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PHYSICOCHEMICAL, FUNCTIONAL PROPERTIES AND MYCOTOXIN OCCURRENCE OF GHANAIAN TIGERNUTS (*Cyperus esculentus L.*)

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DECLARATION

I do hereby declare that this thesis is the result of my own research except for references to works of others that have been duly cited under the supervision of Prof. Firibu Kwesi Saalia, Prof. Agnes Simpson Budu and Prof. Emmanuel Ohene-Afoakwa. This work either in whole or part has not been presented for another degree elsewhere.

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ABSTRACT

In Ghana, tigernut (*Cyperus esculentus L.*) is grossly underutilized in food applications and is mostly consumed raw as a snack. However, the soil and climate conditions of the country are conducive for cultivation of the crop on a large scale for applications in food for local consumption, industrialisation and for the export market. Food applications of tigernut and its derivatives and their possible inclusion as ingredient in the Ghanaian diet would require knowledge on its handling quality and functional properties. The aim of this study was to characterise Ghanaian tigernut as an ingredient for possible food applications.

The study design consisted of two parts: (a) a cross-sectional survey of different categories of stakeholders using pre-tested semi-structured questionnaires (b) followed by laboratory designed experiments to study the quality, physicochemical and functional properties of tigernut as a function of tigernut variety and process conditions. For the surveys, a total of 1277 stakeholders in the value chain, comprising of 711 consumers and 487 traders (wholesalers/retailers) in Greater Accra region and 79 tigernut farmers in the Western and Eastern regions of Ghana, were interviewed using semi-structured researcher-administered questionnaires. The questionnaires sought to gain information on respondents' level of knowledge on mycotoxins, as well as ascertain if measures were in place to mitigate the risk of fungal colonisation of the crop along the supply chain. Additionally, tigernuts collected at various points along the supply chain (farm, wholesale and retail) were analysed for their mycotoxin (aflatoxins and ochratoxin A) levels using reverse phase High-Performance Liquid Chromatography (HPLC), to determine the hot spots of mycotoxin contamination along the value chain. The second part of the study investigated the physical characteristics of tigernut tubers as well as the functional properties of tigernut flour with the aim of determining its suitability in food applications. Additionally, the shelf life of the tigernut flour was determined by accelerated shelf life testing using the Arrhenius model. Fresh tigernut milk is usually

characterised by the sedimentation of starch which influences its flow behaviour as well as the physical stability. Furthermore, heat treatment of the milk leads to gelatinisation of the starches, which also affects the same properties. Consequently, the effects of heat (by roasting tigernuts) and adding α- amylase to the tigernut milk on the physicochemical and functional properties of tiger nut milk were studied. Tigernut oil was extracted and the phenolic and functional properties were determined as well as the effect of heat on these properties. The macro nutritional composition of tigernut tuber, flour, oil and milk were also investigated.

The results of the surveys showed that tigernut farmers and consumers had appreciable knowledge in and displayed better attitude towards the prevention of mycotoxin contamination than the tigernut traders (wholesalers/retailers). The educational level of all stakeholders influenced their attitude and knowledge towards the prevention of mycotoxin contamination. Almost all consumers were willing to try new tigernut products such as the flour, oil and milk and would like to see more of these products on the Ghanaian market.

The number of samples and the levels of mycotoxins (Ochratoxin A and aflatoxins) increased as the value chain progressed, with retail samples containing all the mycotoxins analysed. Total mycotoxins ranged from 0-27 µg/kg at the farm stage to 0-52 µg/kg at the wholesale stage and finally to 7.9 to 1115.48 µg/kg at the retail stage. These highlight post-harvest stage of the value chain as the focal point for mycotoxin prevention programs, although mycotoxin prevention can be agreed as a cumulative process.

Both black and yellowish-brown tigernut flours contained relatively high and comparable amounts of sucrose, glucose and fructose. The relatively high resistant starch content of the tigernut flour makes the flour ideal for diabetics and weight watchers. The yellowish-brown variety had higher total starch content, higher water-retaining ability and viscosity at heating and holding cycles as compared to the black variety. Titratable acidity was found to be the

crucial determinant of spoilage in tigernut flour and higher temperature was observed to increase the oxidation of the tigernut flour. This may imply that tigernut flour should be stored below room temperature.

Heat and the addition of α- amylase increased the total solids, brix and titratable acidity but caused a decrease in the pH of the tigernut milk. Addition of 0.2% of α- amylase to roasted tigernut milk improved its emulsion stability. Heat and addition of α- amylase caused the tigernut milk to become darker in colour. The flow behaviour of the tigernut milk exhibited shear thinning (pseudoplastic) fluid properties. This implies that commercial production of milk from tigernut must control parameters such as speed of machines during processing as well as concentrations of food additives such as α-amylase.

