mastersizer 2000MU with wet sample unit Hydro-G (Malvern Instruments Ltd, Malvern WR14 1XZ, UK). The samples were suspended in water with pump speed set at 2000rpm and stirrer speed at 750rpm. A general purpose analysis model was used with particle refractive and absorption indices of 1.53 and 0.01, respectively, while the refractive index of water as the dispersant was 1.33. Particle size was defined as the volume weighted mean (d[4,3]). Unlike dry or sieve analysis, the reported size is possibly for partially hydrated samples, but all the particles were detected within the range (0.020 –
2000.00 µm) of the Mastersizer. The obscuration in all the measurement ranged from 4.82% to 12% and red laser measurements was at 3000.

3.7. Proximate Composition Analysis

All the methods used in the proximate compositions are based on existing equipment used in the Food and Nutrition Laboratory.

3.7.1. Moisture Content

The moisture and dry matter were determined by measuring the loss of weight after heating the sample in a hot air oven at a temperature of 105°C. A small Aluminium pan filled with sand was glowed in an oven for 3 hours. The pan was cooled to room temperature in a desiccator. The pan and sand were weighed to the nearest 1mg. 5mg of weighed sample was put in the pan and was mixed with the sand. The pan, sand and sample were dried for 2 hours at 105°C. The heated sample was cooled down in a desiccator to room temperature and weighed to the nearest 1mg. Sample were dried again for 30 minutes, cooled down and weighed. This procedure was repeated until a constant weight was reached. Moisture content of the sample was calculated according to the following equation:

\[
\text{% of moisture content} = \frac{\text{Wet weight (g)} - \text{Dried weight (g)}}{\text{Sample weight (g)}} \times 100
\]

3.7.2. Total Solids

The % total solids was calculated by this formula

\[
= 100 - \text{% moisture content}
\]
3.7.3. Ash Content

Prepared samples were weighed into a pre-weighed ceramic crucible and incinerated for 18 hours at 550˚C in a carbolite 11/7 Muffle Furnace Ltd., Bamford, Sheffield, UK. The inorganic residue in the crucible was the ash of the sample.

Ash content of the sample was calculated according to the following equation:

\[
\text{Ash (\%)} = \frac{(\text{Weight of crucible+food before ashing}) - \text{Weight of crucible (g)}}{\text{Sample weight (g)}} \times 100
\]

3.7.4. Mineral contents

To eliminate the risk of contamination, all glassware and polyethylene material was washed with distilled water after each use, soaked in a 2% HNO₃ solution (at least overnight), and rinsed several times with bi-distilled deionised water.

The following mineral contents (Calcium, Chromium, Cobalt, Copper, Iron, Potassium, Magnesium, Manganese, Sodium, Phosphorus, Selenium and Zinc) were determined according to the method described by Nielsen (1998). Borosilicate digestion tube (Kjeldahl digestion tube) was washed, rinsed and soaked in 2% Nitric acid solution. After this, it was rinsed in distilled water and oven dried before used.

The dried samples were weighed into borosilicate digestion tube. Digestion was conducted in Gerhardt Kjeldatherm digestion block which comprised of 40 positions (Gerhardt Laboratory Instruments, Bonn, Germany) at 60˚C for 1 hour, the temperature was then raised to 90˚C for another 1 hour and was raised to 110˚C for 30 minutes, finally it was raise to 140˚C for 2 hours. The digestion process was
performed in the scrubber unit Gerhardt Turbosog unit) in which acid fumes was neutralised through a 15% NaOH. The cooled sample was diluted to 50mls with deionised water in volumetric flask and transferred into 50mls labelled centrifuge tube. Stock solutions from all the required minerals were used to prepare the standard solutions for the analyses using two different types of machines to determine the macro and microminerals Varian 725-ES inductively coupled Plasma Optical Emission Spectrophotometer (ICP-OES) and Thermo Scientific X series Z (ICP-MS, Hemel Hempstead, UK). The layout of a typical ICP-OES instrument is shown in the Figure below with the following operating conditions:

Power = 1400 watts

Coolants gas flow = 15Lmin\(^{-1}\) and 13 Lmin\(^{-1}\) with Argon gas

Auxiliary gas flow = 1.5Lmin\(^{-1}\) and 0.75Lmin\(^{-1}\) with Argon gas

Nebuliser gas flow = 0.68Lmin\(^{-1}\) and 0.70Lmin\(^{-1}\) with Argon gas

Nebuliser type was v-groove, spray chamber type was surman-masters and viewing height = 8mm above load coil.
3.7.5. Protein content

The protein content was determined by Kjeldahl method. Samples were weighed into nitrogen free paper and then transferred to borosilicate digestion tubes. Kjeldahl catalyst tablet (3g K₂SO₄, 105mg CUSO₄.5H₂O and 105mg TiO₂, BDH Ltd UK) and 10ml of concentrated H₂SO₄ were added to each tube. Digestion was conducted in a Gerhardt Kjeldatherm digestion block comprising of 40 positions (Gerhardt Laboratory Instruments, Bonn, Germany) at 105°C for 15minutes, the temperature was then raised to 225°C for a further 60minutes and finally was raised to 380°C for 45minutes. The digestion process was conducted on a digestion block attached to a scrubber unit (Gerhardt Turbosog unit) in which acid fumes was neutralised with 15% NaOH. The cooled sample was distilled using a Gerhardt Vapodest 40 distillation unit where the sample was diluted with distilled water and neutralised with 37% NaOH. The liberated Ammonia in sample was then trapped into 50ml of 4% orthoboric acid.
\((\text{H}_3\text{BO}_3)\) with 4.5 BDH as an indicator) by automatically steam distillation. The distillate was then back-titrated against 0.1M H\(_2\)SO\(_4\).

The Protein content was calculated according to the formula stated below

\[
n = 0.1 \text{ M} \times [(50 \text{ mL} - B) / 1000]
\]

\[
\%N = [(n \times 14.07 \text{ g}) / 0.5 \text{ g}] \times 100
\]

\%
Protein = \%N \times \text{conversion factor}

Where: B = Titration of base; n = mols. of NH\(_3\); \%N = \% nitrogen

6.25 \text{ s conversion factor, protein is considered as 16\% Nitrogen.}

3.7.6. Total lipid

Lipid was estimated by exhaustive extraction of two to three grams of dried samples with petroleum ether using rapid soxhlet extraction apparatus (Soxtherm SE- 416, Gerhardt, Bonn, Germany). Briefly, two to three grams of dried ground samples was weighed into cellulose thimbles and lightly plugged with cotton wool. The thimbles were placed into a wire support and inserted into a pre-weighed extraction. Using a bottle-top dispenser (Eppendorf Varispenser, Eppendorf UK Ltd, Stevenage, UK), 40 mL of petroleum ether was added into the beaker that was properly mounted on the heating plate of the Soxtherm unit. The extraction process was carried out following the instruction from the Multistat unit. At the end of the extraction, the beakers were removed from the unit into a fume cupboard. The thimbles and their holders were removed from the beaker and under full fume extraction traces of solvent were allowed to evaporate before re-weighing the beakers.

The total lipid content was calculated using the following equation:

\[
\text{Lipids} \text{ (\%)} = \frac{\text{Final weight of beaker} - \text{Initial weight of beaker}}{\text{Sample weight}} \times 100
\]
3.7.7. Carbohydrate (CHO)

Two methods were used to calculate the crude carbohydrate of the samples.

- **First method (A):**

  \[
  \text{CHO} = 100 - (\text{Moisture} + \text{Ash} + \text{Protein} + \text{Fat}) \quad \text{(by difference method)} \quad (\text{Galla et al., 2012})
  \]

- **Second method (B):**

  \[
  \text{Energy content} = 4 \times \text{Protein} + 4 \times \text{CHO} + 9 \times \text{Fat} \quad (\text{Galla et al., 2012})
  \]

  \[
  \text{CHO} = \frac{\text{Energy Content} - (4 \times \text{Protein} + 9 \times \text{Fat})}{4}
  \]

3.7.7.1. **Determination of High Molecular Weight Dietary Fibre (HMWDF) from tiger nuts flour and its products using Megazyme integrated total dietary fibre method.**

Samples from the prepared tiger nut flour as an ingredient and the tiger nut bread dietary fibre fractions IDF, SDFP and SDFS were determined using both the Megazyme integrated total dietary fibre assay kit (K-INTDF 06/12 Megazyme international, CO.Wicklow, Bray, Ireland) developed by McCleary (2007) and the integrated enzymatic gravimetric and chromatographic procedures as detailed by AOAC methods 2009.01 and 2011.25 (Association of Official Analytical Chemists (AOAC), 2005) and 2011.25, (Association of Official Analytical Chemists (AOAC), 2012) were used.
Ethanol (or IMS) 95% v/v. was prepared by adding 10mls of deionised water to 190mls of the absolute alcohol (100%). Ethanol (78%) v/v (180 mL deionised water was placed into 1Litre volumetric flask. It was diluted to volume with 95% v/v ethanol and mixed thoroughly.

Stock amyloglucosidase (AMG) solution 3300 Units/mL was dispensed in 50% v/v in viscous solution of glycerol using a positive displacement dispenser.

Pancreatic α-amylase (50 Units/mL)/AMG (3.4 Units/mL) was prepared immediately before use, 0.10g of purified porcine pancreatic α-amylase (150,000 Units/g; AOAC Method 2002.01) was dissolved in 290 mL of sodium maleate buffer (50 mM, pH 6.0 plus 2 mM CaCl$_2$ and 0.02% sodium azide) and stirred for 5 min. 0.3 mL of AMG was added. Protease (50 mg/mL; 350 Tyrosine Units/mL in 50% v/v glycerol). Solution being viscous; was dispensed using a positive displacement dispenser. Protease was devoided of α-amylase and essentially devoid of β-glucanase and β-xylanase.

The LC retention time standard which has the distribution of oligosaccharides (DP > 3) corn syrup solids (DE 25; Matsutani Chemical Industry Co., Ltd., Itami City, Hyogo, Japan; www.matsutani.com) was analysed by LC plus maltose in a ratio of 4:1 (w/w). 2.5 g of oligosaccharide mixture was dissolved in 80 mL of 0.02% sodium azide solution and transferred to 100 mL volumetric flask. 10 mL of internal standard was pipetted into a flask; it was brought to volume with 0.02% sodium azide solution. The solutions were transferred to 50 mL polypropylene storage bottles®.

Ten grams of D-sorbitol (99%) analytical grade was weighed into a 100 mL volumetric flask. It was dissolved in 80 mL of 0.02% (w/v) sodium azide solution and was adjusted to volume with 0.02% sodium azide solution. The solution was mixed well. (NOTE: sodium azide was handled with caution as prescribed after reviewing the MSDS, for example; using appropriate personal protective gear and laboratory
D-Glucose LC standards (5, 10, 20 mg/mL) solution was prepared when 0.5, 1.0, and 2.0 g portions of high purity (> 99.5%) D-glucose (Sigma Chemical Co.; cat. no. G5767) were accurately weighed and transferred to 3 separate 100 mL volumetric flasks. Ten mLs of internal standard (D-Sorbitol) were pipetted to each flask and 0.02% sodium azide solution was used to bring them to volume. The solutions were transferred to 100 mL Duran® bottles. Sodium maleate buffer 50 mM, pH 6.0 plus 2 mM. CaCl₂ and 0.02% sodium azide was prepared by dissolving 11.6 g of maleic acid in 1600 mL of deionised water and adjusted the pH to 6.0 with 4 M (160 g/L) NaOH solution. Calcium chloride dihydrate (CaCl₂.2H₂O) (0.6 g) and 0.4 g of sodium azide were dissolved and the volume was adjusted to 2 L.

Trizma® Base (Sigma cat. no. T-1503), 0.75 M.; 90.8 g of Trizma® base was added to approx. 800 mL of deionised water and dissolved. The volume was adjusted to 1 Litre. Acetic acid solution, 2 M. was prepared by adding an amount of 115 mL of glacial acetic acid (Fluka 45731) to a 1 L volumetric flask and diluted to 1 L with deionised water.

Sodium azide solution (0.02% w/v) was prepared by adding 0.2 g of sodium azide to 1 L of deionised water and dissolved by stirring (Caution: Sodium azide was not added to solutions of low pH, because acidification of sodium azide releases a poisonous gas. This chemical was handled with caution after reviewing the MSDS, using appropriate personal protective gear and laboratory hood). Deionised water containing Na₂CaEDTA (50 mg/L). 50 mg of Na₂CaEDTA was weighed into a 1 L Duran bottle and dissolved in 1 L distilled water. This solution was prepared fresh weekly; and was filtered through 0.45 mm filter before use. The pH standards by preparing buffer solutions at pH 4.0, 7.0 and 10.0.
Celite® was acid-washed and pre-ashed. A Mixed-bed ion exchange resins for each test portion was prepared. (1) m-1. Approximately, 4 g Amberlite® FPA53 (OH-) resin (Rohm and Haas, France S.A.S.) ion exchange capacity 1.6 meq/mL (min) or equivalent (R-OH exchange capacity data supplied by manufacturer) and approximately 4 g Ambersep® 200 (H+) resin or equivalent, (Rohm and Haas, France S.A.S.) ion exchange capacity: 1.6 meq/mL (minimum). The two resins were mixed just prior to use and packed in the improvised columns.

Improvised chromatography columns for analysis of each test portion were used. After mixing and packing, they were washed with 20 mL of milli-Q water. For a concern that carbohydrates may be retained on the resin, a test solution consisting of 1 mL of 100 mg/mL internal standard was prepared and 2.5 mL of 10 mg/mL fructooligosaccharides diluted to 10 mL.

Recovery of the internal standards and fructooligosaccharides was checked to find out if the solution injected matched directly onto the LC.

Ground samples were defatted as per AOAC 985.29 because tiger nuts contain more than 10% fat. All samples were dried in an oven at a temperature of 50ºC overnight. Prepared samples were transferred to a wide mouthed plastic jar, sealed, and mixed well by shaking and it was inverted. They were stored in the presence of a desiccant.

With each assay, two blanks were run along with samples to measure any contribution from reagents to residue.

Duplicate samples 1.000 ± 0.005 g. were weighed accurately into 250 mL Fisherbrand® soda glass, wide mouth bottles.

The prepared samples were wetted with 1.0mL of ethanol (78%) and 40mL of pancreatic α-amylase/AMG mixture was added to each bottle. The bottles were
capped and then transferred to a shaking incubation bath. The bottles were secured in place with the springs in the shaker frame.

The reaction solutions were incubated at 37°C and was set at 150 rpm in orbital motion in the shaking water bath for exactly 16 hours (17:00 to 09:00)

The α-amylase and AMG were deactivated by adjustment of pH to approximately 8.2 (pH 7.9-8.4). After 16 hours, all the sample bottles from the shaking water bath were removed and 3.0mL of 0.75M Trizma® base solution was added to each bottle to terminate the reaction. The caps of sample bottles were slightly loosen and the bottles were immediately placed a water bath (non-shaking) at 95-100°C, and were incubated for 20 minutes with occasional shaking (by hand). The final temperature (≥90°C) of the bottle contents were measured with a thermometer. (Only one bottle was checked).

All sample bottles were removed from the hot water bath and cooled approximately to 60°C. An amount of 0.1mL of protease solution with a positive displacement dispenser and were incubated at 60°C for 30 minutes.

Four millilitres of 2M acetic acid was added to each bottle and mixed well. The final pH was approximately 4.3. For the internal standard, one millilitre of D-sorbitol (internal standard solution 100mg/mL) was added to each bottle and was thoroughly mixed. Crucible containing Celite® was weighed to the nearest 0.1mg. The bed of Celite® in the crucible was wetted and redistributed with 15mL of 78% (v/v) EtOH from a wash bottle. Suction was applied to crucible to draw Celite® onto the fritted glass as an even mat. All the enzyme digested samples were filtered using vacuum, through the crucibles. The incubation bottles were rinsed with 60°C deionised water using a wash bottle with minimum volume of water (approx. 10 mL) and a rubber policeman (spatula) were used to dislodge all particles from the walls of the container.
The suspensions were transferred to the crucibles. The bottles were further rinsed with 10 mL of water at 60°C and again transferred to the crucible. The combined filtrates and washings were collected and the volumes adjust to 70 mL. By using vacuum, the residues were successively washed with two 15 mL portions of the following: 78% (v/v) EtOH (or IMS), 95% (v/v) EtOH (or IMS) and Acetone. All the washings were discarded.

The crucibles containing residue were dried overnight in 105°C oven. The crucibles were cool in a desiccator for approximately 1 hr. The crucible containing insoluble dietary fibre residue and Celite® were weighed to nearest 0.1 mg. Residue masses, were obtained by subtracting tare weight, i.e. weight of dried crucible and Celite®.

The residue from one crucible was analysed for protein, and the second residue of the duplicate was analysed for ash. Protein analysis was performed on residue using Kjeldahl. Factor of 6.25 was used for all cases to calculate mg of protein.

Ash analysis was determined by incinerating the second residue for 5 hours at 525°C. The incinerated samples were cooled in a desiccator and were weighed to nearest 0.1 mg. The crucible and Celite® weight were subtracted to determine ash.

The filtrate of each sample (approx. 70 mL), were pre-heated to 60°C and 280 mL (measured at room temperature) of 95% (v/v) ethanol were added and mixed thoroughly. The precipitate was allowed to form at room temperature (approximately 21°C) for 60 min.

The filtrates from one of the sample duplicates were set aside to use in case of spills or if duplicate SDFS data was desired. One half of filtrate duplicate was transferred to a 500 mL evaporator flask and evaporated to dryness under vacuum at 60°C. Five mL of deionised water was added to the evaporator flask and the flask was swirled for approx. 2 min to dissolve the sample. The solution was transferred to a 20 mL
sealable polypropylene container. Two mL of this solution was transferred to the top of the improvised disposable column containing 4 g each of freshly prepared and thoroughly mixed, Amberlite FPA 53 (OH-) and Ambersep 200 (H+). The columns were eluted at a rate of 1.0 mL/min into a 100 mL Duran® bottle. When the sample has entered the resin, 2 mL of deionised water was added to the resin and allowed to percolate in. Approximately 20 mL of deionised water was added to the top of the column and continued to elute at a rate of 1.0 mL/min. The eluted samples were transferred to a 250 mL round bottom rotary evaporator flasks and evaporated to dryness under vacuum at 60°C. Two mL of deionised water was added to each flask and dissolved the sugars by swirling the flask for approximately 2 min. Pasteur pipettes were used to transfer the solution to a polypropylene storage container. The solution were transferred to a 10 mL disposable syringe and filtered through a 0.45μm. Hundred micro-litre LC glass syringe was used to fill the 50μL injection loop on the LC. All analyses were performed in duplicate.

The Column was prepared by dissolving Sugar-Pak® (6.5 x 300 mm), using distilled water containing Na2Ca-EDTA (50 mg/L). The flow rate was set at 0.5 mL/min and the temperature was at 90°C. Hundred micro-litres LC syringe was used to fill a 50 μL injection loop for each standard D-sorbitol/D-glucose solution. Each was injected in triplicate.

The values for the peak areas of D-glucose and internal standard from the 3 chromatograms were obtained. The reciprocal of the slope was obtained by comparing the ratio of peak area of D-Glucose / peak area of D-sorbitol internal standard (y-axis) to the ratio of the mass of D-glucose / mass of D-sorbitol (x-axis) is the “response factor”. The average response factor was determined (typically 0.97 for D-sorbitol).
The following formula as described in the accompanied manual with assay kit was used to calculate the Response factor \( R_f = \frac{(PA-IS)}{(PA-Glu)} \times \frac{(Wt-Glu)}{(Wt-IS)} \)

where:

- \( PA-IS \) = peak area internal standard (D-sorbitol);
- \( PA-Glu \) = peak area D-glucose;
- \( Wt-Glu \) = mass of D-glucose in standard.

The values obtained for ash and protein were subtracted from average residue weight.

**CALCULATIONS FOR HMWDF, IDF and SDFP:**

**Blank (B) determination (mg):**

\[
= \left( \frac{BR1+BR2}{2} \right) - PB - PA
\]

where:

- \( BR1 \) and \( BR2 \) = residue mass (mg) for duplicate blank determinations respectively, and
- \( PB \) and \( PA \) = mass (mg) of protein and ash determined on first and second blank residues respectively.

**HMWDF, IDF or SDFP (mg/100 g)**

\[
= \left( \frac{R1+R2}{2} \right) - \frac{PB-PA-B}{(M1+M2)/2} \times 100
\]

where:

- \( R1 \) = residue mass 1 from M1 in mg;
- \( R2 \) = residue mass 2 from M2 in mg;
- \( M1 \) = test portion mass 1 in g; \( M2 \) = test portion mass 2 in g;
- \( PA \) = ash mass from R1 in mg; \( PB \) = protein mass from R2 in mg.

**HMWDF (%) = HMWDF (mg/100 g)/1000**
IDF (%) = IDF (mg/100 g)/1000

SDFP (%) = SDFP (mg/100 g)/1000

SDFS (mg/100 g)

= Rf x (Wt-IS, mg) x (PA-SDFS)/(PA-IS) x 100/M

where:

Rf is the response factor

Wt-IS is weight in mg of internal standard contained in 1 mL of internal standard solution pipetted into sample mixture (100 mg).

PA-SDFS is the peak area of the SDFS.

PA-IS is the peak area of the internal standard (D-sorbitol).

M is the test portion mass (M1 or M2) in grams of the sample whose filtrate was concentrated and analysed by LC.

3.8. Determination of gross energy

Gross energy was determined by Isoperibol bomb calorimeter (Parr Instrument, model number: A1136DDEF, Serial number: M4173. Moline, USA). The method is theoretically based on measuring the liberated heat from a complete combustion of the sample by electrical ignition in oxygen rich atmosphere (bomb). 1 gram of dried ground material was pelleted by a Pellet making Die and placed in a crucible. The crucible was placed in the metal loop of the bomb. Fuse wire was connected to the anode and cathode of the bomb allowing the wire to touch the surface of the pellet. One ml of distilled water was pipette into the bomb cylinder. The bomb was filled with oxygen to 30 bars before ignition. The calorimeter bucket was filled with 2000gm of water at a temperature below room temperature by 2-3 degrees. The bucket was placed in the calorimeter chamber and the bomb was lowered down into the water bucket. The two ignition wires were connected into the terminal sockets which
located on the bomb head. The firing chamber was closed with their cover. The bomb was fired and the microprocessor was automatically compared to the temperature rise with a known thermal curve. The result in MJ/kg was printed out. This result was later converted to kilocalorie/kg by this converting factor: 1MJ/kg = 238.8Kcal/kg.

All analyses were carried out in triplicate with all the prepared samples from the various countries.

3.9. Antioxidants

Methanol, Ethanol (95%), deionised water. Schrott bottles, weighing scale, volumetric flask (20 and 50mls), Whatman filter paper (24cm and 0.2µm), centrifugal machine and Rotary Vacuum Evaporator.

Ten grams of each sample from Cameroon, Ghana and UK market were weighed, put in a Schrott bottle and suspended in 20mls of solvents for the extraction. The following solvents were used: Methanol (99%), Ethanol 95%, Ethanol: Water (70:30) and Methanol: Water (70:30).

The Phytochemical compounds were extracted at room temperature for 20 hours. Samples were initially filtered using Whatman filter pore size of 24cm. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40°C) using Büchi 1 Rotary Vacuum Evaporator R3, (Switzerland). After the evaporation process, the concentrates in the flasks were dissolved using 50mls (volumetrically measured) of deionised water. The Solutions were again centrifuged at 2400 rpm for 10 minutes. The centrifuged solutions were filtered using Whatman filter paper with a pore size of 0.2µml. Prepared samples were stored in the deep freezer until ready to be use.
3.9.1. Rancimat method

Tiger nut oil from the three countries, BHT, lard (Purchased from Sainsbury supermarket, Plymouth) and weighing scale.

Lard was spiked with tiger nut oil and BHT and then subjected to oxidation in a Methrom Rancimat model 679 (Herisau, Switzerland). The oxidative stability of lard was determined with or without antioxidants (tiger nut oil and BHT). An amount of 3.0 ±0.01 lard without antioxidants and 0.50 ±0.01 grams of the antioxidants (tiger nuts oil and BHT) were used in all the experiments. The tests were carried out in triplicate. All samples were studied at, 120, 130, 140, and 150°C. The correction temperature of the conductivity tube was constant at 4.2°C. Air flow rate was set at 20 Litres/hour for all determinations. The induction times were printed automatically by apparatus software with precision of 2 decimal places. The averages of 3 measurements were taken and for a given sample at the given temperature the maximal deviation from mean value was less than 1%. At the end of each set of experiment the glassware were thoroughly washed with special rancimat detergent, rinsed with acetone and distilled water respectively. After washing they were dried in an oven overnight at temperature of 50°C. The time taken until there is a sharp increase of conductivity is termed the induction time (IT), and it is expressed in hours. IT was determined by the intersection of the baseline with the tangent to the conductivity curve (Frank et al., 1982). The antioxidant activity index (AAI) was calculated by the measurement of induction time of blank (pure oil) and oil with spice extracts according to (Viuda-Martos et al., 2010b) using via the following formula: AAI = induction period of lard spiked with tiger nut oil samples/BHT / induction period of lard.
3.9.2. Determination of total phenolic contents

Samples as outlined under section 3.2.2.2. Refrigerator, test tubes, incubator, stop clock, vortex, aluminium folio, standard 1cm³ disposable cuvette and Spectrophotometer. All measurements were performed at room temperature 19-21˚C. Folin Ciocalteu's Phenol regent, Sodium Carbonate, distilled water and Gallic acid

Folin Ciocalteu’s Phenol regent was prepared by dissolving 1ml of the regent in 9mls of distilled water. 15gm of Sodium Carbonate salt was dissolved in 200mls of distilled water. The solutions were stored in the refrigerator until they were ready to be use.

Total phenolic content of tiger nuts extracts were determined using a Folin–Ciocalteu (FC) assay based on the method described by Alothman et al. (2009) with some modifications. In brief, different amounts (volume ml) of extracts (0.2, 0.3, 0.5, 0.8 and 1) were put in test tubes and topped up to 1ml with distilled water. Furthermore, resulting solutions were mixed with 2 ml of FC phenol reagent; after standing for 6 min at room temperature, 2.5 ml of (7.5% w/v) Sodium Carbonate solution was added. The solutions were mixed thoroughly using a Vortex at a rate of 1800rpm for one minute. The solution was incubated in a water bath which was covered with Aluminium folio at a temperature of 43°C for 10minutes. Blank solution was prepared using 1ml of distilled water and subjected to the same treatment as the extracted samples. Distilled water was used to zero the spectrophotometer before prepared samples’ absorbance readings were measured. The absorbance was measured at 760 nm, using spectrophotometer (Cecil, CE 1010, Cambridge. UK) and the results were expressed in gallic acid equivalents (GAE; mg/100 g) using gallic acid (0–0.1 mg/mL) standard curve. Gallic acid was used to produce standard calibration curve. The total phenolic content was expressed in mg of gallic acid equivalents (GAE) / g. This following formula was used to convert absorbance into ppm subsequently into
mg/g; \( Y = mx + c \) thus \( X = \frac{Y-c}{m} \), Where \( y \) = absorbance and \( c \) & \( m \) values are obtained from standard curve.

The standard solution was prepared by dissolving 0.05g in 100mL of distilled water.

All measurements were performed in triplicate and means and standard deviation were presented. The blank values were deducted from the actual absorbance values.

### 3.9.3. Determination of antioxidants scavenging properties of tiger nuts sample using DPPH

Samples as prepared under section 3.2.2.2. Absorption spectra were determined using Cecil, (CE1010 Cambridge, UK) Spectrophotometer. Spectrophotometric measurements at 517nm were performed using standard 1cm³ disposable cuvettes. All measurements were performed at room temperature 19-21˚C. Three of the samples used in this experiment were obtained from local vendors in local markets in Accra (Ghana) and Bamenda (Cameroon) and the sample from UK market.

Butylated hydroxytoluene (BHT) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma Chemical Company (St Louis, USA). All other chemicals used in this study were of analytical grade.

Ten millimolar (mM) solution of DPPH was prepared by weighing 0.0039 grams of the chemical and dissolved in 10mls of methanol. Then, 0.6 ml of the methanolic solution of DPPH was further added to 9.4 mls of methanol. All solutions were freshly prepared daily and the container holding the solution was covered with aluminium foil to minimise DPPH decomposition.

The antioxidant activity of the samples was measured in terms of radical scavenging ability of DPPH using the methods proposed by Viuda-Martos et al. (2010a) with
some modifications. Two mls of the methanolic DPPH solution were dispensed into test tubes and different volumes (50 µL, 100 µL, 150 µL, 200 µL and 250 µL) of each sample including the standard (BHT) were added. Each solution was mixed thoroughly for one minute using vortex machine which was set at 1800rpm. The solution was incubated for 30 minutes at a temperature of 50˚C in a water bath. The water bath containing the prepared batch samples were covered with an aluminium foil to create a dark environment. At the end of this period, the incubated sample was put into cuvette (1cm) and the absorbance was determined at 517nm using Spectrophotometer. Methanol was used to zero the spectrophotometer. Absorbance of the radical without sample (control) was incubated simultaneously with the samples and was also assayed after each batch of methanolic solution of DPPH preparation.

The unknown concentration of the samples were determined by measuring the amount of light that samples absorbs by using Beer-Lambert’s law, absorbance \( A = \varepsilon b c \);

\( \varepsilon \) is the extinction coefficient of the analyte with units of \( \text{mM}^{-1}\text{cm}^{-1} \).

\( b \) is the path length (1 cm) of the cuvette in which the sample is contained.

\( c \) is the concentration of the compound in solution, expressed in \( \text{molL}^{-1} \)

The value for the extinction coefficient (\( \varepsilon \) of DPPH (4.09mM\(^{-1}\text{cm}^{-1}) \)) was taken from Sung-Kun et al. (2004) and used to calculate the number of moles of DPPH reduced per volume of samples.

The results were expressed as BHT equivalent in milli Molar (mM).
3.10. Effect of tiger nut bagasse on final bread loaf characteristics

3.10.1. Flour viscosity

The viscosities of the wheat flour and the tiger nut fibre were determined using Rapid Visco Analyser. This was done to determine the level of substitution of tiger nut fibre to that of wheat flour. The moisture content of the wheat flour and the tiger nut fibre were determined in an oven assisted fan as described by American Association of Cereal Chemists (AACC) (2000), 25.0 g of distilled water was dispensed into a new canister and a weighed amount of the samples approximately 5.0 ± 0.1g was transferred onto the surface of water in the canister. The paddle was place into the canister and the blade was jogged through the sample up and down ten times. The paddle was placed into the canister and was inserted firmly into the paddle coupling. The motor tower was pressed down to release the instrument to initiate the measurement cycle.

3.10.1.1. Bread preparation

The bread making performances of wheat flour and the dried tiger nuts bagasse powder were determined using straight dough AACC method (1984) with a slight modification. Strong white bread baking wheat flour and fast action dry yeast were purchased from Tesco (Plymouth). These items were used together with salt and water. Refer to Table 3-1 bread samples formulation.
<table>
<thead>
<tr>
<th>Ingredients</th>
<th>0</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strong white bread flour</td>
<td>300</td>
<td>240</td>
<td>210</td>
<td>180</td>
</tr>
<tr>
<td>Tiger nuts bagasse flour</td>
<td>0</td>
<td>60</td>
<td>90</td>
<td>120</td>
</tr>
<tr>
<td>Water</td>
<td>170</td>
<td>170</td>
<td>170</td>
<td>170</td>
</tr>
<tr>
<td>Fast action dry yeast</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Salt</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Weight in grams

Bread loaves with 0% tiger nuts fibre supplementation were referred to as the controls.

The dried ingredients were sifted together to remove unwanted debris and for aeration purpose. The sifted ingredients were put into the kneading machine, and machine was turned on, whilst the dough hook was in motion, water was gradually added to the mixed dried ingredients. The mixture was kneaded between 7-10 minutes with speed between 92-96 rpm. In a study carried out by Tipples et al. (1996), it was observed that during the kneading process, the starch could absorb up to 46% of water.

After the kneading, the dough was weighed using (electronic weighing balance) and then divided into two equal portions for moulding. The moulded dough were put into pans and proofed in an incubator temperature set at 43°C for 45 minutes. At the end of this period the proofed dough were taken out and baked for 25 minutes in a fan-assisted oven, (Zanussi Combiwave oven with a model number Fcvm/62E) at a temperature of 200°C. After removing from the oven, the baked bread was placed on wire grids to cool at room temperature over a period of less than two hours. Each sample treatment (bread) was made in triplicate so mean results could be calculated. All analysis was done within a period of 48 hours.
3.10.1.2. Physical characteristics of the baked bread

In this study, the physical characteristic of the baked bread have been listed as the Loaf weight, loaf volume, loaf density, bread specific volume, texture and colour.

3.10.1.2.1. Determination of loss of mass upon baking

The physical property of the masses of the uncooked dough and the bread were determined. The density of the bread was calculated according to Equation below.

\[
\text{Loaf density} \left( \frac{g}{mL} \right) = \frac{\text{Weight of bread}}{\text{Loaf volume of bread}}
\]

3.10.1.2.1 Determination of loaf volume

Loaf volume was assessed by the sago seeds displacement methods. Each triplicate was measured for a mean to be obtained for each level of supplementation. (Mariotti et al., 2006) have observed that supplementing wheat flour with low gluten flour tends to reduce bread making potential of the composite flour as a result of gluten dilution. The loaf specific volume was calculated by dividing the loaf volume by loaf weight and the results were expressed as ml/g (Koca and Anil, 2007).

3.10.1.2.2. Determination of texture

Bread firmness was determine according to the standard method published by AACC (74-09, AACC, 2000) using the Texture Analyser (TA-XT2, Stable Micro Systems, Godalming, UK). The texture analyser was calibrated and set up with a 5Kg load cell.

After storage for 1 day, the various samples of bread were sliced by hand into equal slice thickness of 12.5mm thick. The crusts were gently removed from the crumb. Two slices of bread were stacked and measured the force to compress to 40% of the
height by using 36mm diameter cylinder probe with pre-test 1mm/s, test speed of 2mm/s, and post-speed of 2mm/s, respectively. Three measurement per stacked of loaf for a replication were recorded and three replications were done one per batch. Each time of measurement was taken, the maximum peak force value was recorded and the average was calculated in force unit.

3.10.1.2.3. Determination of loaf colour

The colour of bread crust and crumb were measured using (Konica Minolta Spectrophotometer CM-2600d), the L* a* b* colour space system (CIELAB). In this colour space, L* indicates lightness and a* and b* are the chromaticity coordinates. In this system, L* indicates lightness and a* and b* indicate colour directions: +a* is the red direction, -a* is the green direction, +b* is the yellow and -b* is the blue direction. The colour difference of the baked bread was calculated according to this following method (Borsuk et al., 2012).

Whiteness = 100 - [(100 - L*) 2 + a*2 + b*2]0.5

The crust colour was measured from three different places on the surface of the uncut loaves of the prepared samples. Sample measurement was done in triplicates. The crumb colour was measured from the centre of three slices from each triplicate, giving nine measurements in total, and the means calculated.

3.10.1.2.4. Determination of sensory characteristics

In line with the Plymouth University policy, the sensory evaluation protocol received the approval of the Human Ethical Committee of the Faculty of Science and Environment.
Nine untrained panellists were recruited from within the University of Plymouth staff, postgraduate and undergraduate students via e-mail invitation and verbal communication. The volunteers were warned of health risks regarding nuts and wheat allergies.

In the morning (10.00 am to 12.00 noon) of the day of the sensory evaluation, after briefing and signing consent form at the main entrance of the Nutrition Laboratory, the panellists were invited to sit at sensory evaluation booths where the prepared bread samples were presented in random order with a ballot sheet for each sample. Instructions on the ballot sheet directed the panellists to evaluate a range of sensory attributes (suitability for sandwich, colour, taste and overall acceptability). Samples were evaluated and scored on a 9-point hedonic scale, with the degree of likeness of the product attribute expressed as follows: 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 and 9 - like extremely.
3.11. Effect of tiger nut bagasse addition on biscuit characteristics

3.11.1. Biscuit preparation

Table 3 - 2: Tiger nut (whole and bagasse flour) biscuit formulation

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>0</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self-raising flour</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Wholemeal flour</td>
<td>70</td>
<td>56</td>
<td>49</td>
<td>42</td>
</tr>
<tr>
<td>Tiger nut flour</td>
<td>0</td>
<td>14</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td>Unsalted butter</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Salt</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Caster sugar</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Water</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
</tr>
</tbody>
</table>

Biscuits samples were processed from dough containing 0, 20, 30, 40% whole tiger nut and tiger nut bagasse flours as substituting levels for wheat flour according to the method described by (Leelavathi and Rao, 1993) with some modifications. The formula used was as follows: tiger nut flour components 0, 14, 21, and 28g; whole meal wheat flour, 70, 56, 49 and 42g; self-raising flour 90g; Caster Sugar 25g; unsalted butter 70g; sodium chloride 0.25 g; sodium bicarbonate 0.3g and 17 ml water. The caster sugar and fat were creamed in a mixer with a flat beater for 3 min at 61 rpm (speed 1). Sodium bicarbonate and sodium chloride were dissolved in water and added to the mixture. The contents were mixed for 6 min at 125 rpm (speed 2) to obtain a homogenized and creamy texture. Sieved flour was added to the cream and mixed for 2 min at 61 rpm (speed 1). The dough pieces were sheeted to a thickness of 4.6 mm using pastry guides and cut using a circular mould (circumference 207.66mm and baked at
180°C for 15-18 min. After baking, biscuits were left to cool on a wire rack at room temperature. After cooling the biscuits were vacuumed packed and were stored in a refrigerator until further analyses were carried out on them.

3.11.2. Physical measurements

Effect of TG on biscuit Physical properties

3.11.2.1. Diameter

To find the average diameter of the biscuit a caliper was used and measured all of the biscuits. The widest point of each biscuit was recorded to keep it the same each time. Using $\pi \times$ diameter the circumference of the biscuit could be worked out. The average diameter and average circumference could then be found.

3.11.2.1. Average thickness

To find the average thickness of the biscuit, the highest point on each biscuit was measured using a caliper. The highest point on the biscuit was recorded to make it the same each time. The spread ratio and specific volume were calculated by dividing diameter (W) by thickness (T) (Ashoush and Gadallah, 2011) and volume divided by weight of biscuits (Galdeano and Grossmann, 2006) respectively.

3.11.2.2. Weights

The weights of all the biscuits were taken and recorded, before the mean of all the biscuits were calculated. This method was more accurate than just weighing one biscuit. The biscuits were weighed before and after baking so the water content lost by baking could be calculated.
3.12. Sensory analysis

The experimental protocol was approved by the University of Plymouth- Faculty of Science and Technology- Human Ethics committee. Sensory evaluation was based on the British Standard Guidelines ISO 6658-1995 and 6564-1985 (British Standards 5929 1986).

3.12.1. Bread

Bread test samples were subjected to sensory evaluation within a week after production. The following attributes namely, taste, aroma, flavour, colour, texture, appearance and overall acceptability were assessed on the product samples using a 7-point hedonic scale with 7 as like extremely and 1 as dislike extremely (Meilgaard et al., 1999). Non-trained 23 panellists from University of Plymouth (both students and staff) and were neither sick nor allergic to baked products, were involved in the assessment. The panellists were instructed to rinse their mouth with water after tasting each bread and biscuit test samples. Participants were allowed to opt out from this study if they felt uncomfortable from any of the procedures.

Evaluation sheets were created and the products to be evaluated were randomly coded so the participant didn’t know which bread or biscuit was being taken.

3.12.2. Biscuits

The biscuits prepared by incorporating whole tiger nut (WTN) (with fats) and tiger nut bagasse (TNB) (without fat) flour were evaluated for their sensory characteristics: appearance, colour, flavour, aroma, texture, taste and overall acceptability, by a panel consisting of forty non-trained judges. The judges included the students, and staff from University of Plymouth. The seven point Hedonic scale score-card method
was used to determine the sensory characteristics of the biscuits made. All samples of biscuits (prepared from 0-40% WTN and TNB flours substitution) were given different code letters. The quality factors such as appearance, colour, flavour, texture, taste and overall acceptability were allotted a maximum score of 7 each. The scoring scale was adapted from Amerine et al. (1965.): 1 (Dislike extremely), 2 (Dislike moderately), 3 (Dislike slightly), 4 (Neither dislike nor acceptable), 5 (Slightly acceptable), 6 (Moderately acceptable) and 7 (Extremely acceptable).

3.13. Evaluation of glycaemic index for whole tiger nut and bagasse

Study design

This work was conducted in three sessions. (For the Standard diet (control), Whole and bagasse tiger nut bread).

Healthy, non-diabetic, Undergraduates, postgraduates’ students and staff from the University (n = 11) were recruited to participate in this work. The meals were prepared in the Food and Nutrition kitchen, meal formulation was based on the previous work done on the bread formulation developed in the Food and Nutrition Laboratory, University of Plymouth. White slice bread was used as the standard and the test meals were wheat substituted with whole and bagasse tiger nut bread. The test meals were based on the 20% tiger nut flour substitute bread as it was the only bread that ran the control (white bread) in close seconds during the sensory analysis. The available carbohydrate and dietary fibre in tiger nut and wheat flour based on the FAO food composition table are 47.8, 75.3, 10.2 and 3.1g/100 respectively. However, the available carbohydrates and dietary fibre in the control bread, the whole nut bread and bagasse bread are 40.1, 40.91, 45.54, 7.68, 9.69 and 10.42 g/100 respectively. An amount of 66.4g, 80g, 70g of control, bagasse and whole nut bread
respectively were served with a glass of water/tea, 12g butter and 15g cheese to each participant on each session.

Participants were advised to fast overnight (10-12 hours) on each session. They were asked to arrive at the Nutrition Clinical assessment room around 9.00am for the baseline measurements.

Participants were asked to prick their finger with a lancet (Safe-T-Pro Plus) manufactured by Roche Diagnostics GmbH, (Mannheim, Germany) for blood sample (time 0) and the blood glucose was determined using blood glucose monitor Accu-check Performa glucose monitor and Accu-check strip, these were manufactured by the same company named above. After this a standard meal, (a weighed amount of white bread containing 50 grams of available carbohydrate, a standard drink of water (150ml) or tea and cheese) were given, food was eaten within 5 -10 minutes). Blood glucose level was checked within these periods 15, 30, 45, 60, 90 and 120 minutes after first bite of meal. Ideally, the standard food test for the blood glucose response was supposed to have been repeated three times and averaged as suggested by (Food and Agriculture Organisation (FAO) and World Health Organisation (WHO), 1998). Day-to-day variability in glycaemic response is normally managed by testing the reference food on 2 or 3 separate occasions. But in this study it was done only once due to the unwillingness of participants to be subjected to pain for five different days of pricking fingers for blood (invasive nature of study).

The incremental area under the glucose response curve (IAUC) was calculated geometrically by applying the trapezoid rule as described by (Wolever, 2006) for each prepared sample meal using the fasting level as the baseline and ignoring any area below the baseline. The GI was defined as the IAUC of the blood glucose response curve of a 50 g available carbohydrate portion of a test meal expressed as
a percentage of the response to the same amount of available carbohydrate from the reference food. The reference value and each subject’s individual GI for each cereal were calculated. The GI for each cereal was taken as the mean of all eleven individual values. The GI was calculated using this formula \((\text{IAUC test food/IAUC reference food}) \times 100\). The GI of each test food was taken as the mean for the whole group.

To while away time between blood samplings, participants’ anthropometric measurements were taken to determine their body mass index. A stadiometer (Seka Ltd., Birmingham, UK) was utilised to measure height of the participants with no shoes. The weight of the participants was determined using a Tanita BC-418MA (Tanita UK Ltd., Yiewsley, Middlesex, UK) and body fat content and body fat index was calculated.

The sample size was determined by the ISO/BSI standard methods. Participants were recruited via e-mail invitation and orally. Individuals on medication, any known self-declared allergies to the test diet, diabetes, obesity, kidney problems or with any blood disorders for example, haemophilia, were excluded from the experiments. Participants were made aware that if they feel uncomfortable with any of the procedures, they have every right to stop the experiment. In order to maintain confidentiality, participants were assigned codes which were not made known to anybody. Individual information has been kept out of reach to anybody except the Principal Investigator.

The protocol used to determine the GI value of the tiger nut flour and bagasse flour bread samples follows the International Organization for Standardization ISO 26642:2010 for the determination of the glycaemic index (GI) (International Standards Organisations (ISO), 2010). The study undertaken was a single-blinded cross-over design and all test samples including the control (white bread) were
randomised throughout the three testing sessions. All three testing sessions were completed within a two-week period (Monday, Wednesday and Friday on both weeks). The Human Ethics Committee of the University of Plymouth approved the study.

3.14. Ethical approval

The Human Ethics committee of the Faculty of Science and Technology, University of Plymouth approved the sensory evaluation and the glycaemic index protocols. Each participant was provided with a consent form. The consent form stated that each participant has the ability to withdraw from the panel at any time, without penalty or having to give a reason during the experiment.

3.15. Data analysis

All the numerical data were analysed using Microsoft Excel 2010 and MINITAB version 16 (Minitab Ltd, Coventry, UK), one-way analysis of variance (ANOVA), and general linear model (GLM) were used to compare different treatment groups followed by one of the appropriate multiple comparison test using Tukey (Zar, 1999) and two tail t-test. Results are presented with their Means, Standard deviation and confidences (P ≤ 0.05) were considered to be statistically significant.

3.16. Health and safety

Risk assessments for this project work were carried out as per University protocol and the Food and Nutrition protocol. See appendix for sample filled health and safety forms.
CHAPTER 4

PHYSICAL PROPERTIES OF TIGER NUT’S TUBERS COLLECTED FROM CAMEROON, GHANA, UK MARKET (SPAIN)

4.0. Results and Discussions

4.1. Determination of the hydration properties of tiger nut tubers at room temperature (19 -21˚C)

Figure 4-1: Water uptake of tiger nut tubers soaked at room temperature 19-21 °C

Figure 4-1 shows the relationship between the weights of 40 randomly selected counted nuts and time variation in water uptake of tiger nut tubers at room temperature for a period of eight days. From the graph, the increased in weight of tiger nut during water absorption can be predicted as quadratic and linear functions of soaking time with an approximate maximum time of three days at room temperature.
For time between 0 and 3, Water uptake of nuts $= -2.2 x^2 + 12.9 x + 30.7$ and $3 > \text{time (day)} > 8$, Water uptake $= 50.3$; $R^2 = 0.98$. The effect of soaking time on the weights of tiger nuts for the first three days was statistically significant ($P < 0.5$). The graph shows that water diffused from a lower concentration to a higher concentration of nutrients in the nut’s cells in the first two days and this reached and maintained equilibrium gradually for a day. The water uptake for the tubers depended on the dryness of the samples. From the graph, the maximum water uptake per tuber is estimated to be 0.47ml per tuber for three days approximation. After this period the pattern changed linearly and begins to drop, this may be due to change in pH of the soaking water or the period that the sugars and other nutrients migrate from the cells to the freshly changed water in the beaker. The change in graph shape is consistent to what Chen et al. (1999) have postulated, water diffuses into food product and some components leach out during soaking. The leaching out components may include soluble protein, sugars, and non-starch bound lipids. Again the change follows the same pattern as described by Djomdi et al., (2007) and Kashaninejad and Kashiri (2007), however the methods used by these authors are different from this experiment. In their case, they investigated the relationship between different soaking temperatures and water uptake whilst in this experiment the relation between water up-take and time (days) was investigated.
### 4.2. Determination of unit mass

Table 4-1: Summary of unsoaked, soaked unit mass (g) and water uptake of tiger nut tubers from Cameroon, Ghana and Spain (n= 20) at room temperature (19-21°C) for three days

<table>
<thead>
<tr>
<th>Country of sample origin</th>
<th>Weight (g) of unsoaked nut</th>
<th>Weight (g) of soaked nut</th>
<th>Water uptake per tuber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cameroon</td>
<td>0.95 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.07 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.13 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ghana (brown)</td>
<td>1.44 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.60 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.12 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ghana (black)</td>
<td>1.32 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.66 ± 0.17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.26 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Spain</td>
<td>0.76 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.03 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.36 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values represent the mean ± standard deviation of five replications with twenty sample size. <sup>a-d</sup> significant difference (p < 0.05) between means bearing different letters in the same.
From Table 4-1, the average weight of samples as collected from the three countries ranges between 0.76 to 1.44 g. The samples from Spain were of uniform weights as the standard deviation depict. Samples from Ghana showed significantly higher tuber weights both in the soaked and un-soaked and least values in samples collected from Spain. Coskuner et al. (2002), have stated that the hydration capacity per tuber is directly related to the tuber size; the bigger the tuber, the higher the hydration capacity. But the data obtained from this current studies show otherwise. From Table 4-1, samples from Spain have lower average unit weight for the unsoaked and the water uptake was the highest whereas Ghana (brown) samples have higher average weight for the unsoaked but recorded a lower water uptake. This pattern shows that the moisture content before soaking has a greater effect on hydration rather than the tuber size. From Figure 4-2, results obtained for Ghana (black) falls within values obtained by Abano and Amoah (2011) when they used the same species in their studies. The soaked samples have an average weight ranging between 1.07 to 1.66g per tuber (nut). The values for the un-soaked tubers are significantly different from each other whereas the soaked tubers from Ghana (brown and black) were significantly different from Spain and Cameroon. Factors that may have contributed to the significant differences among samples may include; drying state of samples, for example the Cameroon samples were fresh. The samples from Spain were well dried and sorted with somehow regular shape. Samples from Ghana were unsorted, irregular shape and semi dried), maturity before harvested, packaging methods and storage environment.
### 4.3. Determination of dimensions of tiger nut’s tubers

Table 4-2: Summary of the physical properties of tiger nuts sampled from Spain, Ghana and Cameroon

<table>
<thead>
<tr>
<th>Origin</th>
<th>Length (mm)</th>
<th>Width (mm)</th>
<th>Thickness (mm)</th>
<th>Geometric mean diameter ($D_g$) (mm)</th>
<th>Sphericity (Φ)</th>
<th>Surface Area (mm$^2$)</th>
<th>Volume (mm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td>14.71±2.41$^a$</td>
<td>9.81±1.11$^a$</td>
<td>7.99±1.58$^a$</td>
<td>10.39±1.16$^a$</td>
<td>0.72±0.11$^a$</td>
<td>297.9±67.8$^a$</td>
<td>452.7±161.9$^a$</td>
</tr>
<tr>
<td>Ghana (brown)</td>
<td>18.93±5.24$^b$</td>
<td>12.16±1.63$^b$</td>
<td>11.06±1.95$^b$</td>
<td>13.46±1.74$^b$</td>
<td>0.75±0.15$^a$</td>
<td>511.3±131.4$^b$</td>
<td>1019.0±401.1$^b$</td>
</tr>
<tr>
<td>Cameroon</td>
<td>12.58±1.65$^c$</td>
<td>11.06±1.26$^c$</td>
<td>9.86±1.37$^c$</td>
<td>11.06±1.16$^a$</td>
<td>0.89±0.09$^b$</td>
<td>361.4±78.6$^c$</td>
<td>644.4±208.8$^c$</td>
</tr>
<tr>
<td>Ghana (black)</td>
<td>17.98±3.01$^b$</td>
<td>11.96±2.05$^b$</td>
<td>9.64±2.72$^c$</td>
<td>12.6±2.08$^c$</td>
<td>0.71±0.14$^a$</td>
<td>593.8±185.2$^d$</td>
<td>1352.4±648.2$^d$</td>
</tr>
</tbody>
</table>

All values represent the mean ± Standard deviation of three replications with twenty determinations. $^abcd$ significant difference ($p<0.05$) between means bearing different letters in the same column. $n=$number of observation per mean.
Table 4-2 shows the summary of the physical dimensions of tiger nuts selected from three different countries. The linear dimensions (length, width, and thickness) and shapes (sphericity and geometric mean diameter) of the four samples were found to be significantly different from each other at $P < 0.05$. The significant difference or variations between some of the samples are consistent with the finding of Gamage and Rahman, (1999), they asserted that the physical properties could be the result of the individual properties of tiger nuts’ tubers such as: genetic, growing conditions, environment, harvesting, storage and variety.

4.4. Tuber Length and Width

The length ranges between 12.58mm to 18.93mm, and falls within the findings of (Coskuner et al., 2002). As can be seen, samples selected from Cameroon and Spain show significantly different lower values as compared to samples selected from Ghana which show no significant difference between them and show the highest average length. The length and width dimensions recorded for Spain indicate that the measurements show uniform values and may be said that the tiger nut industry is well developed as compared to the other two countries. The Spanish samples may have been sorted into different sizes before they were dehydrated and packaged. The length values obtained for the black nut in this study confirms the studies carried out by Abano and Amoah, (2011).

The width’s dimension of the tubers ranges from 9.81 to 12.16mm. In all, the tiger nuts’ tubers width from Spain had a lower value as compared to the others. The Ghana1 (brown) had the highest value.
4.4.1. Geometric Mean Diameter and Sphericity

The geometric mean diameter and sphericity of the samples range between 10.39 to 13.5 and 0.72 to 0.89 respectively. Samples from Cameroon had a sphericity value which was closer to one than the other samples. This result indicates that tubers from Cameroon are more spherical and therefore will flow with ease on a conveyer belt during sorting and processing. The sphericity value obtained for the sample (Ghana black) is different from the value obtained by Abano and Amoah (2011) using the same species of sample.

The surface area and volume have a range between 361.4 to 593.8mm$^2$ and 416.9 to 919.3mm$^3$ respectively. Again, the two samples collected from Ghana recorded highest values and also biggest standard deviations. This may indicate that the tiger nut’s tuber industry is not well developed in Ghana as compared to Spain. The differences between the means and large values for the standard deviation of the volume and surface areas should be taken into account during packaging, transporting and processing. Tiger nut from Ghana should be graded or sorted before they are package for export.
### 4.4.2. Dimensional Ratios

#### Table 4-3: Correlation coefficient of tiger nut tubers dimensions (n=20)

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Cameroon</th>
<th>Ghana (brown)</th>
<th>Ghana (black)</th>
<th>Spain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length : Width</td>
<td>1.14±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6±0.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.54±0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.52±0.32&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.83</td>
<td>-0.99</td>
<td>0.45</td>
<td>0.83</td>
</tr>
<tr>
<td>Length : Thickness</td>
<td>1.3 ± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.78 ± 0.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.01± 0.61&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.92 ± 0.52&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.71</td>
<td>-0.85</td>
<td>0.41</td>
<td>0.71</td>
</tr>
<tr>
<td>Length : GMD</td>
<td>1.14 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.4 ± 0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.45 ± 0.25&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.42 ± 0.22&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.91</td>
<td>0.98</td>
<td>0.81</td>
<td>0.91</td>
</tr>
<tr>
<td>Length : Sphericity</td>
<td>14.44 ± 2.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.91±14.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.39 ± 7.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.3 ± 6.57&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>-0.81</td>
<td>-1.0</td>
<td>-0.73</td>
<td>-0.81</td>
</tr>
<tr>
<td>Length : Surface Area</td>
<td>0.04&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.04 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.05 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.89</td>
<td>0.95</td>
<td>0.94</td>
<td>0.89</td>
</tr>
<tr>
<td>Length : Volume</td>
<td>0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.90</td>
<td>0.98</td>
<td>0.98</td>
<td>0.9</td>
</tr>
</tbody>
</table>

All values represent the mean ± Standard deviation of three replications with twenty determinations. <sup>abcd</sup> Significant difference (p<0.05) between means bearing different letters in the same row. n=number of observation per mean.

Table 4-3 shows the highest ratio in Length/Sphericity, followed by Length/Thickness, Length/Width and Length/Geometric mean diameter in descending order. The ratio for the Length and width indicates that samples from Spain and Cameroon have regular shapes. The black tubers from Ghana have irregular shapes as depicted by the ratio. The Ghana (brown) has a strong negative correlation. This means that as the length of tubers increases the width decreases. The ratios of the linear dimensions of the tubers show almost similar
result as reported by Abano and Amoah (2011) and Özan and Haciseferogullari (2004).

4.5. Particle size distribution of tiger nut flours

Size distribution of a potential binding has been shown to affect various formulation characteristics like flowability, compactibility, water binding capacity, and drug release (Agrawal et al., 2003, Li et al., 2004). The water medium in which the samples were suspended has an influence on absorption samples constituents for example the amount of polar groups, the surface hydrophobicity (the insoluble fibres in samples), the pH and the protein composition (Huyghebaert, 1999).

The standard percentile and particle size distribution of tiger nuts are shown in Tables 4.4 to 8 and Figure 4-2 (A-D).

Table 4-4 shows summary of results on all samples used in the particle size distribution analysis. Values shown in this table indicate that some of the samples exhibited clear cut of shapes; mono-modal, bimodal or tri-modal.

The whole nut dried at 50°C and milled with coffee grinder has two distinct bimodal particle size distributions. The first mode range 1-100µm, with the mode occurring at 15µm. the second mode which is the biggest occurring between 150- 2000µm. The whole nut dried at 40°C and milled with coffee grinder again showed a bimodal psd. The hammer milled whole tiger nut flour exhibited tri-modal psd; the first peak has a narrow range of 1-20µm, second peak occur between 30-200µm and the third peak between the range 200-2000µm.
Table 4-4: Summary of results on Particle size distribution of samples

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>≤50µm</th>
<th>50-100µm</th>
<th>100-250µm</th>
<th>250-500µm</th>
<th>≥500µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK market (Dried sample)</td>
<td>49.34</td>
<td>22.91</td>
<td>14.09</td>
<td>86.34</td>
<td>2.72</td>
</tr>
<tr>
<td>Ghana (Black and dried sample)</td>
<td>43.58</td>
<td>24.66</td>
<td>23.56</td>
<td>91.80</td>
<td>1.03</td>
</tr>
<tr>
<td>Ghana (Brown and Dried sample)</td>
<td>38.08</td>
<td>15.46</td>
<td>28.96</td>
<td>82.50</td>
<td>1.95</td>
</tr>
<tr>
<td>Cameroon (Dried sample)</td>
<td>17.83</td>
<td>14.88</td>
<td>45.12</td>
<td>77.83</td>
<td>1.42</td>
</tr>
<tr>
<td>Bagasse Bread for Glycaemic index test</td>
<td>6.72</td>
<td>4.61</td>
<td>21.02</td>
<td>32.34</td>
<td>17.12</td>
</tr>
<tr>
<td>Whole Nuts Bread for Glycaemic index test</td>
<td>8.94</td>
<td>6.27</td>
<td>26.79</td>
<td>42.00</td>
<td>12.69</td>
</tr>
<tr>
<td>Whole Nuts Flour (Dried at 50ºC)</td>
<td>41.73</td>
<td>4.26</td>
<td>8.72</td>
<td>54.70</td>
<td>17.01</td>
</tr>
<tr>
<td>Bagasse Flour (Dried at 50ºC)</td>
<td>34.38</td>
<td>12.68</td>
<td>19.93</td>
<td>66.99</td>
<td>8.60</td>
</tr>
<tr>
<td>Whole Nut Flour (Dried at 40ºC)</td>
<td>41.79</td>
<td>12.70</td>
<td>17.91</td>
<td>72.40</td>
<td>7.73</td>
</tr>
<tr>
<td>Bagasse Flour, First extraction (Dried at 40ºC)</td>
<td>42.97</td>
<td>10.51</td>
<td>15.11</td>
<td>66.59</td>
<td>9.13</td>
</tr>
<tr>
<td>Bagasse Flour, Second extraction (Dried at 40ºC)</td>
<td>44.37</td>
<td>11.09</td>
<td>16.95</td>
<td>72.38</td>
<td>6.73</td>
</tr>
<tr>
<td>Whole Nut Flour (Hammer milling)</td>
<td>36.02</td>
<td>16.09</td>
<td>20.54</td>
<td>72.65</td>
<td>6.53</td>
</tr>
<tr>
<td>Whole Nut Flour (Hammer milling)</td>
<td>35.44</td>
<td>15.73</td>
<td>20.62</td>
<td>71.78</td>
<td>7.44</td>
</tr>
<tr>
<td>Elephant Atta (Medium Chapati Flour)</td>
<td>40.19</td>
<td>32.73</td>
<td>19.42</td>
<td>92.3</td>
<td>2.67</td>
</tr>
</tbody>
</table>
### Table 4- 5: Comparative study on Particle size distribution of selected tiger nuts flours samples from three different countries.

<table>
<thead>
<tr>
<th>Country of origin</th>
<th>Particle size Parameter (v/v, µm)</th>
<th>Specific surface area (m² g)</th>
<th>Surface weight D [3, 2] (µm)</th>
<th>Volume weight D [4, 3] (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cameroon</td>
<td></td>
<td>0.043 ± 0.003</td>
<td>53.45 ± 3.76</td>
<td>281.62 ± 3.14</td>
</tr>
<tr>
<td>Ghana (Brown)</td>
<td></td>
<td>0.096 ± 0.003</td>
<td>23.61 ± 0.63</td>
<td>219.85 ± 15.26</td>
</tr>
<tr>
<td>Ghana (Black)</td>
<td></td>
<td>0.113 ± 0.002</td>
<td>20.003 ± 0.26</td>
<td>148.44 ± 12.26</td>
</tr>
<tr>
<td>UK market</td>
<td></td>
<td>0.109 ± 0.004</td>
<td>20.76 ± 0.73</td>
<td>175.78 ± 14.10</td>
</tr>
</tbody>
</table>

abcd means that do not share a letter are significantly different in the same column. Result are expressed as a Mean ± standard deviation, n=5

* This indicate 10th, 50th (median) and 90th Percentiles, X Sauter mean diameter and Z mean particle diameter.

### Table 4- 6: Comparative study on Particle size distribution of bread used in the glycaemic index test

<table>
<thead>
<tr>
<th>Type of bread</th>
<th>Particle size Parameter (v/v, µm)</th>
<th>Specific surface area (m² g)</th>
<th>Surface weight D [3, 2] (µm)</th>
<th>Volume weight D [4, 3] (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Nuts Bread</td>
<td></td>
<td>0.019 ± 0.002</td>
<td>120.60 ± 9.99</td>
<td>549.55 ± 9.58</td>
</tr>
<tr>
<td>Bagasse Bread</td>
<td></td>
<td>0.016 ± 0.002</td>
<td>146.02 ± 15.83</td>
<td>630.85 ± 8.60</td>
</tr>
<tr>
<td>White Bread</td>
<td></td>
<td>0.023 ± 0.004</td>
<td>102.35 ± 15.87</td>
<td>787.18 ± 25.10</td>
</tr>
</tbody>
</table>

abc means that do not share a letter are significantly different in the same column. Result are expressed as a Mean ± standard deviation, n=5

* This indicate 10th, 50th (median) and 90th Percentiles
Figure 4-2: Conditions for particle size distribution of samples.
<table>
<thead>
<tr>
<th>Type of milling</th>
<th>Parameter (v/v, µm)</th>
<th>Particle size Parameter (v/v, µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d (0.1)¹</td>
<td>d (0.5)¹</td>
</tr>
<tr>
<td>Elephant Atta (Medium chapati flour (Roller)</td>
<td>16.51 ± 0.65ᵃ</td>
<td>82.96 ± 4.83ᵇ</td>
</tr>
<tr>
<td>Whole Nuts Flour Dried at 40ºC (Coffee machine)</td>
<td>9.73 ± 0.57ᵇ</td>
<td>132.95 ± 4.2ᵇᵃ</td>
</tr>
<tr>
<td>Whole Nuts Flour Dried at 50ºC (Coffee machine)</td>
<td>9.54 ± 0.54ᵇ</td>
<td>139.83 ± 6.68ᵇᵃ</td>
</tr>
<tr>
<td>Whole Nut Flour (Hammer milling)</td>
<td>8.83 ± 0.40ᵇᶜ</td>
<td>109.51 ± 12.53ᵇ</td>
</tr>
</tbody>
</table>

abc means that do not share a letter are significantly different in the same column. Result are expressed as a Mean ± standard deviation, (n=5)

¹ This indicate 10th, 50th (median) and 90th Percentiles
Table 4-8: Effect of drying temperature and extraction step on Particle Size Distribution (PSD) of tiger nut flour

<table>
<thead>
<tr>
<th>Sample (extraction)</th>
<th>Drying temperature (°C)</th>
<th>d (0.1) $^\gamma$</th>
<th>d (0.5) $^\gamma$</th>
<th>d (0.9) $^\gamma$</th>
<th>Specific Surface area (m$^2$/g)</th>
<th>Surface weight D (3, 2) µm</th>
<th>Volume weighted D (4, 3) µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bagasse flour (First)</td>
<td>40</td>
<td>8.60 ± 0.32$^b$</td>
<td>132.15 ± 15.48$^{ab}$</td>
<td>966.96 ± 30.8$^b$</td>
<td>0.092 ± 0.004$^a$</td>
<td>24.6 ± 1.01$^b$</td>
<td>343.11 ± 16.00$^{bc}$</td>
</tr>
<tr>
<td>Bagasse flour (Second)</td>
<td>40</td>
<td>8.02 ± 0.28$^b$</td>
<td>96.17 ± 9.77$^b$</td>
<td>852.98 ± 21.8$^d$</td>
<td>0.100 ± 0.004$^a$</td>
<td>22.52 ± 0.9$^b$</td>
<td>292.34 ± 12.16$^c$</td>
</tr>
<tr>
<td>Bagasse flour (First)</td>
<td>50</td>
<td>12.71 ± 0.96$^a$</td>
<td>181.55 ± 16.85$^{ab}$</td>
<td>942.03 ± 36.8$^{bc}$</td>
<td>0.064 ± 0.006$^b$</td>
<td>22.20 ± 4.35$^b$</td>
<td>350.04 ± 15.46$^b$</td>
</tr>
<tr>
<td>Whole Nut flour</td>
<td>40</td>
<td>8.83 ± 0.4$^b$</td>
<td>109.51 ± 12.53$^b$</td>
<td>894.09 ± 33.5$^{cd}$</td>
<td>0.091 ± 0.004$^a$</td>
<td>24.85 ± 1.26$^b$</td>
<td>303.93 ± 16.72$^{bc}$</td>
</tr>
<tr>
<td>Whole Nuts flour</td>
<td>50</td>
<td>7.68 ± 1.02$^b$</td>
<td>266.23 ± 164.71$^a$</td>
<td>1210.38 ± 53$^a$</td>
<td>0.105 ± 0.018$^a$</td>
<td>35.87 ± 3.34$^a$</td>
<td>461.03 ± 59.84$^a$</td>
</tr>
</tbody>
</table>

$^{abcd}$ means that do not share a letter are significantly different in the same column. Result are expressed as a Mean ± standard deviation, n=5

$^{\gamma}$ This indicate 10$^{th}$, 50$^{th}$ (median) and 90$^{th}$ Percentiles
4.5.1. Sample origin

Table 4-5 illustrates the granule (particle) size distribution of tiger nut samples from three different countries. The \( D(v, 0.1) \) values for the Cameroon, Ghana (brown), Ghana (black) and UK market (Spain) are 20.65, 7.99, 7.07 and 7.58 \( \mu m \) respectively. This implies that 10% of the samples are below 7.07 and 20.65 \( \mu m \). The Cameroon sample was significantly different (\( P < 0.05 \)) from the rest of the samples whilst the remaining samples were not significantly different at this percentile. The mean granule size \( D(v, 0.5) \) values for the Cameroon, Ghana (brown), Ghana (black) and UK market (Spain) are 232.35, 127.73, 81.56 and 63.93 \( \mu m \) respectively. This implies that 50% of the samples are smaller than 63.93 \( \mu m \) and 50% of the sample is larger than 232.35 \( \mu m \) for Ghana (black) and Cameroon tiger nuts samples, respectively. Similarly, the \( D(v, 0.9) \) values indicate that 90 percent of samples from Cameroon, Ghana (brown and black) and UK market (Spain) are 594.31, 572.2, 372.5 and 547.4 \( \mu m \) respectively. Statistically, Ghana (black) was significantly different from the other three samples, whilst these three samples did not show any differences between them. \([D_{4,3}]\) is higher for the Cameroon sample (281.62 \( \mu m \)) and a lower value was recorded for the Ghana (black) sample (148.44 \( \mu m \)), whereas values for Ghana (brown) and UK market (Spain) falls within the values recorded for Cameroon and Ghana (black).

Figure 4 - 2A indicates that Samples from Ghana (black), Ghana (brown) and UK market (Spain) exhibited a bimodal and tri-modal particle size distribution with the first peak size of small granule fractions ranging from 0.5 to 20 \( \mu m \) respectively; the samples from Cameroon exhibited a semi-bimodal 0.5 to 20 \( \mu m \). Second peak for the particle distribution for Cameroon, Ghana (black),
Ghana (brown) and UK market (Spain) are within these ranges 30-900, 30-400, 50-900 and 20-200 µm respectively. The third peak for the UK market occurred between 300-900 µm. In a study carried out by Joshi et al. (2013b), they identified that among three starches, potato and corn exhibited bimodal particle size distribution with the size of small granule fractions ranging from 0.8 to 5 µm and 0.5 to 3 µm respectively. Lentil starch granules showed monomodal size distribution with narrow size range (10–45 µm). Values obtained in their study fall within the range obtained in this current study.

Although from literature reviewed on particle size technology so far, there seems to be no documentation on precise categorisation of granule size, yet starch granules have arbitrarily been classified as large (>25 µm), medium (10–25 µm), small (5–10 µm), and very small (<5 µm) (Lindeboom et al., 2004). Tiger nut starch predominantly consists of small- to medium-sized granules (percent volume basis) with a mean particle size of 8.25 µm. The particle size distribution of tiger nut starch, shown in Fig. 4-2A, depicts granules ranging from 0.5 to 20 µm with a mean of 10 µm, this is fairly consistent with the studies reported range of 3–12 µm (Umerie et al., 1997) and 2-17 µm (Manek et al., 2012). These particle size and size distribution data are within range of other published data (Hoover et al., 2010, Tester et al., 2004). Typical particle size distributions for commercially used starches include maize starch (2–32 µm), potato starch (10–100 µm), rice starch (2–20 µm), tapioca starch (5–35 µm), and wheat starch (2–45 µm) (Rowe et al., 2003).

4.5.2. Type of Bread

Table 4 - 5 and Figure 4-2B illustrate the granule (particle) size distributions (PSD) of tiger nut bread (whole and bagasse flours) and wheat bread (white
flour). All the three samples show bi-modal distributions that have similarly developed shoulders that are skewed towards the left. The first peak which is far smaller than the second peak occurs within the range 10-100µm. The means for these samples all occurred at 35 µm; whereas the bigger peak ranged between 300-2000µm and 150-2000µm with a mean particle size 900, 600 and 700 µm for white bread (control), whole and bagasse tiger nut flour bread respectively. The 10th percentile or D (v, 0.1) values for the wheat, whole and bagasse tiger nut bread are 54.37, 74.87 and 120.43µm respectively. This means that 10 percent of the wheat bread (white) has particle size below 54.37µm. Similarly, 10 percent of the whole and bagasse tiger nut bread have below 74.87 and 120.43µm respectively. Statistically (P<0.05), there was no significant difference between the particle size for wheat and whole nut bread. However, the bagasse sample was found to be different from the two types of bread.

Joshi et al. (2013b), have posited that larger granules tend to swell more on cooking and hence granule size is an important factor affecting the starch functionality. The bigger particle size distribution may be due to the retrogradation of starch, interaction between protein, fat and starch and the hard crust formed on the bread as suggested by (Devaux et al., 1998).

4.5. 3. Effect of drying temperature

Particle size distributions of whole and bagasse tiger nut flours with different extraction rate and dried at temperatures 40 and 50ºC are shown in Fig.4-2D and Table 4-6. Analysis of variance showed that extraction and drying temperatures were a significant factor to particle size in all modes. All the treated samples depict bimodal distribution. The bagasse flour with one
extraction that was dried at 50ºC was significantly different from the remaining samples at the 10 percentile. The bagasse flours extracted once and dried at 40 and 50ºC showed no significant difference from each other at the 50th percentile. The whole nut flours that were dried at 40 and 50ºC had particle size distribution of 894.09 and 1210.38µm at the 90th percentile respectively. The whole nut dried at 50ºC was significantly different from the rest of the treated samples. At this drying temperature, the whole nut flour became visibly brown (non-enzymatic browning) and lumpy.

The volume weighted D (4, 3) µm for the whole nut flour that was dried at 50ºC was significantly different from the rest of the treated samples. It has the highest particle size distribution of 461.03µm, whilst the bagasse flour with twice extracted milk and dried at 40ºC had the lowest particle size distribution of 292.34µm. Even though Devaux et al. (1998) used different crops to do their studies, yet their findings are more consistent with the current studies.

4.5.4. Type of milling method for flour production

A reduction in the size of grains by milling increases the surface area per unit volume, which can result in a marked increase in the rate of macronutrient (carbohydrate, protein) digestion and fermentation in the mono-gastric digestive tract (Al-Rabadi et al., 2009, Stewart and Slavin, 2009), with consequences for human nutrition and the efficiency of animal growth (Wondra et al., 1995). Reduction of particle size can also facilitate handling and improve mixing of ingredients, but risks unwanted effects from very small particles due to dust and gastric ulcer-inducing properties in e.g. pigs (Ayles et al., 1999).

Particle size distributions of milling methods used in the production of flours are depicted in Table 4-7 and Figure 4.2C; from this figure, it is observed that the
Elephant Atta flour which was roller milled, show a bimodal distribution. The mean diameter $D (4, 3)$ for the flour ranged between 149.79 - 325.74µm. The Elephant Atta flour was different from all the rest of the flours; however, there were no significant difference between the tiger nut flours which were dried at 40, 50°C and coffee and hammer milled.

The whole nut dried at 50°C and milled with coffee grinder has two distinct bimodal particle size distributions. The first mode range 1-100µm, with the peak mode occurring at 15µm. The second mode which is the biggest peak occurring between 150- 2000µm. The whole nut dried at 40°C and milled with coffee grinder again showed a bimodal psd. The hammer milled whole tiger nut flour exhibited tri-modal psd; the first peak has a narrow range of 1-20µm, second peak occur between 30-200µm and the third peak between the range 200- 2000µm.
CHAPTER 5

PROXIMATE ANALYSIS AND ANTIOXIDANT PROPERTIES OF TIGER NUTS’
(CYPERUS ESCULENTUS) TUBERS SELECTED FROM CAMEROON,
GHANA AND UK MARKET (SPAIN)

Results and Discussion

The summarised proximate nutrient composition analyses of samples selected
from Cameroon (yellow species), Ghana (black species), Ghana (brown species)
Spain (brown species) and From FAO food composition table for West Africa
are presented in Table 5.1 The proximate composition obtained for the four
analysed samples were: moisture 7.72-32.18g/100g, total solids 68.04-
92.66g/100g, crude lipid 20.92–36.62/100g, protein 3.27–6.70g/100g, ash 1.57–
2.14g/100g, carbohydrate (by difference method A) 39.30–53.84g/100g, second
method (B) 50.46-82.06g/100g and caloric value method (A) 371.81–546.00
kcal/100g dry matter, (B) 509.69-565.33kcal/100g .
Table 5-1: Proximate Composition on dry basis (db) (g/100g) of tiger nuts from the three countries and FAO

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Cameroon</th>
<th>Ghana (black)</th>
<th>Ghana (brown)</th>
<th>Spain</th>
<th>FAO **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>9.09 ± 0.13(^a)</td>
<td>26.84 ± 0.15(^b)</td>
<td>31.66 ± 0.3(^c)</td>
<td>8.66 ± 1.32(^a)</td>
<td>9 - 38.7</td>
</tr>
<tr>
<td>Total solids</td>
<td>90.91 ± 0.13(^a)</td>
<td>73.16 ± 0.10(^b)</td>
<td>68.34 ± 0.30(^c)</td>
<td>91.34 ± 1.32(^a)</td>
<td>71.30 -91.0</td>
</tr>
<tr>
<td>Ash</td>
<td>2.01 ± 0.08(^a)</td>
<td>2.02 ± 0.12(^a)</td>
<td>1.70 ± 0.11(^b)</td>
<td>1.69 ± 0.12(^b)</td>
<td>1.1 -1.8</td>
</tr>
<tr>
<td>Crude Carbohydrate A</td>
<td>53.0 ± 0.84(^a)</td>
<td>39.59 ± 0.29(^c)</td>
<td>41.50 ± 0.86(^c)</td>
<td>47.50 ± 1.56(^a)</td>
<td></td>
</tr>
<tr>
<td>Crude Carbohydrate B</td>
<td>60.18 ± 1.34(^c)</td>
<td>74.41 ± 1.55(^b)</td>
<td>80.81 ± 1.25(^a)</td>
<td>52.16 ± 1.70(^d)</td>
<td>41.2 – 58.0</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>4.5 ± 0.40(^b)</td>
<td>3.69 ± 0.75(^c)</td>
<td>3.52 ± 0.25(^c)</td>
<td>6.31 ± 0.39(^a)</td>
<td>3.5 – 4.9</td>
</tr>
<tr>
<td>Crude Lipid</td>
<td>31.35 ± 0.66(^b)</td>
<td>27.87 ± 0.75(^c)</td>
<td>21.71 ± 0.79(^d)</td>
<td>35.53 ± 1.09(^a)</td>
<td>15.3 – 26.3</td>
</tr>
<tr>
<td>Energy (kcal) A</td>
<td>512.31 ± 3.57(^b)</td>
<td>423.9 ± 4.06(^c)</td>
<td>375.44 ± 3.63(^d)</td>
<td>535.02 ± 10.10.98(^a)</td>
<td></td>
</tr>
<tr>
<td>Energy (kcal) B</td>
<td>540.95 ± 1.33(^c)</td>
<td>563.24 ± 2.09(^a)</td>
<td>512.40 ± 2.71(^d)</td>
<td>553.66 ± 4.96(^b)</td>
<td>304 - 468</td>
</tr>
<tr>
<td>pH</td>
<td>6.47 ± 0.03(^a)</td>
<td>6.71 ± 0.04(^b)</td>
<td>6.86 ± 0.2(^b)</td>
<td>6.11 ± 0.04(^c)</td>
<td>NP</td>
</tr>
</tbody>
</table>

All values represent the mean ± Standard deviation of three replications. \(^a\)-\(^c\) significant difference (p ≤ 0.05) between means bearing different letters in the same row. Mean (n = 5)

** (Food and Agriculture Organisation (FAO), 2012) Food Composition values from West Africa

NP Not Provided
5.1. **Determination of Moisture Content**

The moisture content in the tiger nuts ranged from 7.72 to 32.18g/100g. There were no significant differences (P ≤ 0.05) between samples from Ghana, nor between Spain and Cameroon. However, moisture of the two samples from Ghana is significantly different from samples from Spain and Cameroon. The differences in the moisture content among the tiger nut sample can be attributed to the difference in variety, place of origin, the agro-cultural practices and processing factors. In contrast to other roots and tubers the tiger nuts has a low moisture content resulting in relatively high dry matter content. However, the obtained value is closer to the values obtained for other nuts samples (Ros, 2010, Rose and Vasanthakaalam, 2011, Venkatachalam and Sathe, 2006) but in sharp deviation from the value (26.0 g/100g) obtained by Sanchez-Zapata et al. (2009). On the other hand, this study might have recorded lower values for samples from Spain and Cameroon due to loss of volatile constituents, degradation of unsaturated fats, caramelisation of sugars and maillard (reaction between reducing sugars and amino acid lysine) in these samples during the drying process. From the table, the values obtained for Cameroon, Ghana (black) and Ghana (brown) falls within the values obtained from the FAO food composition values from West Africa (Food and Agriculture Organisation (FAO), 2012).

Rose and Vasanthakaalam (2011) posited that the average dry matter content for roots and tubers (sweet potato) is approximately 30% but may varies widely depending on factors such as cultivar, location, climate, day length, soil pest diseases, harvesting, storage and cultivation practices.
Low moisture content is important for the preservation of quality and shelf life of seeds, decreasing the probability of microbial growth, unwarranted fermentation, premature seed germination and undesirable biochemical changes (Venkatachalam and Sathe, 2006).

3.17. Ash Content

The ash content of a food is the inorganic residue that is left after the organic matter has been incinerated. It is usually not the same as the inorganic matter present in the original food as there may be losses due to volatilisation or chemical interaction between the constituents (Pearson, 1975).

In Table 5.1, the mean range of ash content for the selected sampled tiger nut flour falls between 1.57-2.14g/100g. It has been posited by Pomeranz and Clifton (1981) that ash contents of nuts, seed and tubers should fall in the range 1.5-2.5% in order to be suitable for animal feeds. The ash content of all the tiger nut samples fell within this range hence it can therefore be recommended for animal feeds. The value obtained falls within the value quoted for nuts and nut products (0.8-3.4%). There is no significant difference (P ≤ 0.05) between samples from Ghana (black) and those from Cameroon. Samples from Spain and Ghana (brown) show no significant difference (P ≤ 0.05). The analysed results from the selected samples reaffirms the findings of the following researchers (Coskuner et al., 2002, Eteshola and Oraedu, 1996, Linssen et al., 1989, Mokady and Dolev, 1970, Sanchez-Zapata et al., 2012, Temple and Ojobe, 1990).
5.3. Determination of minerals content

The mineral profile of the four selected samples from three countries is presented in Table 5.2. Twelve different minerals were detected in all the four samples analysed in this study.

Four (4) tiger nut flour samples were analysed for a total of 12 elements. The results of the analysed macro-mineral and the micro-mineral contents obtained from Cameroon, Ghana and in trade from UK market are detailed as mg/100g and µg/100g respectively on dry weight basis. The results obtained after analysing the data statistically indicate that the concentrations of macro-minerals in the tiger nut flour are in the descending order of K, P, Mg, Ca and Na. The micro-elements again follow this pattern, Fe> Zn> Mn> Cr> Cu> Co >Se. On the face of Table 5.2, the statistically analysed results show that the coefficient of variation were higher in the micro-elements than in the macro-minerals. The sodium has a high coefficient of variation as a result of the analysed Ghana (black) sample result as compared to the sample coming from the same origin (Ghana). This sample is normally grown in the coastal (Gulf of Guinea) region whilst the brown is grown in the Kwahu Rocky Mountains in Ghana (Anonymous, 2013). Even though the two samples are from the same country, yet the place of cultivation is different. The selenium was found to exhibit a higher coefficient of variation for the microelements. This shows that the element is not easy to determine instrumentally as this researcher was made to understand by Fisher (2012), who took the researcher through the experimental instrumentation.
Table 5-2: The dietary minerals (macro-minerals (mg/100g) and micro-elements (µg/100g) composition of tiger nuts samples selected from 3 countries on dry weight basis.

<table>
<thead>
<tr>
<th>Macro-minerals</th>
<th>Country of origin</th>
<th>Cameroon (yellow)</th>
<th>Ghana (black)</th>
<th>Ghana (brown)</th>
<th>Spain (UK market) (brown)</th>
<th>C.V</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td></td>
<td>16.4 ± 0.2</td>
<td>26.5 ± 1.9a</td>
<td>23.1 ± 5.2ab</td>
<td>22.2 ± 1.6ab</td>
<td>20.5</td>
<td>0.016</td>
</tr>
<tr>
<td>Magnesium</td>
<td></td>
<td>73.9 ± 3.9a</td>
<td>78.4 ± 4.8a</td>
<td>80.4 ± 1.9a</td>
<td>77.90 ± 11.8a</td>
<td>12.9</td>
<td>0.462</td>
</tr>
<tr>
<td>Sodium</td>
<td></td>
<td>16.4 ± 5.2b</td>
<td>62.5 ± 0.2a</td>
<td>17.9 ± 3.5b</td>
<td>18.5 ± 3.9b</td>
<td>71.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Potassium</td>
<td></td>
<td>6927 ± 26ab</td>
<td>695 ± 19ab</td>
<td>802 ± 143a</td>
<td>555 ± 96b</td>
<td>17.2</td>
<td>0.051</td>
</tr>
<tr>
<td>Phosphorus</td>
<td></td>
<td>182.3 ± 13.7a</td>
<td>185.5 ± 5.7a</td>
<td>106.4 ± 24.1b</td>
<td>199.4 ± 32.4a</td>
<td>25.1</td>
<td>0.003</td>
</tr>
<tr>
<td>Iron</td>
<td></td>
<td>8.7 ± 2.7a</td>
<td>3.8 ± 0.4b</td>
<td>7.87 ± 1.9ab</td>
<td>10.8 ± 1a</td>
<td>39.0</td>
<td>0.007</td>
</tr>
</tbody>
</table>

| Micro-minerals | | | |
|----------------|-------------------|-------------------|---------------|---------------|---------------------------|-----|---------|
| Chromium       |                   | 637.6 ± 92.8a     | 199.4 ± 52.8b | 389.5 ± 90.4a| 203.5 ± 35.5b            | 54.3| 0.0001 |
| Cobalt         |                   | 14.6 ± 0.7a       | 6.8 ± 1.3b    | 16.6 ± 3.8a   | 15 ± 0.9a                | 33.2| 0.0001 |
| Copper         |                   | 270 ± 50a         | 330 ± 8a      | 320 ± 77a     | 270 ± 6a                 | 18.7| 0.462  |
| Manganese      |                   | 764.3 ± 25.8a     | 294.5 ± 83c   | 836.3 ± 6.4b  | 1250.7 ±1.3b             | 47.3| 0.0001 |
| Selenium       |                   | 2.6 ± 3.7b        | 0.5 ± 8b      | 22.1 ± 8.3a   | 4.8 ± 4.7b               | 133.5| 0.0001 |
| Zinc           |                   | 1,636 ± 127b      | 1,877 ± 175b  | 3,073 ± 666a  | 1,639± 293b              | 34.1| 0.005  |

\[a-c\] means that do not share a letter in the same row are significantly different (p ≤ 0.05). Values for the individual samples are mean and standard deviation of triplicate determination using ICP-OES and ICP-MS instruments.
Table 5-3: Comparison of mineral content (mg/100g) between the current study and reported data for African tiger nuts. Values on means (standard deviation).

<table>
<thead>
<tr>
<th>Minerals</th>
<th>Current study</th>
<th>Niger (Glew et al., 2006)</th>
<th>Nigeria (Ekeanyanwu and Ononogbu, 2010)</th>
<th>Egypt (Arafat et al., 2009)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>22.02 (1.3)</td>
<td>18.8 (0.44)</td>
<td>100 (2.65)</td>
<td>152</td>
</tr>
<tr>
<td>Chromium</td>
<td>0.36 (1.9)</td>
<td>0.29 (0.04)</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>Cobalt</td>
<td>0.13 (0.04)</td>
<td>0.023 (0.004)</td>
<td>NP</td>
<td>12</td>
</tr>
<tr>
<td>Copper</td>
<td>0.29 (0.16)</td>
<td>0.24 (0.046)</td>
<td>0.92 (0.05)</td>
<td>1.3</td>
</tr>
<tr>
<td>Iron</td>
<td>7.8 (0.88)</td>
<td>5.3 (0.1)</td>
<td>4.12 (0.1)</td>
<td>2</td>
</tr>
<tr>
<td>Magnesium</td>
<td>77.6 (2.9)</td>
<td>76.3 (1.3)</td>
<td>94.4 (1.28)</td>
<td>53.3</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.79</td>
<td>1.2 (0.03)</td>
<td>0.26 (0.01)</td>
<td>41</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>168.4 (12.2)</td>
<td>193.7 (6.4)</td>
<td>219.0 (10)</td>
<td>123</td>
</tr>
<tr>
<td>Potassium</td>
<td>686.2 (34.1)</td>
<td>557.3 (20.7)</td>
<td>486 (59.9)</td>
<td>NP</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.074 (0.1)</td>
<td>0.028</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>Sodium</td>
<td>28.8 (5.9)</td>
<td>8.2 (0.8)</td>
<td>34.3 (1.53)</td>
<td>140</td>
</tr>
<tr>
<td>Zinc</td>
<td>2 (0.2)</td>
<td>1.12 (0.02)</td>
<td>3.98 (0.31)</td>
<td>1</td>
</tr>
</tbody>
</table>

NP Not Provided
5.3.1. Calcium

The analysed concentrated calcium content found in tiger nut flour samples was generally low. The result falls within the ranged of 16.3 – 28.6 mg/100g. From Table 5.2, it could be observed that, there was no significant difference between samples from the UK and the Ghana (brown) market. The samples from Cameroon and the Ghana (black) were different from each other in terms of this element. The highest amount of calcium from the selected samples in this study was found in Ghana (black) which was around 26.5mg/100g. The p-value obtained for the analysis carried out on this element was 0.016 which is less than p-value (0.05) meaning there were significant differences between means of the analysed samples. The analysed results are at the same levels as samples from Niger as reported by (Glew et al., 2006), but higher than studies carried out in Nigeria and Egypt as reported by Ekeanyanwu and Ononogbu (2010) and Arafat et al. (2009). The calcium content investigated by Luis et al. (2011) in potatoes is far below that of this current work and other research carried out on tiger nuts.

5.3.2. Chromium

The chromium content of the samples ranged between 146.9 – 824.7µg/100g and the calculated mean for all the four samples pooled together was 357.5 ± 194.2µg/100g. The investigated concentration of samples from Cameroon recorded the highest value 637.6 ± 92.8µg/100g while sample Ghana (black) had the lowest recorded concentration 199.4 ± 52.8µg/100g. The calculated p-value (0.001) for the samples mean is less than the P-value (0.05) and coefficient of variation is 14.56%. Statistically, there are significant differences
between the samples, but investigated samples from Ghana (black) and UK are significantly the same.

5.3.3. Cobalt

The dietary mineral concentration of this element as shown in Table 5-2 and 3 in this investigation ranged from 5.3 – 22.7µg/100g. The minimum value recorded in this work was found to be in samples obtained from Ghana (black), whilst samples (Ghana (brown) coming from the same origin had the highest value 16.6 ± 3.8µg/100g. Apart from sample Ghana (black), all the other investigated samples for this element have the same concentration statistically. The p-value obtained was <0.05 meaning there are significant differences in this element. The findings identified in this studies are in agreement to reported results from Egypt by Arafat et al. (2009). Investigated results from Niger have lower value as compared to current studies and from Egypt.

5.3.4. Copper

The copper concentration of the investigated samples ranged from 215.5 – 372.7µg per 100g on dry weight basis. From Table 5.2, it is observed that statistically the concentration of copper in all the samples are the same, the calculated p value was 0.462 which means the p >0.05. By comparing current work to work carried out by Ekeanyanwu and Ononogbu (2010) and Glew et al. (2006) using samples from Nigeria and Niger respectively indicate that the findings are the same, however, the reported results on samples (Egypt) worked on by Arafat et al. (2009) exceeds that of the current work and that of samples from Niger and Nigeria.
5.3.5. Iron

The iron content of the analysed samples ranged from 3.3 – 11.8mg/100g and the mean of this mineral is 7.8 ± 0.88mg/100g. The analysed concentration result of samples followed in this ascending order Ghana (black) 3.8 ± 0.4mg/100g, Ghana (brown) 7.9 ± 1.9mg/100g, Cameroon 8.7 ± 2.7mg/100g and UK market 10.8 ± 9.6mg/100g. The calculated p-value was 0.007 and was less than the alpha value (0.05). The coefficient of variation for the samples was 39%.

5.3.6. Magnesium

Magnesium concentrations in the investigated four samples ranged between 62 to 98.8 mg/ 100mg. The investigated result from the table shows that all the samples are the same. The calculated p-value which is 0.462 is greater than the alpha (α) value 0.05. This indicates that, the place of origin have no effect on the concentration of magnesium. Probably, this particular element is an inherent to nuts in general as indicated by (Gropper et al., 2005). Investigated report on Table 2 is consistent with the results obtained in this study.

5.3.7. Manganese

The investigated manganese concentration range in this study is 240 - 1355µg/100g. The mean for all the samples as indicated in Table 5.3 is 790µg/100g. From Table 5.2, the sample Ghana (black) had the least recorded concentration (294.5 ± 83µg/100g), whilst the highest concentration was found in UK market sample (1250.7 ± 133.3µg/100g). The calculated p-value was 0.0001 which indicates that the value is less than the α-value (0.05). From
Table 2, samples from Egypt as indicated by (Arafat et al., 2009) the mineral concentration exceeded more than that of Niger and current studies.

5.3.8. Phosphorus

Phosphorus was found to be the next highest minerals in tiger nuts. The concentration of phosphorus in the sampled tiger nut ranged from 82.2 to 230.1 mg/100g. Samples from the UK market recorded the highest (199.4), followed by the black specie from Ghana (185.5), Cameroon (182.3) and the brown specie from Ghana (106.4) mg/100g. Statistically there was no difference between the following samples; UK, Ghana (black) and Cameroon markets however the Ghana (brown) was different from all the other three samples. The calculated p-value was 0.003 which is less than the p-value 0.05 meaning there is significant differences between means of one of the samples and three remaining samples and (Ekeanyanwu and Ononogbu, 2010) found that analysed phosphorus content (193.7mg/100g) selected from Niger are consistent with the current studies.

5.3.9. Potassium

Potassium content of the samples was found to range between 469.4 – 936.3mg/100g. Ghana (brown) recorded the highest value among the selected analysed samples. Whilst the sample obtained from UK market recorded the least. There was no difference between samples obtained from Ghana (black), Cameroon and UK market statistically. The coefficient of variation and the p-value which is greater than or equal to 0.05 for the investigated samples are 17.2% and 0.051 respectively, this indicate that there is significant difference between means of samples.
5.3.10. Selenium

The concentration of dietary element of Selenium ranged from 0.0 - 34µg/100g. The concentration of sample Ghana (black) is 0.5 ± 0.8µg/100g and the sample Ghana (brown) had the highest concentration 22.1 ± 8.3µg/100g. The calculated p-value 0.0001 which is less than the alpha value 0.05 indicates that three of the investigated analysed concentration of this particular element is significantly different from one of the samples. Even though samples Ghana (black) and Ghana (brown) were picked from the country, yet the identified concentrations of this element are significantly different from each other. Studies carried from Nigeria and Egypt did not provide values for this elements, however, Glew et al. (2006) investigated sample from Niger agrees with this studies.

5.3.11. Sodium

The dietary mineral concentration of sodium had a range of 13.3 – 62.7mg per 100g. The analysed Ghana (black) sample recorded the highest value 62.4 ± 0.2 mg per 100g, whilst samples from Cameroon recorded the lowest value (16.4 ± 5.2 mg/100g). Maintaining the ratio of sodium to potassium < 1 in the body is one of the major strategies in the prevention of hypertension (Du et al., 2014, Nieman et al., 1992). The sodium and potassium ratio in tiger nuts is less than one, and therefore from the Authors assertion, the nuts could probably be used to reduce high prevalent rate of high blood pressure in Ghana. Studies conducted and reported by the following authors Ekeanyanwu and Ononogbu (2010), Arafat et al. (2009) and Glew et al. (2006) are in agreement to this studies. When samples from Ghana and Niger are compared, the results followed this pattern, Ghana (black), Ghana (brown) and Niger (studies). The
Ghana (black) is cultivated around the coast of Gulf of Guinea, followed by Ghana (brown) normally found around the middle belt of Ghana and the Niger's studied samples which are cultivated far away from the sea.

5.3.12. Zinc

As shown in the Table, Zinc levels in the different selected samples are within the range 1,333 μg/100g and 3,736 μg/100g. Zinc levels in Cameroon, Ghana (black) and UK market tiger nut samples appear to be comparable (1,635.9 ± 127.1μg/100g, 1,876.8 ± 174.6μg/100g and 1,638.5± 292.8μg/100g whilst the Ghana (brown) samples show the highest concentration 3,072.5 ± 666μg/100g). The investigated results in all the samples exceeded values as reported by the following authors; (Arafat et al., 2009, Ekeanyanwu and Ononogbu, 2010, Glew et al., 2006). This observed variability of Zn in the nut samples could be a consequence of the variability of Zn in the soil or due to the differences in the nature of the nuts. In a study carried out by Moodley et al. (2007), posited that nuts and legumes are relatively good plant sources of Zn, but plant Zn concentrations may get enhanced, if grown in Zn-rich soils or treated with Zn-rich fertilizers. In Ghana, tiger nuts are grown without fertilizer and may indicate that zinc is an intrinsic property of tiger nut.
Table 5- 4: Macro-minerals, micro-elements and the covering (%) of RNI for adults from tiger nut flour (dry weight basis)

<table>
<thead>
<tr>
<th>Macro-minerals</th>
<th>Contents (mg/100g)</th>
<th>RNI (RDA/AI) (mg/day)</th>
<th>Covering of RNI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum - Maximum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>16.3 – 28.6</td>
<td>900 (700)</td>
<td>1.8 – 3.2</td>
</tr>
<tr>
<td>Potassium</td>
<td>469.4 - 936.3</td>
<td>3,400 (4700)</td>
<td>13.8 – 27.5</td>
</tr>
<tr>
<td>Magnesium</td>
<td>62 – 98.8</td>
<td>300 (420)</td>
<td>20.7 – 32.9</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>82.2 – 230.1</td>
<td>3,200 (700)</td>
<td>2.6 – 7.2</td>
</tr>
<tr>
<td>Sodium</td>
<td>13.3 – 62.7</td>
<td>3,800 (1500)</td>
<td>0.35 – 1.65</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Micro-mineral</th>
<th>Content (µg/100g)</th>
<th>RNI (RDA/AI) (µg/day)</th>
<th>Covering of RNI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum - Maximum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromium</td>
<td>146.9 – 824.7</td>
<td>100 (35)</td>
<td>146.9 – 824.7</td>
</tr>
<tr>
<td>Cobalt</td>
<td>5.3 – 22.7</td>
<td>10</td>
<td>53 – 227</td>
</tr>
<tr>
<td>Copper</td>
<td>215.5 – 372.7</td>
<td>1,310 (900)</td>
<td>16.4 – 28.5</td>
</tr>
<tr>
<td>Iron</td>
<td>3,300 – 11,800</td>
<td>13 (18)</td>
<td>25.4 – 90.8</td>
</tr>
<tr>
<td>Manganese</td>
<td>240 – 1,355</td>
<td>3,400 (2,300)</td>
<td>7.1 – 39.9</td>
</tr>
<tr>
<td>Zinc</td>
<td>1,333 – 3,756</td>
<td>123.4 – 347.8</td>
<td>1.080 (1100)</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.0 - 34</td>
<td>56 (55)</td>
<td>0 – 60.7</td>
</tr>
</tbody>
</table>

**Total mineral content** 648.4 – 1,375mg/100g

Reference Nutrients Intake (RNI) quoted from Manual of nutrition (Davies, 2012)
5.4. Implications of mineral content on Reference Nutrient Intake (RNI) and Recommended Daily Intake (RDA/AI)

Table 5.4 shows the range of mineral element composition determined in this study, as well as the contribution (per cent) of the elements investigated, the micro-elements had the highest covering of the RNI as compare to the macro-minerals. Among the macro minerals investigated, magnesium had the highest covering of the RNI, ranging from 20.7 to 27.5 per cent contribution to the RNI, followed by potassium (13.8 to 27.5 per cent RNI), Phosphorus (2.6 to 7.2 per cent RNI), Calcium (1.8 to 3.2 per cent RNI) whilst sodium provides the least covering (0.35 to 1.65 per cent RNI). Davies (2012), posits that macro-minerals such as calcium, phosphorus, potassium, magnesium and sodium in moderation are required in amounts greater than 100mg per day. Intake of calcium, phosphorus, magnesium, and potassium, together with a moderate sodium intake, is associated with protection against bone demineralisation, arterial hypertension, insulin resistance, and overall cardiovascular risk (Segura et al., 2006). The coverings of RNI among the micro elements follow this order chromium, zinc, cobalt, iron, selenium, manganese and copper. The chromium, Cobalt and zinc are found to exceed way beyond the RNI. A feature of the evaluated four sampled nuts was the low Ca : P, Ca : Mg and Na : K ratios with respective means of 0.13, 0.28 and 0.04, these ratios are far above the value as obtained by Vanhanen and Savage (2013).
5.5. **Crude Carbohydrate (CHO)**

The Carbohydrate content for the two methods yielded two different results, because the obtained results depend on the methods used to assess this nutrient, the highest value falls with method B, within the range of 50.46 - 82.06, whilst method A had the least range 39.30 - 53.84g/100g. the result obtained with method A is consistent with the findings of (Oladele and Aina, 2007). The method B recorded higher values probably the system (bomb calorimeter) combusted all the nutrients and phytochemical components (organic materials) to the inorganic composite which is the ashes. The values obtained are higher than those reported by (Sanchez-Zapata et al., 2012). The carbohydrate content of the analysed samples accounts for 39.33-49.31% of energy and it falls within the report proposed by Food and Agriculture Organisation (FAO) and World Health Organisation (WHO) (1998). During their consultation meeting, members proposed that carbohydrates which have an energy value of 4kcal/g and account for 40–75% of energy intake depending on social and economic factors.

5.6. **Determination of High Molecular Dietary Fibre (HMDF) and sugars in tiger nuts flour and its products using Megazyme integrated total dietary fibre method**

The sugars obtained during the hydrolysis of the samples were used to calculate the available carbohydrate base on the information provided below. Available carbohydrate is the sum of the free sugars (glucose, fructose, galactose, sucrose, maltose, lactose and oligosaccharides) and complex carbohydrates (dextrins, starch and glycogen). These are the carbohydrates which are digested and absorbed, and are glycogenic in man. This corresponds
to the term “glycaemic carbohydrates” proposed in the FAO/WHO report on Carbohydrates in Human Nutrition (Food and Agriculture Organisation (FAO) and World Health Organisation (WHO), 1998).

Carbohydrate values expressed as monosaccharide equivalents can exceed 100g per 100g of food because on hydrolysis 100g of a disaccharide such as sucrose gives 105g monosaccharide (glucose and fructose). 100g of a polysaccharide such as starch gives 110g of the corresponding monosaccharide (glucose). Thus white sugar appears to contain 105g carbohydrate (expressed as monosaccharide) per 100g sugar.

Tables 5.5, 5.6 and 5.7 show the analysed results of the studies carried out on the dietary fibre and sugars from country of origin, tiger nut processing method and the type of bread used in the glycaemic index samples respectively.

From Table 5.5, the means for IDF, SDFP, HMWDF, ITDF, Glucose, Maltose and xylose for the analysed samples when pooled together are 17.9g/100g, 1.27g/100g, 19.18g/100g, 19.2g/100g, 44.87g/100g, 0.47g/100g and 5.47g/100g respectively. Among the four samples, the ITDF and IDF contents were low in Ghana (brown) (17.22% and 15.76%), followed by Cameroon (19.14% and 17.3%), Ghana (black) (19.86% and 19.17%), and UK market (Spain) (20.56% and 19.41%) in that ascending order. The values obtained in this study is consistent with the findings of Linssen et al. (1989) but lower than values obtained by Alegria-Toran and Farre-Rovira (2003) as cited by Sanchez-Zapata et al. (2012). The total dietary fibre content is higher than that of nuts, roots and tubers as indicated in chapter two’s literature review. The total dietary fibre in oats varies between 7.1% and 12.1%, the range attributed to differences in cultivars. The typical values for TDF, IDF and SDF in oats are
18.6%, 10.6% and 8.0% respectively as reported by (Kalhlon and Chow, 1997). The IDF content in tiger nuts is higher than in oats; however, the SDF is lower.

In the case of soluble dietary fibre in water but precipitated in 78% aqueous ethanol produced the following results; Cameroon > Ghana (brown) > UK market > Ghana (black). The results for the high molecular weight dietary fibre followed the same patterned result as the insoluble dietary fibre.

The soluble dietary fibre in water and not precipitated in 78% aqueous ethanol was not detected in all the samples apart from Ghana (brown). This may be due to the high nature of starch content in the other three samples as determined in the proximate analysis and high sugar (glucose) content obtained for Ghana (brown). In a study carried out by Brunt and Sanders (2013), they did indicate that there is an imperfection in the AOAC2009.01 method for high starch containing matrices. They go further to state that it appeared that in those matrices the available starch and maltodextrins were not fully converted into glucose and maltose by the enzymatic hydrolysis. This results in minor amounts of residual malto-oligosaccharides still present in the LMWDF fraction, which will erroneously be quantified as LMWDF. Brunt and Sanders (2013) have advised that this error could easily be eliminated by introducing an extra hydrolysis step in the analytical protocol.

The total dietary fibre (Integrated Method) analysed result was in this ascending order Ghana (brown), Cameroon, Ghana (black) and UK market.

The sugars analysed in this study were glucose, xylose (monosaccharide) and maltose (disaccharide). The conversion factors indicated in table 5.4 was used to determine the carbohydrate equivalent in g/100g.
The average available carbohydrate content of the selected sample is 46.66g/100g. The range falls between 39.28 to 48.74g/100g. Samples selected from Ghana (brown) had the highest value (48.34 ± 0.4g/100g) whilst the Cameroon samples recorded the least value result. From the analysed result, there were no significant differences (P ≤ 0.05) between Ghana (brown), Ghana (black) and UK market but the Cameroon sample was different from the Ghana (brown). With the maltose, all the samples are statistically the same (P = 0.068) this is greater than the theoretical value (P ≤ 0.05). The xylose was found to be abundant in the Cameroon samples (6.90 ± 0.30g/100g) whereas the UK market (Spain) (4.68 ± 0.25g/100g) had the least value. The obtained xylose content in tiger nut in this study is lower than the value (9.37%) quoted by Linssen et al. (1989).

The available carbohydrate content for the processing method (water extraction) obtained in this study was 28.37g/100g with a range of 14.26-38.96g/100g. The result obtained for the sample which was extracted twice had the least value (14.32 ± 0.06 and the whole nuts had 38.54 ± 0.42g/100g. The obtained results indicate that extracting tiger nut flour with water has an influence on the dissolve available carbohydrate. The available carbohydrate in the tiger nut bagasse formulated bread used in the glycaemic index test yielded the highest whilst the control bread which was white wheat flour yield the least. The theoretically available carbohydrates that was obtained from food composition tables Food and Agriculture Organisation (FAO) (2012) and Food Standard Agency (FSA) (2004) are higher than results obtained for the wheat bread (white).

From Table 5.6, the two separate water extraction yielded the highest result value for insoluble dietary fibre, followed by the one extraction and whole nuts. With the SDFP a different yield pattern ensued; second extraction > whole nut >
first extraction. The HMWDF and ITDF followed the same pattern as described under IDF. The SDFS was detected in the whole nut whilst there was no detection in the first and second extraction. All the sugars yielded the same pattern; whole nuts > first extraction > second extraction.

From Table 5.7 the type of processed bread yielded different result for all the dietary fibre components. With the IDF, the whole nut flour bread yielded the highest value followed by the bagasse flour bread and the control bread (wheat flour bread). The yield for SDFP was as follows control > bagasse bread > whole nut flour bread. HMWDF had this pattern of yield whole nut > bagasse > control. The ITDF and all the sugars had the same pattern of yield; bagasse > whole nut > control. The results obtained in this table 5.7 are lower than table 5.5 and 5.6 because of the following reasons; heat treatment (baking) of the tiger nut product (bread) rendered it more digestible and the yeast in the products did use up the carbohydrate fraction prior to this work. In bread-making, the yeast breaks down the sugars that are formed from the starch in the dough to alcohol and then to carbon dioxide and water.
Table 5: Dietary fibre fractions and sugars (mean ± standard deviation) obtained in tiger nuts samples selected from Cameroon, Ghana (black), Ghana (brown) and UK market (Spain) in (g/100g) Dry basis

<table>
<thead>
<tr>
<th>Sample origin</th>
<th>Dietary fibre</th>
<th>Sugars</th>
<th>Available carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IDF SDFP HMWDF SDFS ITDF Glucose Maltose Xylose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cameroon</td>
<td>17.3 1.84 19.14 ND 19.14 41.18 ± 2.36b 0.54 ± 0.1a 6.90 ± 0.33</td>
<td>41.75 ± 2.47</td>
<td></td>
</tr>
<tr>
<td>Ghana (black)</td>
<td>19.17 0.69 19.86 ND 19.86 47.31 ± 0.37ab 0.35a 4.89 ± 0.03b</td>
<td>47.68 ± 0.37</td>
<td></td>
</tr>
<tr>
<td>Ghana (brown)</td>
<td>15.76 1.41 17.17 0.05 17.22 47.78 ± 0.38a 0.53 ± 0.02a</td>
<td>48.34 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>UK market (Spain)</td>
<td>19.41 1.15 20.56 ND 20.56 43.19 ± 1.94ab 0.45 ± 0.02a</td>
<td>43.67 ± 1.96</td>
<td></td>
</tr>
</tbody>
</table>

a, b Means that do not share a letter are significantly different

Table 5: Content of dietary fibre fractions and sugars of processed tiger nuts in (g/100g) Dry basis

<table>
<thead>
<tr>
<th>Tiger nut flour processing method</th>
<th>Dietary Fibre Fraction</th>
<th>Sugar</th>
<th>Available carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IDF SDFP HMWDF SDFS ITDF Glucose Maltose Xylose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole nut Flour</td>
<td>17.57 2.19 19.76 0.49 ± 0.02 20.27 38.12 ± 0.36a 0.40 ± 0.06a</td>
<td>38.54 ± 0.42</td>
<td>4.79 ± 0.1a</td>
</tr>
<tr>
<td>1st Extraction</td>
<td>23.72 1.97 25.69 ND 25.69 31.21 ± 0.13a 0.4a</td>
<td>31.63 ± 0.13</td>
<td>4.79 ± 0.02a</td>
</tr>
<tr>
<td>2nd Extraction</td>
<td>28.87 3.17 32.04 ND 32.04 14.18 ± 0.04c 0.13 ± 0.02b</td>
<td>14.32 ± 0.06</td>
<td>1.94 ± 0.06b</td>
</tr>
</tbody>
</table>

Means that do not share the same letter are significantly different
<table>
<thead>
<tr>
<th>Type of bread used in G.I test</th>
<th>Dietary Fibre</th>
<th>Sugars</th>
<th>Available carbohydrate</th>
<th>Xylose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IDF&lt;sup&gt;A&lt;/sup&gt;</td>
<td>SDFP&lt;sup&gt;B&lt;/sup&gt;</td>
<td>HMWDF&lt;sup&gt;C&lt;/sup&gt;</td>
<td>SDFS&lt;sup&gt;D&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>4.1</td>
<td>2.78</td>
<td>6.88</td>
<td>0.80</td>
</tr>
<tr>
<td>Bagasse</td>
<td>6.77</td>
<td>2.42</td>
<td>9.19</td>
<td>ND</td>
</tr>
<tr>
<td>Whole nut flour</td>
<td>8.94</td>
<td>0.75</td>
<td>9.69</td>
<td>1.17 ± 0.06</td>
</tr>
</tbody>
</table>

Means that do not share the same letter are significantly different ($P < 0.05$). Values are means of duplicates and ± standard deviation.
5.7. Crude Total Lipid

Crude total lipid was the second predominant component, ranging from 20.92g per 100 g DM in Ghana (brown) to 36.62g per 100 g dry matter (dm) in Spain (brown), with an average of 29.94g per 100 g DM. The Ghana (brown) recorded the lowest value and statistically there were no significantly difference between samples from Cameroon, Spain and Ghana (black). These results are consistent with several studies that have been reported in literature. It has been reported to range between 22.8 and 32.8g/100g (dm) (Coskuner et al., 2002, Eteshola and Oraedu, 1996, Linssen et al., 1989, Mokady and Dolev, 1970, Sanchez-Zapata et al., 2012, Temple and Ojobe, 1990). The wide range may be attributed to the fact that some of the lipid was bound to the protein and therefore an alkali or acid should first be used before applying Petroleum ether in the final extraction.

From this study, the crude lipid content seems to be fairly constant and independent of origin as suggested by (Nasri et al., 2005), and the small variations may be attributed to environmental factors, growing conditions and postharvest handling (Sagredo-Nieves, 1992).

The high amount of lipids in tiger nuts is comparable to the content found in seeds used for production of commercial vegetable oils such as sunflower seed (22–36%) and rapeseed (22–49%) (Salunkhe and Kadam, 1989, Belitz et al., 2004). The amount of lipids is almost twice that of soybeans (17–20% (dm) (Belitz et al., 2004).

The crude lipid content of 36.62g/100g was higher than for most nuts and tubers: potatoes, Cassava and Fenugreek seeds but lower than for peanuts 49.7g/100g, Conophor nut 46.5g/100g and walnut 58g/100g (Sanchez-Zapata
The high lipid content for tiger nuts obtained in this study affirms the statement made by Manek et al. (2012) that tiger nut tubers are the only known roots and tubers to have substantial amount of oil.

The extracted tiger nut oil had a golden yellowish colour, bland flavour and neither solidified when stored in a deep freezer nor crystalized in a refrigerator (4.0°C).

5.8. **Crude Protein Content**

The present study revealed low levels of crude protein in tiger nuts, with a range between 3.27 – 6.70g/100g (dm) irrespective of the origin and varieties. The crude protein content was high in the Spain (brown) sample 6.31 ± 0.39g/100g (db), while protein content was least in the Ghana (brown) sample variety with 3.52 ± 0.25. The results findings in this study is consistent with the findings of Sanchez-Zapata et al. (2012). Analysis of variance showed that the geographic origin of samples had a significant effect on protein content ($P < 0.0001$), that is, samples selected from Cameroon, Ghana (black) and Ghana (brown) (West Africa) show no significant difference ($P ≤ 0.05$) between them. In Spain, cultivation of tiger nuts is a well-developed industry where fertilisers are used to improve soil fertility whereas in Ghana, the peasant farmers have no money to buy fertiliser to manure the land. This may account for the high protein content recorded for samples from Spain.

Protein content in the diets of low income groups in developing countries like Ghana and Cameroon is derived mostly from foods of vegetable origin. The average total protein content of tiger nut is low as compared to other nuts (Erdogan and Aygun, 2005, Freitas and Naves, 2010, Panahia and Khezri,
2011, Ros, 2010, Venkatachalam and Sathe, 2006), however, it is superior to other roots and tubers such as cassava, plantains, taro and inferior to potato, yams and cereals even those cooked as porridges (Rose and Vasanthakaalam, 2011).

5.9. Caloric content

The caloric value of tiger nuts (A) and for (B) as shown in Figure 5.1 was estimated to be within the range of 371.81-546.0 kcal/100g (db) and 515.11-565.33 kcal/100g (db) respectively, which is an indication that it could be an important source of dietary calories. The high caloric content of tiger nuts is attributed to high carbohydrates and oil contents.

From the figure below it could be observed that the Atwater method (A) produced lower energy values when compared to the bomb calorimetric method.(B) In method A, all selected analysed samples indicate statistically that they are significantly different (P≤0.05) from each other.

Again, the method B results indicate that they are statistically different from each other. However, the two methods gave two different results, the method B result are higher than the calculated method (Atwater) probably from the reasons as Livesey et al. (2000) has indicated: (i) errors introduced when estimating carbohydrate by difference; (ii) the relative proportions of monosaccharides, disaccharides and polysaccharides in the food; and (iii) the way in which dietary fibre or unavailable carbohydrate is taken into account.
Figure 5-1: Energy content of tiger nut selected from 4 samples types. Means that do not share a letter are significantly different (p ≤ 0.05). (n=3)

Deep green colour (By calculation (Atwater’s method)) and light green (Bomb calorimetric method)

5.10. Determination of pH of selected tiger nuts sample

The pH of tiger nuts’ milk falls within the range of 6.11-6.86. From the graph, samples from Ghana had the highest values followed by Cameroon and Spain in that descending order. Samples from Spain are more acidic than the rest of...
the selected samples. This finding is quite consistent with the pH value 6.3-6.8 quoted by Sanchez-Zapata et al. (2009). Again, this result is consistent with the findings for Spanish samples under section water uptake, the pH influences the absorption of water by influencing the molecular charges (Huyghebaert, 1999). The pH values indicate that the tuber especially the ones obtained from Ghana will be more prone to microbial growth and therefore need to be stored well. McClendon and Sharp (1919) have indicated that pH in foods are all on the acid side of neutrality no matter what the condition of freshness, mode of storage, stage in the preparation (cooking) or dilution with water. Potatoes, sweet potato juice, carrot juice like all root and tuber crops have a pH range of 5.80 to 9.44

5.11 Antioxidant properties of tiger nuts

5.11.1 Rancimat method

The two most important types of chemical reactions identified in the food industries responsible for quality loss in processed foods are the browning and oxidation (Kulisic et al., 2005). Brand-Williams et al. (1995), have indicated that the process of lipid oxidation in foods is responsible for the formation of off-flavours and undesirable chemical compounds, which may or not to be hazardous to health. In order to counteract these problems it is necessary to choose the best conditions, Rancimat method is normally used to determine the antioxidant capacity of lipids and also provide relatively good results regarding shelf-life prediction of the lipid systems; especially when the levels of the operational parameters of the instrument are suitably chosen. It is used in the food industries and academic world to obtain result within a few hours instead of weeks and months.
An antioxidant activity index higher than one indicates the prevention of lipid oxidation by the antioxidant sample. Even though the use of this method has been questioned (Frankel, 1993), yet it is a procedure commonly used in the food industry and governmental analytical laboratories (Parejo et al., 2003).

The antioxidant activity of the samples (Ghana (black), Ghana (brown), Cameroon, UK market (Spain), and BHT) at four different temperatures (120, 130, 140, and 150ºC) were tested on lard. Table1 and 2 show the induction time and related antioxidant activity index (AAI) of lard spiked with the selected samples respectively. By this method, all test samples mixed with the lard have significantly increased the oxidation time of lard (p ≤ 0.05). The control sample (lard) had an induction time of 4.47 hours but when it was spiked with the test samples at different temperatures, the induction time increased more than thrice. At all the chosen temperatures the UK market (Spain) had the highest induction but the rest of the test samples were statistically the same. The BHT is highly volatile at high temperatures as it was observed condensed on the glassware used in the test, hence the result obtained.

As expected the induction time decreased as the experimental temperature was increased. Heat processing (i.e. frying) could also reduce the levels of natural and added antioxidants in fats, and this seems to be dependent on the saturation level and heating regimes, as studied Barrera-Arellano et al. (2002) for tocopherols and by Gomez-Alonso et al. (2003) for phenolic compounds. The matrix may then play a relevant role on the antioxidant or prooxidant activity, as it may influence interactions of compounds and possibly interfere with reaction kinetics. No statistically different results were found when experiment was conducted at temperature of 130ºC. The AAI for all the tiger nut oil and the BHT had values more than 1. At 120ºC, tiger nut oil had values
ranging from 3.16 to 3.98 and the BHT artificial antioxidant which is normally used in the food industries had a value of 3.39. The BHT may be small because of its volatility and might have not mixed with the lard well during the experiment, this findings is confirmed by observation made by Gramza-Michalowska et al. (2011), that results on sunflower oil oxidative stability in the Rancimat test indicate a limited antioxidant potential of examined spice extracts and synthetic BHT.

The value obtained for the tiger nut oil and BHT are higher than values quoted by Sanchez-Zapata et al. (2012) when they use tiger nut liquid co-product (TNLC) (2.32) and BHT (2.42). In previous experiment that was carried out by Sanchez-Zapata et al. (2010) using TNLC and ascorbic acid (1.44), the AAI for TNLC was higher than the ascorbic acid and again the AAI for TNLC (1.30) was higher than lemon extracts (1.19) (Kuri et al., 2005). The tiger nut sample used in their experiment was different from the samples used in this current study and this may account for the disparities in the use of the same biological sample.
Table 5-8: Rancimat induction times (hours) (Ț Rancimat) for the oxidation of lard spiked with BHT and tiger nut oil

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Temperature (ºC)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>120</td>
<td>130</td>
<td>140</td>
<td>150</td>
</tr>
<tr>
<td>Lard (Control)</td>
<td>4.47 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.75 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.9 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.55 ± 0.11&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>BHT</td>
<td>15.13 ± 0.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.41 ± 1.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.39 ± 0.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.75 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cameroon</td>
<td>14.73 ± 0.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.36 ± 0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.48 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.61 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ghana (black)</td>
<td>15.67 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.37 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.36 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.75 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ghana (brown)</td>
<td>14.70 ± 0.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.85 ± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.70 ± 0.12&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.89 ± 0.38&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>U.K market (Spain)</td>
<td>17.37 ± 0.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.40 ± 0.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.96 ± 0.53&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.48 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-c</sup> Mean values within a column with the same letter are not significantly different at (P≤0.05) according to Tukey’s test. Values are shown as mean ± standard deviation of three replicates.
Table 5-9: Antioxidant Activity Index (AAI) for lard spiked with BHT and tiger nut lipid measured at different temperatures by Rancimat method

<table>
<thead>
<tr>
<th>Sample</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>120</td>
</tr>
<tr>
<td>Lard (Control)</td>
<td>1.00</td>
</tr>
<tr>
<td>BHT</td>
<td>3.39 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cameroon</td>
<td>3.30 ± 0.09&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ghana (black)</td>
<td>3.51 ± 0.13&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ghana (brown)</td>
<td>3.29 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>UK market (Spain)</td>
<td>3.88 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>ab</sup> Mean values within a column with the same letter are not significantly different at (P≤0.05) according to Tukey's test. Values are shown as mean ± standard deviation of three replicates.
5.11.2. Determination of free radical scavenging activity by DPPH method

DPPH radical is a stable organic free radical with an absorption band at 517 nm. It loses this absorption on accepting an electron or a free radical species, which results in a visually noticeable discolouration from purple to yellow. It can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentration (Hseu et al., 2008).

The absorbance profiles of DPPH in ethanol (95%), ethanol (70%), methanol (absolute) and methanol (70%) are shown in Figure 1. The order of antioxidant scavenging of DPPH was methanol (absolute) = ethanol (70%) > methanol (70%) > ethanol (95%). From Table 0-1 and Figure 0-1, it could be observed that there is no significant variation (P < 0.05) between methanol and ethanol (70%) with the solvents methanol (70%) and ethanol (95%). Among the solvents, the ethanol (70%) extracts show consistency in scavenging activity. The Ghana (black) exhibit the highest antioxidant value compared to the other samples.

According to the following researchers (Gülçin et al., 2004, Hinneburg et al., 2006, Lu et al., 2011, Viuda-Martos et al., 2010b), a positive relationship between total phenol and antioxidant capacity was found in many plants, however, in this study there was no correlation between total phenolic content and the antioxidant activity of the selected tiger nut samples.
Table 5-10: DPPH radical scavenging activities of tiger nut samples using different solvents for extraction calculated with Lambert-Beer law.

<table>
<thead>
<tr>
<th>Sample origin</th>
<th>Solvents</th>
<th>Ethanol (95%)</th>
<th>Methanol (70%)</th>
<th>Ethanol (70%)</th>
<th>Methanol (abs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cameroon</td>
<td></td>
<td>1.01 ± 0.14bc</td>
<td>2.3 ± 0.19ab</td>
<td>3.70 ± 1.17ab</td>
<td>1.12 ± 0.19bc</td>
</tr>
<tr>
<td>Ghana (black)</td>
<td></td>
<td>2.04 ± 0.28bc</td>
<td>3.99 ± 1.24ab</td>
<td>7.89 ± 0.61ab</td>
<td>7.98 ± 0.73ab</td>
</tr>
<tr>
<td>Ghana (brown)</td>
<td></td>
<td>1.42 ± 0.39bc</td>
<td>2.66 ± 0.27ab</td>
<td>3.67 ± 1.20ab</td>
<td>1.40 ± 0.23bc</td>
</tr>
<tr>
<td>UK market</td>
<td></td>
<td>1.04 ± 0.18bc</td>
<td>2.00 ± 0.40bc</td>
<td>4.35 ± 1.16ab</td>
<td>1.39 ± 0.14bc</td>
</tr>
</tbody>
</table>

Values are shown as mean ± standard deviation of five replicates. ab and A-C Means followed by the same letter in the same column and row respectively are not significantly different (P < 0.05).

5.12.3. Determination of Total Phenolic Contents

Extraction conditions and characteristics of the sample can affect the efficiency of the extraction, independently or interactively (Liyana-Pathirana and Shahidi, 2005).

Natural phenolic compounds as asserted by Fang et al. (2002) contribute and exert their health beneficial effects mainly through their antioxidant activity. These compounds are capable of decreasing oxygen concentration, quenching singlet oxygen, preventing first chain initiation by scavenging initial radicals such as hydroxyl radicals, binding metal ion catalysts, decomposing primary products of oxidation to non-radical species and breaking chains to prevent continued hydrogen attraction from substances (Shahidi and Naczk, 2003).

The total phenolic content yielded of crude extracts obtained using ethanol absolute, ethanol 70%, methanol (absolute) and methanol 70% are presented
both in graphical and table forms. In Figure 1 and 2, the amount of extracted total phenolic content varied among samples’ place of origin and type of solvent use. From the graphs, the samples obtained from Cameroon and the Ghana (brown) yielded different values with the types and concentration of solvents, whereas the Ghana (black) and the UK market (Spain) samples followed the same pattern in this order; ethanol (absolute), ethanol (70%), methanol (absolute) and methanol (70%). Using ethanol absolute, the black specie from Ghana yielded the highest TPC. This finding is different from the findings reported by Samarin et al. (2012) and Tatiya et al. (2011) when they studied the TPC yield in potatoes and a stem bark respectively. In their studies they reported that the total phenolic content from the extracted prepared samples at concentration of 2g per litre differ significantly, ranging from 94.52mg GAE g\textsuperscript{-1} in Cameroon samples and 393.03mg GAE g\textsuperscript{-1} in Ghana (black) as depicted in (Table 1-4). The total phenolic content of Ghana (black) was significantly higher \((P < 0.05)\) than for all the rest of samples. The samples obtained from UK market (Spain) yielded values ranging between 128.93 to 182.74mg GAE g\textsuperscript{-1}, samples from Ghana (brown) yielded 97.86 to 171.79mg GAE g\textsuperscript{-1} whereas the Cameroon samples yielded the least value 94.52 to 163.54mg GAE g\textsuperscript{-1}. Samples obtained from Ghana were significantly different from each other; this may be due to species variation. However, the samples from UK market (Spain) and Ghana (brown) were of the same species, but again differ significantly from each other. Results reported by (Sanchez-Zapata \textit{et al.}, 2012) is within the range of values obtained in this current study. Even though tiger nut was used by these authors and present study, yet different components were used. In this study the whole tiger nut flours were used whereas, the above authors used the
bagasse. The two identical results may suggest that the TPC were located in the cell walls of the bran as it has earlier been reported by (Parker et al., 2000).
Figure 5-3: Total Phenolic contents yield per average concentration (0.112 mg (GAE g⁻¹)) with different extraction solvents. 

a-d Means follow by the same letter are not significantly different (P ≤ 0.05)
Figure 5-4: Effect of sample origin, type of extraction solvents and concentration on TPC yield.
Table 5-11: Total phenol content of tiger nut extracts measured in gallic acid equivalent (mM) at different concentrations of extracting solvents

<table>
<thead>
<tr>
<th>Sample origin</th>
<th>Solvent</th>
<th>Solvent concentration (mg/l)</th>
<th>0.04</th>
<th>0.06</th>
<th>0.1</th>
<th>0.16</th>
<th>0.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cameroon</td>
<td>Ethanol (absolute)</td>
<td></td>
<td>42.02 ± 0.21^c</td>
<td>55.12 ± 0.55^b</td>
<td>65.83 ± 0.21^a</td>
<td>123.45 ± 0.41^c</td>
<td>163.33 ± 0.21^a</td>
</tr>
<tr>
<td>Ghana (brown)</td>
<td></td>
<td></td>
<td>39.05 ± 0.41^a</td>
<td>44.29 ± 0.36^c</td>
<td>70.83 ± 0.21^a</td>
<td>116.55 ± 0.21^d</td>
<td>148.91 ± 0.00^a</td>
</tr>
<tr>
<td>Ghana (black)</td>
<td></td>
<td></td>
<td>71.91 ± 0.21^a</td>
<td>116.67 ± 0.21^a</td>
<td>203.10 ± 0.41^a</td>
<td>314.64 ± 0.62^a</td>
<td>392.62 ± 0.41^a</td>
</tr>
<tr>
<td>Spain</td>
<td></td>
<td></td>
<td>45.36 ± 0.71^b</td>
<td>55.36 ± 0.36^b</td>
<td>80.00 ± 0.00^d</td>
<td>142.62 ± 0.74^b</td>
<td>178.69 ± 0.21^b</td>
</tr>
<tr>
<td>Cameroon</td>
<td>Methanol (absolute)</td>
<td></td>
<td>17.26 ± 0.21^c</td>
<td>46.91 ± 0.21^b</td>
<td>63.81 ± 0.21^c</td>
<td>69.17 ± 1.03^d</td>
<td>94.52 ± 0.21^a</td>
</tr>
<tr>
<td>Ghana (brown)</td>
<td></td>
<td></td>
<td>17.38 ± 0.21^c</td>
<td>29.64 ± 0.36^a</td>
<td>48.10 ± 0.21^c</td>
<td>73.93 ± 0.36^c</td>
<td>97.86 ± 0.00^c</td>
</tr>
<tr>
<td>Ghana (black)</td>
<td></td>
<td></td>
<td>47.50 ± 0.00^a</td>
<td>126.07 ± 0.00^a</td>
<td>175.48 ± 0.55^a</td>
<td>196.43 ± 0.00^a</td>
<td>242.50 ± 0.00^a</td>
</tr>
<tr>
<td>Spain</td>
<td></td>
<td></td>
<td>26.07 ± 0.00^c</td>
<td>43.93 ± 0.00^c</td>
<td>75.71 ± 0.36^c</td>
<td>110.36 ± 0.00^c</td>
<td>128.93 ± 0.00^c</td>
</tr>
<tr>
<td>Cameroon</td>
<td>Ethanol (70%)</td>
<td></td>
<td>23.69 ± 0.81^a</td>
<td>37.14 ± 0.00^a</td>
<td>65.12 ± 0.74^a</td>
<td>108.45 ± 0.21^a</td>
<td>133.45 ± 0.21^a</td>
</tr>
<tr>
<td>Ghana (brown)</td>
<td></td>
<td></td>
<td>26.07 ± 0.00^c</td>
<td>45.36 ± 0.62^c</td>
<td>77.5 ± 0.36^c</td>
<td>137.02 ± 0.21^c</td>
<td>171.79 ± 0.00^c</td>
</tr>
<tr>
<td>Ghana (black)</td>
<td></td>
<td></td>
<td>63.45 ± 1.69^a</td>
<td>100 ± 0.36^a</td>
<td>175.36 ± 0.36^a</td>
<td>271.31 ± 0.74^c</td>
<td>335.71 ± 0.00^a</td>
</tr>
<tr>
<td>Spain</td>
<td></td>
<td></td>
<td>30.60 ± 0.41^b</td>
<td>48.69 ± 0.55^b</td>
<td>85.48 ± 0.21^c</td>
<td>142.50 ± 0.36^b</td>
<td>176.31 ± 0.55^b</td>
</tr>
<tr>
<td>Cameroon</td>
<td>Methanol (70%)</td>
<td></td>
<td>19.41 ± 0.41^a</td>
<td>27.50 ± 0.62^a</td>
<td>46.74 ± 0.62^a</td>
<td>78.21 ± 0.71^a</td>
<td>96.31 ± 0.21^a</td>
</tr>
<tr>
<td>Ghana (brown)</td>
<td></td>
<td></td>
<td>28.69 ± 0.90^c</td>
<td>41.19 ± 0.74^c</td>
<td>69.76 ± 0.83^c</td>
<td>116.43 ± 0.36^c</td>
<td>126.43 ± 0.36^c</td>
</tr>
<tr>
<td>Ghana (black)</td>
<td></td>
<td></td>
<td>50.24 ± 0.21^a</td>
<td>72.62 ± 0.55^a</td>
<td>108.57 ± 0.36^a</td>
<td>186.55 ± 0.21^a</td>
<td>238.21 ± 0.95^a</td>
</tr>
<tr>
<td>Spain</td>
<td></td>
<td></td>
<td>37.74 ± 0.55^b</td>
<td>56.55 ± 0.20^c</td>
<td>102.38 ± 0.41^b</td>
<td>148.21 ± 0.00^b</td>
<td>181.79 ± 0.95^b</td>
</tr>
</tbody>
</table>

*Mean values with the same letter for each solvent within a column are not significantly different at (P ≤ 0.05) according to Tukey's test.*
CHAPTER 6

NEW FOOD PRODUCTS DEVELOPMENT AND HEALTH BENEFITS

Results and Discussion

6.1. Effect of tiger nut bagasse flour (TNBF) on bread loaf characteristics

6.1.1. Effect of TNBF on loaf volume and specific volume

The effect of the TNBF substitution on bread quality characteristics is summarised in Table 6-1 and the pictures could be observed from Figure 6-1 and Figure 6-2. Table 6-1 shows the mean results obtained for the dough weight, bread weight, volume, bread specific volume and the bread yield.

The incorporation of TNBF substitution at all levels decreased the volume of the breads significantly (p < 0.05), but the loaf weight of the bread were somehow insignificantly different from each other (p < 0.05). Ragaee et al. (2011) and Hathorn et al. (2008) reported that partial substitution of wheat flour with barley, oat, rye, cellulose and sweet potato flour has resulted in reduction of loaf volume in bread. It was observed during the kneading and fermentation of the dough that partially substituted TNBF for wheat flour diluted the protein and interfered with the optimal gluten matrix formation. The viscosity and texture of the dough formed were lost as the levels increased. From the findings of Hung et al. (2007), this effect could have been counteracted by increasing the water content of the dough.

The loaf bread volume for the control bread was 403 ± 17.1 ml. However, a significant reduction in loaf volume was observed as the level of substitution with tiger nuts fibre was
increased. For example when the fibre was increased from 0 to 40g/100g, the loaf volume reduced by almost 56%. This effect may be due to the reaction between fibre and gluten, this interaction caused decreased gas retention ability of the formed dough which led to cracks and reduction of the volume of the bread. Chen et al. (1988) and Sangnark and Noomhorm (2004) reported similar findings in their studies. The specific volumes of the breads ranged from 0.74 to 2.03 ml/g. In this study, substitution at 20, 30 and 40 percent of TNBF to wheat flour yielded reductions in the specific volumes from 0.74–1.69 ml/g, compared with the control. The substitution at 40 percent resulted in the lowest specific volume, compared with the other bread samples. In terms of loaf yield, bread containing 30 percent TNBF, with the exception of 20 and 40 percent substitution, were significantly (p < 0.05) higher than the control. The calculated bread density, of the baked bread containing TNBF substitution at all levels, were significantly (p < 0.05) denser than the control. This indicates that the TNBF bread had a tightly packed crumb structure; the density reflects the size and ratio of air cells to the solid product. Possible explanations for the high density of TNBF substitution may include factors such as the dilution of the gluten network and the water holding capacity of the bagasse flour.
Figure 6-1: Bread with tiger nut bagasse nut substitution at different levels

Figure 6-2: Appearance of tiger nuts bread

0% Wheat flour bread without tiger nut bagasse flour; 20% (20% bagasse substitution); 30% (30% bagasse substitution); 40% (40% bagasse substitution)
Table 6-1: Effect of tiger nuts bagasse flour substitution on the physical characteristics of bread

<table>
<thead>
<tr>
<th>TN substitution level (g/100g)</th>
<th>Dough weight (g)</th>
<th>Bread weight (g)</th>
<th>Volume (ml)</th>
<th>Density of bread</th>
<th>Bread Specific volume (ml/g)</th>
<th>Bread Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0**</td>
<td>241.05 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>207.07 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>402.9 ± 17.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.51&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.95 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.9 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>241.38 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>210.35 ± 0.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>349.38 ± 7.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.60&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.66 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.1 ± 0.3&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>241 ± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>211.53 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>238.9 ± 15.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.13 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>87.6 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>40</td>
<td>241.76 ± 0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>208.66 ± 1.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>171.1 ± 15.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.82 ± 0.08&lt;sup&gt;d&lt;/sup&gt;</td>
<td>86.3 ± 0.5&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>abcd</sup> means bearing different superscripts within each column differ significantly.

** Control (white bread)  Values are the Mean ± SD (n=3).
6.1.2. Effect of TG on loaf texture

Figure: 6.3 and Table: 6. 3 indicate that tiger nut’s bagasse has effect on the bread crumb firmness above 30% substitution. The calculated p-value (0.001) shows that there is a strong positive relationship between bread crumb firmness and levels of tiger nuts addition. There was no significant change at level below 20% substitution. This finding is reflected in studies carried out by Greene and Bovell-Benjamin (2004) they reported that there is a positive relationship between bread crumb hardness/firmness and loaf volume in gluten-free breads. Conforti and Davis (2006), found that bread crumb texture was firmer in bread containing non-wheat flours than only wheat flour.

Figure 6-3: Texture profile of tiger nut’s bagasse substitution level (0, 20, 30 and 40) on bread firmness.
Table 6-2: Texture profile of tiger nuts bagasse substitution level (%) (0, 20, 30, and 40) on bread firmness.

<table>
<thead>
<tr>
<th>Sample treatment (g/100g)</th>
<th>Firmness (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0)</td>
<td>11.68 ± 1.01a</td>
</tr>
<tr>
<td>20</td>
<td>11.29 ± 1.05a</td>
</tr>
<tr>
<td>30</td>
<td>16.79 ± 1.56b</td>
</tr>
<tr>
<td>40</td>
<td>17.52 ± 1.7c</td>
</tr>
</tbody>
</table>

abc Mean values with the same letter for each solvent within a column are not significantly different at (P ≤ 0.05) according to Tukey’s test.

6.1.3. Effect of TG on loaf colour

The effect of tiger nuts bagasse addition on the bread colour is summarised in Figure 6-1 & 2 and Table 6-4 & 5. All colour data were expressed by Hunter $L^*$, $a^*$, and $b^*$ values corresponding to lightness, redness, and yellowness, respectively. The crust colour of samples was affected by the replacement of wheat flour with tiger nut bagasse flour. In general, as tiger nut bagasse flour level increased, the crust colour became darker as measured by the colorimeter. The crust of the control was lighter and less yellow than any of the other samples. Although the original colour of ingredients may have some influence on the bread crust colour due to association to Millard and caramelisation as reported by Therdthai and Zhou (2003) yet, the colour difference $\Delta E^*_{ab}$ on the bread crust supplemented with tiger nuts bagasse (taking the normal bread colour as reference) shows that there was no significant difference between control and the three treated samples. However, taking a critical look on the bread crust colour of the three samples found in Figure 6.1 & 6.2 show that the samples containing the 30 and 40 percent tiger
nut fibre were darker than the control and the 20 percent fibre substitution. In terms of the a* and b* crust colour values, breads with 0 and 20 percent fibre substitution gave a higher values than breads with 30 and 40 percent fibre substitution, the a* and b* values differ significantly at p < 0.05. This finding is in total agreement with what have been reported by Anil (2007) and Mohammed et al. (2012), however, the reported finding for the L* value for the control bread crust colour differs from the study conducted by Anil (2007).

From Table 6-4 and Figure 6-4, the L values for the bread crumb colour in the samples, decrease as the level of substitution of wheat flour with fibre increase, this indicates that the colour changes from white to grey. The a* and b* on the other hand increased as the level of substitution goes up. The obtained results are consistent with the findings of Hooda, (2005) and Miguel, (1999). The crumb colour difference, $\Delta E_{ab}$ (taking the control bread colour as reference) shows the influence of tiger nut substitution on bread crumbs. The darkening of bread containing tiger nut bagasse flour may be attributed to an increased Maillard reaction taking place during baking due to the presence of amino acid lysine and reducing sugar. In the Maillard reaction, reducing carbohydrates react with free amino acid side chain of protein mainly lysine and lead to amino acid–sugar reaction products (polymerized protein and brown pigments).
Table 6- 3: Crust colour of bread prepared from wheat flour (control) and wheat flour substituted at 20, 30 and 40 percent with tiger nut bagasse flours.

<table>
<thead>
<tr>
<th>Sample treatment (g/100g)</th>
<th>Bread Crust Colour</th>
<th></th>
<th></th>
<th>ΔE&lt;sub&gt;ab&lt;/sub&gt;*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lightness (L*)</td>
<td>Redness (a*)</td>
<td>Yellowness (b*)</td>
<td></td>
</tr>
<tr>
<td>0 (Control)</td>
<td>54.68 ± 0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.89 ± 0.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.01 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>58.71 ± 2.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.51 ± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.57 ± 0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.98 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>54.54 ± 2.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.22 ± 0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.44 ± 0.64&lt;sup&gt;ba&lt;/sup&gt;</td>
<td>6.43 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>40</td>
<td>55.55 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.50 ± 0.09&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>22.53 ± 0.38&lt;sup&gt;cb&lt;/sup&gt;</td>
<td>5.86 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

p- Value | NS | 0.006 | 0.026 | NS |

Values are the Mean ± SD (n=3), NS = Not Significant. <sup>abcd</sup> means bearing different superscripts within each column differ significantly.

Table 6- 4: Crumb colour of bread prepared from wheat flour (control) and wheat flour substituted at 20, 30 and 40 percent of tiger nut bagasse flours.

<table>
<thead>
<tr>
<th>Sample treatment (g/100g)</th>
<th>Bread Crumb Colour</th>
<th></th>
<th></th>
<th>ΔE&lt;sub&gt;ab&lt;/sub&gt;*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lightness (L*)</td>
<td>Redness (a*)</td>
<td>Yellowness (b*)</td>
<td></td>
</tr>
<tr>
<td>0 (Control)</td>
<td>72.05 ± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.96 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.66 ± 0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>62.24 ± 0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.70 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.21 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.34 ± 0.38&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>59.99 ± 0.65&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>4.33 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.32 ± 0.33&lt;sup&gt;ba&lt;/sup&gt;</td>
<td>12.66 ± 1.13&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>40</td>
<td>57.53 ± 0.76&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>4.63 ± 0.04&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>19.91 ± 0.19&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>15.13 ± 1.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

p- Value | 0.001 | 0.001 | 0.039 | 0.027 |

Values are the Mean ± SD (n=3),
<sup>abcd</sup> means bearing different superscripts within each column differ significantly.
This reaction may affect the nutritional value of foods through the blocking and destruction of essential amino nutrients as reported by Hurrell (1990). In a study conducted by Mohammed et al. (2012), they reported that colour of wheat flour bread was light brown which increased significantly upon increasing the level of substitution and that typical loaves were obtained with substitution of wheat flour by chickpea flour at 10% levels.

![Figure 6-4: Comparison of colour difference between bread crust and crumb at different levels of tiger nuts flour substitution (%), taking the Control (0%) bread colour as the reference. For either crust or crumb, means with different letter are significantly different (p ≤ 0.05). (n = 3)](image-url)
6.1.4. Effect of TG on loaf sensory characteristics

The sensory evaluation scores for colour, overall taste, overall appearance and overall suitability for sandwich were obtained from semi-trained panellists and are presented in Figure 6.5. The substitution of wheat flour with tiger nut bagasse flour at the 20% level did not affect all the sensory properties of the bread as compared to the control. In general, the panellists preferred the control bread. There was a clear cut relationship between the sensory scores and forms of tiger nuts fibre as can be seen in total sensory evaluation scores in the above figure. At the 20% tiger nut fibre substitution, the blend of colour was more appealing to the panellists as compared to levels substituted at 30 and 40% as seen in Figure. As indicated by Hathorn et al. (2008), the colour of bread is an important sensory characteristic for consumers. According to these researchers, consumers perceived breads as either light or dark and have preferences depending on which type the bread appears to be. For example, in
a research carried out by these researchers, they found out that consumers expect rye bread to have a dark colour and may reject a light-coloured rye bread. Apart from the official panellists used in this study, people who have seen these products always preferred the bread crust colour at the 20% concentration. Based on their findings and findings in this sensory evaluation of tiger nut bagasse flour substitution, consumers may prefer bread with the incorporation of tiger nut bagasse flour, which would be light in colour. This findings is consistent with studies carried out by the following authors, Hsu et al. (2004) reported that yam could be used to substitute wheat flour at levels up to 20% to produce acceptable bread and Afolabi et al. (2001) also found that up to 20% of beniseed and cassava flour could be supplemented with non-wheat flour to produce acceptable bread. This was however not supported by Conforti and Davis (2006) who found soya flour and flaxseed supplementations of more than 5% to be unacceptable.
6.2. Effect of Tiger Nut Flours (whole and bagasse) addition on characteristics biscuits

Results and Discussion

6.2.1. Effect of TG bagasse flour on Biscuit Physical properties

The present investigation was planned to develop high fibre and caloric wheat biscuit products with whole and bagasse tiger nut flours with different substitution levels. Biscuits, according to Pratima and Yadava (2000) have always been one of the most popular and appealing food products due to its superior nutritional, sensorial and textural characteristics, ready to eat convenience as well as cost competitiveness.

Physical characteristics of biscuits, such as weight, volume, specific volume, thickness, circumference and spread ratio, were affected with the increase in the level of both the whole tiger nut flour and the tiger nut bagasse flour in wheat flour blends used in biscuit making as shown in Table 6.5 and Figure 6.6.

It is evident that the circumferences of biscuits prepared by incorporating tiger nut flours (whole and bagasse) up to 40% level apart from 20% bagasse substitution were lower than the control sample. The range of biscuit weight was 19.2 to 23.67 g with maximum value in 20% tiger nut bagasse biscuits. The weight and volume of the control biscuit were lower than the rest of the substituted tiger nut flour component apart from the 20% and 30% whole tiger nut flour respectively. However, this result is inconsistent with the findings made by Baljeet et al. (2010) in which the weight of biscuits increased as the concentration of buckwheat increased in the blends. This difference may be due
to the prepared dough not well sheeted and cut into shapes. Again, this may be due to the fact that two different biological samples are being compared.

A slight increase in spread ratio of supplemented samples occurred after baking at all replacement levels, when compared with the control sample. The specific volumes of the tiger nut flour component replacement at level 30 and 40% were lower than the control and the 20% replacement of the whole and bagasse tiger nut flours. The changes in width and thickness are reflected in spread ratio and percent spread ratio which was calculated by dividing the width (W) by thickness (T) of the biscuit samples (Mildner-Szkudlacz et al., 2013). The wheat sample (control) had the spread ratio of 4.92 as compared to the other samples used in this study. From the Table it could be observed that there was a slight increase in spread ratio of substituted samples after baking at all replacement levels, when compared with the control sample. It has been reported by Agu et al. (2007) and Mildner-Szkudlacz et al. (2013) that the spread ratio for wheat without any substitution are 4.57 and 3.9 respectively. Agu et al. (2007), have posited that the low spread ratio values of the control (100% wheat) sample as compared to a wheat biscuit that has been substituted with different flour ingredient. And that it may be due to the fact that, starch polymer molecules are highly bound with the granules and swelling is limited when heated. On cooling, the starch rapidly forms a rigid gel with capacity characteristics of large molecular aggregates. The improvement in spread ratio of the biscuits as compared to the control biscuits were 12.8, 10.0 and 5.3 at 20%, 30% and 40% substitution levels when whole tiger nut flour components were used whereas the bagasse flour had an increase scored 21.3% and 19.1% at 20 and 40% replacement level respectively. The positive values means that the presence of
the tiger nut flour components results in weaker dough which is more suitable for biscuit manufacture as posited by Yaseen et al. (2007).

Saxena et al. (1992), reported that biscuits prepared from soft and medium triticale flours had a significantly higher spread ratio than those prepared from hard triticale flour. The reduced spread ratios of samples; soy, fenugreek and buckwheat flours studied by different researchers in fortified biscuits were attributed to the fact that composite flours apparently form aggregates with increased numbers of hydrophilic sites available that compete for the limited free water in biscuit dough (McWatters, 1978). This is consistent with the bagasse flour at 20% and the whole nut at 40%.

Figure 6-6: Biscuit with Tiger nut flour substitution at different levels
From top to bottom; 20% of whole tiger nut flour, 40% of whole tiger nut flour, 0% (control) 20% tiger nut bagasse flour and 40% tiger nut bagasse flour
**Table 6-5:** Physical characteristics of tiger nut flour (whole and bagasse) incorporated biscuits at different levels of substitution

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Control</th>
<th>20%</th>
<th>30%</th>
<th>40%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>Circumference (mm)</td>
<td>217.29 ± 3.11</td>
<td>203.85 ± 3.09</td>
<td>209.45 ± 3.06</td>
<td>213.54 ± 3.16</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>21.85 ± 1.01</td>
<td>19.5 ± 0.99</td>
<td>23.75 ± 1.01</td>
<td>21.97 ± 0.97</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>35.87 ± 0.02</td>
<td>36.90 ± 0.99</td>
<td>42.17 ± 0.99</td>
<td>32.91 ± 1.03</td>
</tr>
<tr>
<td>Specific volume (ml/g)</td>
<td>1.64</td>
<td>1.89</td>
<td>1.78</td>
<td>1.5</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>69.16 ± 0.99</td>
<td>64.92 ± 0.98</td>
<td>66.71 ± 0.98</td>
<td>68.00 ± 1.01</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>14.06 ± 1.12</td>
<td>11.70 ± 1.12</td>
<td>11.18 ± 0.52</td>
<td>12.57 ± 0.45</td>
</tr>
<tr>
<td>Spread ratio</td>
<td>4.92</td>
<td>5.55</td>
<td>5.97</td>
<td>5.41</td>
</tr>
<tr>
<td>Spread ratio (± %)</td>
<td>0</td>
<td>12.8</td>
<td>21.3</td>
<td>10.0</td>
</tr>
</tbody>
</table>

A Whole tiger nut flour substituted biscuit
B Tiger nut bagasse flour substituted biscuit
** Not evaluated
Mean ± Standard deviation (n = 5)
6.2.2. Effect of TG bagasse flour on Sensory characteristics of biscuits

Biscuits with two types of tiger nut flours at different substitution levels with white wheat flour were sensorial evaluated and compared with control biscuits 100% whole wheat flour.

Data obtained in this study are presented graphically in Figure 6.7 and 6.8. The two figures 6-7 and 6-8 show the comparison among the biscuits of their organoleptic quality factors and attributes respectively.

The figures show that, there weren’t much difference between people perceptions about the aroma of the biscuits. The panellists did not feel that changing the concentration of tiger nut flour in the biscuits really changed the aroma. The aroma of the biscuit was between neither like, dislike and like moderately.

The fibre content was the attribute which was ranked highest among all the baked biscuits this may be due to people’s knowledge in the health benefits of dietary fibre (Elleuch et al., 2011) and the publicity that dietary fibre has attractive both in the print and electronic media. Again, the biscuit’s hardness which gave crispy sensation was the whole nut flour at 40%, this may be due to the interaction between the fats and dietary fibre at the baking temperature.

Due to the presence of carbohydrate in wheat and tiger nuts products, some Maillard browning would be expected due to the potential reaction between exposed amino groups on amino acids and reducing sugars, especially where product processing temperatures exceed 150°C (Cheftel et al., 1985). The WTNB and TNBB at 40% substitution scored highest value on the Hedonic scale for all the chosen attributes.
From figure 6.8, the sensory evaluation of the WTN and TNB flours incorporated biscuits showed that the overall acceptability limit was control (0%) up to the level of 40% incorporation. An increased trend in the level of acceptability from dislike slightly to extremely acceptable was observed when the level of WTN and TNB incorporation increasing from 0% to 40%. Control biscuits (0 percent WTN and TNB) and WTN (20%) had the lowest score for all the characteristics, however scores at 20 percent WNB levels was found to be at par with the control for all the sensory characteristics. A significant rise in acceptable level for TNB (20%) incorporation for all the sensory attributes are observed. Regarding the overall acceptability, samples WTN and TNB (40%) incorporation scored the highest on the Hedonic scale. Scores of the other sensory attributes like flavour, colour, texture, aroma, and appearance also
revealed that acceptability level of the respondent was higher for the 40% of the sample flours incorporation level when compared to control (0%), WTN and TNB (20%). All the products samples were liked by the panellists. Alam et al. (2014) have stated that appearances of biscuits depend on appearance of wheat and other raw appearance of ingredients and on processing technique.

Figure 6-8: Sensory evaluation of two types of prepared tiger nut flour (WTN and TNB) substituted and a control biscuit using Hedonic scale of 7. Number of Panellists recruited for the study (n = 40)

Perhaps, the nutty and coconut flavour are some of the attributes that contributed to the overall acceptability of the prepared biscuits. The colour, texture and flavour scores of biscuits with 40% tiger nuts’ flours substitution reached maximum than to the rest of the proportions which were similar to the control sample. The score of colour increased significantly, this was due to increasing level of tiger nut flour incorporation which gave a dark golden brown colour to the biscuits.
Changes in colour and taste in the baked biscuits containing 20%, 30% and 40% for both whole and bagasse tiger nut flour may be due to aromatic volatile compounds in the raw materials used and also due to caramelisation and maillard reactions, as the amino acid (lysine) contributed by the flours might have reacted with the reducing sugar during the baking process, this findings are also consistent with the findings of (Dhingra and Jood, 2001, Mohsen et al., 2009) when they substituted wheat flour with soy flour at different concentration levels in biscuit production. For example, in their findings the buckwheat biscuit tasted bitter probably due to flavonoid compound (rutin) at higher concentration whereas the tiger nut had a good taste due to the fat, vanillic aroma and sugar present at higher concentrations. Alam et al. (2014), have stated that appearances of biscuits depend on appearance of wheat and other raw appearance of ingredients and on processing technique.

The least preferred biscuit were the control (0%) and the WTNB 20% concentration. The TNBB (20%) was in the middle. This is in contrast to the findings made by Baljeet et al. (2010) when they uses buckwheat flour in making biscuits. In their findings the acceptability of biscuits colour was best for control sample and at lower concentration of buckwheat incorporation.

According to research carried out by Seevaratnam et al. (2012), biscuits made from three different proportions of composite flour of wheat and potato (0, 20 and 30% potato flour) revealed that biscuits containing 20% potato flour as a supplement secured the highest score in terms of all sensory attributes among others, though all samples are acceptable. These differences between the current study and that of the researchers may have been observed due to the fact that two different types of samples were used even though they are all
classified as tubers. Misra and Kulshrestha (2003), formulated biscuits by incorporating potato flour in 6 different proportions to replace wheat flour at levels of 0, 10, 20, 30, 40 and 50 percent. Results of the sensory evaluation revealed that until the 20% level, there was no significant difference observed in acceptability of the product and all three levels had similar sensory scores, being judged as good for appearance, taste and colour and fair for flavour, texture and overall acceptability. The sensory evaluation finding in this study is inconsistent with the previous finding with the tiger nut bread. In the bread the overall acceptability was at 20% tiger nut flour substitution.
6.3. EVALUATION OF GLYCAEMIC INDEX OF WHOLE AND BAGASSE TIGER NUTS SUBSTITUTED WHEAT BREAD

Results and Discussion

The individual and general characteristics of the studied subjects are presented in Table 6.6. There were 11 healthy subjects (5 females and 6 males) with a mean (± SD) age of 41.2 ± 12.62 years and BMI (± SD) of 24.86 ± 4.0kg/m², fasting blood glucose (time 0), 5.23 ± 0.53 mmol/L; and body fat composition 25.55 ± 10.39%. All participants completed the study. Apart from one participant, all the participants had blood glucose responses to all the test sample meals in the ‘normal’ range for non-diabetic subjects.

The results obtained in this study are presented in Table 6.6, 6.7, Figure 6.9 and 6.10. From Table 6.6, the calculated blood glucose incremental area under the curve (IUAC) for the control, whole and bagasse tiger nut bread using the mean fasting glucose as the baseline are; 162.3 ± 98.1, 136.51 ± 86.0 and 119.1 ± 63.4 respectively. Their coefficient of variations are; 60.4, 63.0 and 53.2%. Using the mean fasting blood glucose (5.23 ± 0.5) in calculating the GI for the whole nut and bagasse bread are; 79.9 ± 43.2 and 85.9 ± 38.3 respectively, the overall GI for the whole nut bread is lower than the bagasse bread. From the calculated result, according to Foster-Powell et al. (2002) the whole tiger nut and bagasse substituted wheat bread fall under the same GI classification “high GI food”. However, when the GI was calculated using individual fasting blood glucose, slight different results were obtained as indicated by Figure: 6-9. This is consistent with the findings on the dietary fibre and available carbohydrate determination that was carried out in this thesis work. In a review carried out by Frost and Dornhorst (2000), they have indicated
that diets rich in soluble fibre such as guar gum, pectin and sugar beet fibres result in lower post-prandial blood glucose but non-soluble dietary fibre such as cellulose have no effect on dietary glycaemic index. The results obtained in this study differ from the result obtained by El-Shebini et al. (2010) when they used raw tiger nut in their study. This difference might have occurred as a result of the use of wheat as a vehicle for the tiger nuts prepared flour. This goes to confirm that carbohydrate foods consumed in isoglucidic amounts produce different glycaemic responses depending on many factors, such as physiological state of subject, particle size, cooking and food processing, other food components (e.g. fat, protein, dietary fibre) and starch structure (Henry et al., 2005).

Figure 6.9 shows the incremental blood glucose response curves for the white bread (control), whole nut and bagasse tiger nut bread. This shows a typical blood glucose profile in response to high and low GI foods.

Figure: 6.9 shows a gradual rise in blood glucose level for the first 15 minutes, and reached a maximum peak between 30 to 45 minutes after ingesting the TNBB meal. After this period, the blood glucose level declined gradually till the 120 minutes period.

Participants who ingested the WFB and WTNB showed the same blood sugar response after 15 minutes of taking in the test meals, even though the participants peaked after 45 minutes after taking the meals, it was the WFB that elicited the highest peak. After this period there was a gradual drop in blood glucose levels till the 120 minutes allotted time for the experiments reached.
Figures: 6.9 shows individual calculated incremental area under the curve of ingested meals at 15, 30, 45, 60, 90 and 120 minutes. The findings are inconsistent and inconclusive because each individual digested and absorbed the available glucose differently. This again, shows that each individual is unique and react to situations differently. For example during the test, people were seen walking around instead of sitting down quietly.

From Figure 6.9 and 6.10 some participants' blood glucose response levels dropped in the first 15 minutes of test meals ingestion before it began to rise gradually. By the end of the two hour allotment time of test meal ingestion (whole tiger nut bread) some subjects' blood sugar had almost dropped to the baseline and they were complaining of intense hunger. This occurrences indicate that they might have secreted excessive amount of insulin as indicated by (Insel et al., 2006a). This observation is consistence to the findings made by Parkin and Brooks (2002 ), they stated that glucose blood levels peak at approximately 1 hour after the start of the meal and then return to pre-prandial levels within 2–3 hours. This rise and fall of postprandial glucose levels is mediated by the first-phase insulin response, in which large amounts of endogenous insulin are released, usually within 10 min, in response to nutrient intake. High glycaemic diets have been linked with higher insulin levels and an elevated risk of developing diabetes (Hodge et al., 2004). Hodge et al. (2004) have posited that two mechanisms that may have impart on intake of high GI carbohydrates are the increase risk of type 2 diabetes by over-stimulating insulin secretion or contributing to pancreatic β-cell dysfunction which can result in impaired glucose tolerance and eventually type 2 diabetes.
Again, the mineral analysis determined in the tiger nut samples show that they are rich in chromium. This element as indicated by Cheng et al. (2004) acts both as an insulin potentiating factor and antioxidant and could, therefore, counteract the increased oxidative stress associated with insulin resistance in the elderly.

Table 6.7 and Figure 6.10 show how individual participants reacted to the test meals both before and after meals ingestion. As already stated, individuals reacted differently to the meals, but apart from participant 10 whose blood sugar level went beyond the normal range as indicated in the literature review under section glycaemic index all participants fell under category normoglycaemia (American Diabetes Association, 2001, Diabetes.co.uk, 2014, Parkin and Brooks, 2002).
Table 6- 6: Characteristics of individual subjects and their overall IUAC and Glycaemic Index

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Gender</th>
<th>Age</th>
<th>BMI</th>
<th>Body Fat Composition (%)</th>
<th>Fasting Sugar Level (mmol/L) (n=3)</th>
<th>Overall IUAC</th>
<th>Overall Glycaemic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>35</td>
<td>21</td>
<td>26.6</td>
<td>5.07 ± 0.31</td>
<td>87.38</td>
<td>113.63</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>30</td>
<td>18.5</td>
<td>7.2</td>
<td>5.40 ± 0.3</td>
<td>154.13</td>
<td>101.63</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>35</td>
<td>29.8</td>
<td>41.2</td>
<td>4.70 ± 0.44</td>
<td>96.37</td>
<td>30.23</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>30</td>
<td>21.4</td>
<td>13.8</td>
<td>4.90 ± 0.36</td>
<td>135.38</td>
<td>178.88</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>60</td>
<td>29.2</td>
<td>37.9</td>
<td>5.70 ± 0.0</td>
<td>102.68</td>
<td>97.12</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>29</td>
<td>21</td>
<td>30.2</td>
<td>5.07 ± 0.23</td>
<td>112.13</td>
<td>25.43</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>45</td>
<td>25.2</td>
<td>22.6</td>
<td>6.23 ± 0.61</td>
<td>316.13</td>
<td>208.13</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>53</td>
<td>28.8</td>
<td>26.9</td>
<td>5.23 ± 0.06</td>
<td>136.13</td>
<td>99.37</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>59</td>
<td>23.2</td>
<td>34.5</td>
<td>5.17 ± 0.21</td>
<td>136.13</td>
<td>195.38</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>49</td>
<td>27.5</td>
<td>24.2</td>
<td>5.27 ± 0.74</td>
<td>391.13</td>
<td>193.88</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>26</td>
<td>25.2</td>
<td>16</td>
<td>4.77 ± 0.21</td>
<td>117.75</td>
<td>108.15</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>41 ± 12.6</td>
<td>24.9 ± 25.6 ± 0.5</td>
<td>5.23 ± 0.5</td>
<td>162.3 ± 136.51 ± 119.1 ± 79.9 ± 43.2</td>
<td>85.9 ± 38.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IUAC (Incremental Area under the Curve)

**WFB** (Control; white wheat bread), **TNBB** (tiger nut Bagasse flour substituted bread) and **WTNB** (Whole tiger nut flour substituted bread)
Figure 6- 9: Blood glucose response curves after consumption of Control white wheat without tiger nut flour substitution (Blue colour, (WFB)), Tiger nut bagasse flour substitution (Red colour, (TNBB)) and Whole tiger nut flour substitution (Green colour, WTNB) equivalent to 50 g available carbohydrate. (n =11)

Table 6- 7: Percentage change in individual's blood sugar level over fasting blood sugar level after two hours test meal ingestion

<table>
<thead>
<tr>
<th>Individual Subjects</th>
<th>Fasting blood glucose level (mmol/L)</th>
<th>Post prandial blood glucose level (mmol/L)</th>
<th>% Change in blood glucose level (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WFB</td>
<td>TNBB</td>
<td>WTNB</td>
</tr>
<tr>
<td>1</td>
<td>5.00</td>
<td>5.4</td>
<td>4.8</td>
</tr>
<tr>
<td>2</td>
<td>5.70</td>
<td>5.4</td>
<td>5.1</td>
</tr>
<tr>
<td>3</td>
<td>5.20</td>
<td>4.5</td>
<td>4.4</td>
</tr>
<tr>
<td>4</td>
<td>4.60</td>
<td>4.8</td>
<td>5.3</td>
</tr>
<tr>
<td>5</td>
<td>5.70</td>
<td>5.7</td>
<td>5.7</td>
</tr>
<tr>
<td>6</td>
<td>5.20</td>
<td>5.2</td>
<td>4.8</td>
</tr>
<tr>
<td>7</td>
<td>6.10</td>
<td>6.9</td>
<td>5.7</td>
</tr>
<tr>
<td>8</td>
<td>5.20</td>
<td>5.3</td>
<td>5.2</td>
</tr>
<tr>
<td>9</td>
<td>5.10</td>
<td>5</td>
<td>5.4</td>
</tr>
<tr>
<td>10</td>
<td>6.10</td>
<td>4.7</td>
<td>5</td>
</tr>
<tr>
<td>11</td>
<td>4.70</td>
<td>5</td>
<td>4.6</td>
</tr>
</tbody>
</table>

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Figure 6-10: Comparison of individual participant calculated IAUC of blood glucose response at 15, 30, 45, 60, 90 and 120 minutes after the test meals ingestion; Blue colour; Control (White wheat bread), Red colour; Whole tiger nut bread and Green colour; tiger nut bagasse bread.
CHAPTER 7

CONCLUDING DISCUSSION

In this chapter, conclusions to all the work carried out in this study and recommendations for future work have been discussed.

7.1. Concluding remarks for chapter 4

Data obtained in Chapter 4 indicate that water uptake for tiger nuts tubers during soaking at room temperature shows two different relationships (quadratic for three days and linear after this period). Soaking is a slow process controlled by the diffusion of water in the grain and therefore soaking at room temperature may allow microbial growth, which affects quality attributes (such as colour, taste and flavour) of the product. In view of this, it is recommended that soaking water for tiger nuts should be changed every day during this process. Water uptake depends on the initial moisture content of samples, the size of tubers and temperature. The size and linear dimensions of samples from Ghana (brown and black tubers) were observed to be higher than those from Cameroon and Spain. And within Ghanaian samples, apart from the linear dimensions in which the brown had higher values than the black, the black variety had higher values for the surface area and volume. The shape of tiger nut samples from Cameroon (yellow variety) were the most regular compared to the other samples, this indicates that they will flow with less frictional force on conveyor belt. The black variety from Ghana had a higher unit mass, followed by the brown from Ghana, Cameroon and Spain in descending order. The
physical dimensions of tiger nuts selected from the same country were not significantly different; this may be due to farming and postharvest practices.

Sample origin does not have any effect on particle size; however, the variety and hardness seem to have effect as exhibited by the black samples from Ghana. Particle size analysis on the tiger nuts flour show different types of distributions depending on place of origin. Samples from Ghana show a bimodal distribution, the UK market sample show a tri-modal whilst the Cameroon sample was indistinct bimodal. The first mode occurred between 0.5 to 20µm. This result fall within the range reported for potato, corn and lentil starches. Whole nut and bagasse flours dried at temperature of 40°C produced finer and smoother flours as compared to the whole nuts and bagasse flours dried at 50°C. Roller milling method produced finer and more uniform particle size as compared to the hammer and coffee grinding machine methods.

7.1.1. Practical implications of these findings

- Knowledge gained in the investigations of the physical properties of the tiger nuts’ tubers from Ghana, Cameroon, Spain and Turkey can therefore be used to design equipment for harvesting, sorting or grading, transporting and processing.

- The rate of water absorption has significance in the formulation of foods. In the canning industry, knowledge in hydration characteristics of grains prior to further processing is necessary to know since changes can occur such as leaching losses and grain expansion in the can during a thermal process. In order to control and predict the process, optimizing the hydration condition is vital since hydration governs the subsequent operations and the quality of the final product.
• For commercialisation of tiger nut flour, the nuts should be soaked for maximum period of three days, roller milled and dried at 40°C before packaging.
• Modification could be made through the inclusion of size fractions with desirable characteristics in special diets, or the exposure of undesirable size fractions to additional processing to improve their characteristics. Examples could be seen from the types of bread developed for the glycaemic index test. However, it must be noted that even within the same nut type, grinding under similar conditions may result in different particle size distributions as a consequence of variations in mechanical properties, duration of grinding, and the pericarp and endosperm hardness.

7.2. Concluding remarks for chapter 5

Chapter 5 compares the chemical compositions of the selected samples. The ash content in tiger nut from Ghana (black variety) and Cameroon (yellow variety) were highest, compared to that of Ghana (brown variety) and Spain (brown variety). The country of origin does not have an effect on the ash content but rather the specie or variety. The pH of the selected samples tiger nut from Ghana was more on the neutral side than Cameroon and Spain which were more acidic, thus the pH of tiger nut depends on the place of origin. Generally, tiger nuts as product ingredients could therefore be used in different food formulations without altering the acidity of final food product. Using bomb calorimeter in the determination of energy content in this study, tiger nut samples from Ghana recorded the highest values compared to Cameroon and Spain. The carbohydrate content of the analysed samples accounts for 39.33-49.31% of energy and it falls within the report proposal by Food and Agriculture
Organisation (FAO) and World Health Organisation (WHO) (1998). During their consultation meeting, members proposed that carbohydrates which have an energy value of 4kcal/g and account for 40–75% of energy intake depending on social and economic factors. The protein and lipid contents were higher in Cameroon and Spain, from this it may be concluded that place of origin has an effect on the nutrient contents of tiger nut; however, the farming practices and postharvest handling may also be contributory factors. Tiger nut is a good source of lipid which has a bland flavour.

The results obtained in this study show that, the analysed samples indicate that four elements Cu, K, Mg, P, Fe, Zn and Se are inherent to tiger nuts independent of place of origin and variety. Some of the factors that may have affected the variation in the analysed results are place of origin (soil type), genetic (species) and farming practice. Even though the Ghana (black) and Ghana (brown) species were obtained from the same country yet the sodium contents were different because of difference in cultivation area. The four selected analysed tiger nuts in this study show that tiger nuts are good source of dietary minerals, particularly; K, Mg, P, Co, Cr, Cu, Fe, Mn, Se and Zn but fall below dietary minerals commonly eaten tree-nuts (Alexiadou and Katsilambros, 2011), however the selenium content far exceeded the values found in tree nuts. The tiger nuts showed significant values of the minerals studied, particularly with regard to Cr, Co, Fe, Zn and Se content corresponding to 147-825%, 53-227%, 25-91%, 1,000% and 61% respectively of recommended daily intake for adults. From this data it may be concluded that most of the unsubstantiated health claims may be attributed to the high amount of some of the dietary minerals as discussed in the literature review on some functions of specific minerals in the body.
The origin of the samples does not have an effect on dietary fibre yield. Dietary fibre content is an inherent property of tiger nuts. The black variety has low dietary fibre. Tiger nut flours, fractions and enriched products are rich in insoluble and soluble fibre. Extracted fractions (bagasse) are far higher in insoluble fibre than in soluble fibre. From the findings, dietary fibre obtained from tiger nut (whole nuts and bagasse) denotes a potential material for use as product ingredient in the food industry due to the high total dietary fibre content (soluble and insoluble dietary fibre), which in effect has the potential health benefits as good sources of dietary fibre. The processing method used in product preparation has an effect on dietary fibre results. Low molecular weight soluble dietary fibre (LMWSDF) (oligosaccharides) analysed in tiger nut flour were inconclusive. The Ghana (brown) and wheat bread samples contain LMWSDF whereas it was not detected in the other samples. The tiger nut dietary fibre content determined in this work is greater than what is reported in FAO food composition tables (Food and Agriculture Organisation (FAO), 2012). The bagasse bread has more available carbohydrate than the whole nut bread. Theoretically, it should have been the other way round; however, it is consistent with the findings in the bagasse bread dietary fibre which again was greater than both the control and whole nut bread.

Tiger nut has a high caloric content as compared to other roots and tubers but a little lower than of the tree nuts and some legumes. The high content of carbohydrate and lipid are the driving forces behind its high caloric content. The high lipid content is a great advantage to tiger nuts, especially in regards to producing healthy products that may improve the nutritional status of undernourished people in Ghana and Cameroon or use as a biodiesel.
The study addressing the effect of type of solvent extraction and origin of samples on the extractability of phenolic compounds from tiger nut revealed that the tiger nut variety (black >brown >yellow) and extraction process conditions had a significant effect on the content of phenolic compounds in the nuts. A higher ethanol (absolute) to water ratio has a positive influence on the extractability of phenolic compounds than that of methanol (absolute) to water ratio.

In view of the results obtained in the rancimat methods, DPPH Antioxidants scavenging and Total Phenolic content studies, it may be concluded that tiger nut irrespective of sample origin can be considered as a natural source of antioxidants; however, variety of tiger nut has a greater influence on quantity of TPC and antioxidant capacity yield. Phenolic extracts from tiger nut can be used as alternatives to synthetic antioxidants in order to increase the stability of foods by preventing lipid peroxidation, and protect living systems from oxidative damage by scavenging oxygen radicals. Results from the rancimat method in which extracted lipid was used, suggest that the antioxidant activities may be due to both the total phenolic content and tocopherol as indicated by Yeboah et al. (2012).

Tiger nut especially the black variety obtained from Ghana can be used to prepare ‘Functional Foods’ or used as product ingredient to substitute synthetic antioxidants like BHT or BHA that have carcinogenic properties.

7.2.1. Practical implications for findings in chapter 5

- Tiger nut is rich in carbohydrate, lipid and protein and could therefore be added to the Ghanaian and Cameroonian national food baskets
This holds great potential for addressing mineral deficiencies in the developing countries (Ghana and Cameroon).

The high content of dietary fibre (soluble and insoluble) make tiger nut a potential crop in production of ‘functional bread/biscuit’.

Dietary mineral health claims may be made on food products prepared with tiger nut flour as product ingredients.

7.3. Concluding remarks for chapter 6

In the new food product development the following were developed with tiger nut (whole nuts, bagasse and extracted milk); as product ingredients in formulation of bread, biscuits yoghurt, ice cream, beverage (both fermented and unfermented) and alcoholic beverage (poly herbal) were investigated, however, in chapter 6, the application of tiger nut as a product ingredient in the formulation of bread, biscuits, and health implication were reported.

Substitution of wheat flour with tiger nut fibre from 20 to 40% levels affected the quality of bread.

Increases in tiger nut’s bagasse negatively affected the loaf volume.

Increased levels of bagasse addition were linked to decreased acceptability, but bagasse at 20% was still considered suitable for sandwiches, and the taste and colour were only slightly affected.

No significant differences were recorded (p> 0.05) between mechanical and sensory attributes of 0 and 20% samples.

Adding tiger nut bagasse to bread could increase the dietary fibre content if used at adequate levels.
• The 20% of bagasse substitution appeared to have no effect on the dough weight. The addition of tiger nut bagasse flour in the formulation of bread dough made the baked bread looked fresh; the bread crumb was soft and firm.

• Substitution of wheat flour with tiger nut fibre from 20 to 40% levels affected the physical properties (specific ratio and volume) of biscuits

• Texture and flavour were the most preferred attributes for the biscuits, and this was at 40% substitution (whole nuts and bagasse flour).

• The hardest biscuit was the whole nut at 40% substitution level

• The overall acceptability for the biscuits were the 40% substitution (whole nut).

From the results on the health benefits of tiger nuts, it could be concluded that the two types of bread could be classified as having different blood glucose response. The bagasse bread has a higher glycaemic index whilst the whole tiger nut bread could be classified as having medium glycaemic index according to the glycaemic index classification table. These results are consistent with the results obtained in the analysis on dietary fibre and available carbohydrate prior to this study.

7.3.1. Practical implications for chapter 6

• The knowledge gained in this study will in future equip researchers to give or make well inform decisions and advise governments or clients.

• Based on the two sensory analyses carried out on the bread and biscuit, it is recommended that 20 and 40% substitution levels of whole and bagasse tiger nut flour could be used respectively.
• Investigation into substitution of wheat flour with tiger nut’s bagasse, extracted milk or whole nuts (milled) could lead to increase usage in other food products, which would improve the utilisation of the crop, hence boosting its profitability whilst benefiting the health of the consuming public.

• Tiger nut as a bakery product ingredient will also have the advantage of enriching the bread with dietary fibre.

• The agro–industrial exploitation of these products would permit the use of a marginal species in the country with adequate nutritional value, an important product with potential health benefits, in addition to reducing the cost of making pastry and bread products.
7.4. Future work

- The non-starch polysaccharide should be characterised into the various components; cellulose, hemicellulose, resistant starch, lignin, beta-glucans and pectin in tiger nut. This will help food processors or food scientists to develop healthier and acceptable food products (as food ingredients) from this underutilised crop that make up the hard enclosed outer layer. The knowledge gained in the characterisation of the bran will help researchers to understand how structure affects the functionality of the nuts as a whole. Commercialisation of production and applications of fibre fractions from tiger nut have not been studied unlike wheat or oats; therefore further research would be needed if tiger nut dietary fibre is to be produced on a wider commercial scale.

- A better understanding of the physicochemical properties of these fibre fractions is also needed to optimise the use of tiger nut dietary fibre in processing foods like bread, muffins and biscuits. Sample extract should be identified by matching the retention time and their spectral characteristics against those of the standards; p-hydroxybenzoic acid, vallon, ferulic acid, vanillic acid, coumaric.

Ethanol instead of methanol at different concentrations should be used to extract antioxidants in fruits, vegetables and nuts from the considerations on the safety for human consumption and compatibility with food systems. Defatted and non-defatted samples should be studied thoroughly.

- The high content of xylose detected in this crop could be exploited in the production of xylitol which could further be used as sweetener in the food industry.
• Tiger nuts may be used in the bio-fortification strategy based on crop breeding targeted genetic manipulation and/or the application of mineral fertilizers.

• Clinical trials should be conducted on the mineral bioavailability for these nuts in humans.

• Different food vehicles for tiger nut test meals; (gari, yoghurt, porridge or weighed amount of raw tiger nuts as used by the Egyptian’s researchers) apart from bread should be used if white bread is to be used as control. Preferably glucose solution should be used as the standard test for measuring glycaemic index.

• In order to avoid more variability, there is a need to include in the method a standardised carbohydrate quantity in the evening meal before the experiment.
CHAPTER 8

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