Chemical qualities such as iodine value, peroxide value, ester value, saponification value, free fatty acids and acid value of oil extracted from tigernut tubers, all increased at higher temperatures whilst antioxidant activity and phenolic content decreased. The functional properties of tigernut oil suggested that the oil is good for frying at lower temperatures and for shorter periods.

The carbohydrate component of the tigernut tuber was mainly made up of starch and dietary fibre (resistant starches) which reduced in the milk and oil. Crude fat was the second most abundant component in the tigernut tuber. Quercetin and gallic acid were found in appreciable amounts in the tigernut oil. Although, the protein content in the tigernut milk was lower compared to the tuber, it was probably enough to impart desirable functionality to help stabilize the tigernut milk.

Tigernut tuber and its derivatives can offer various options in food products. The safety of the tuber and its products can however be improved when stakeholders of the supply chain are educated and supported to implement strategies that prevent mycotoxin contamination.

DEDICATION

I dedicate this work to my parents Mr. A.S. Battuta and Mrs Gertrude Adorkor Battuta, my husband, Lawrence Dawlah and my lovely kids, Jayda, Jynelle and Jevon for their support and love throughout this programme. I also dedicate it to my siblings, Rashid, Khalid and Trudy for their prayers and support.

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LIST OF ABBREVIATIONS

AACC - American Association for Clinical Chemistry

AFB1- Aflatoxin B1

AFB2- Aflatoxin B2

AFG1- Aflatoxin G1

AFG2- Aflatoxin G2

AOAC - Association of Analytical Chemist

AOCS - American Oil Chemists' Society

FUMs - Fumonisins

GDP - Gross Domestic Product

GEPC -Ghana Export Promotion Council

GIPC - Ghana Investment Promotion Centre

HPLC - High Performance Liquid Chromatography

1D1F - One District One Factory

OTA - Ochratoxin A

WHO - World Health Organisation

CHAPTER ONE

1. Introduction

This chapter presents the background and rationale of the study. The problem statement is also presented together with the justification of the study. The concluding section contains the study objectives and significance.

1.1 Development of the Agricultural Sector in Ghana

It is estimated that the agricultural sector provides employment to about 60% of the Ghanaian population, thereby placing second to the service sector, in order of contribution, to the nation's Gross Domestic Product (GDP) (Sam & Dzandu, 2015). In 2018, the country enjoyed 34% increase in export earnings from the Agricultural sector, which was valued at US$591.036 million (Ghana Export Promotion Authority, 2019). These earnings were mainly from a few crops such as cashew nuts, banana, shea nuts, mangoes, medicinal plants, pineapples, yams, rice and seafoods such as cuttlefish, squid and tuna.

In Ghana, the Agricultural sector is regarded as vital to the alleviation of poverty and improvement in food security. In congruence with this, the Ghanaian Government has directed its efforts to the enhancement of the sector through the improvement of agricultural practices to increase yield, and the industrialisation of food crops, under the One District One Factory (1D1F) Initiative, amongst others. The 1D1F initiative seeks to industrialise the major agricultural produce in every district to ensure supply, create jobs and improve the nation's export prospects (Dzansi et al., 2018). The Government of Ghana, under the 1D1F initiative, has identified tigernut as an underutilized crop with huge potential (Agoo Fm, 2017). With the 1D1F, the nation intends to increase the availability of the tubers to Ghanaians, especially since

the crop is not new to most of the population, considering the occasional media awareness made on the goodness of the tigernut tuber (Yeboah, 2014).

1.1.1 Tigernuts

Tigernut (*Cyperus esculentus L.*), which has its roots in Egypt, is a tuber, contrary to popular beliefs that it is a nut (Negbi, 1992). Nonetheless, its chemical composition has both properties of tubers and nuts (Sánchez-Zapata et al., 2012). It is a perennial grass-like plant with sweet nut-like rhizome/tubers. Tigernut has many local names such as *"chufa"* (in Spanish), *"souchet"* (in French) and *"ermandeln"* (in German), "earth almonds, earth nut, edible galingale, yellow nut sedge, ground almond, and rush nut (Coşkuner et al, 2002). In Ghana, tigernut is commonly called *"atadwe", "atangme", "nansaxa" and "fie"* by the Akans, Gas, Dagombas and Ewes respectively (Dokosi, 1998). It is classified under the division of *Magnoliophyta* and the class of *Liliopsida.* Furthermore, it belongs to the order *Cyperales* and family of *Cyperaceae* (Orhevba & Bankole, 2019).

1.1.2 Farming/Cultivation of Tigernuts in Ghana

Tigernut is regarded as one of the underutilised crops in Ghana. It is a food commodity cultivated in many parts of Ghana in very small scales, with the Kwahu East district being one of the major hubs for tigernut cultivation. For this reason, tigernut has been considered as the major raw material for a factory in that district, as per the 1D1F initiative (Dzansi et al., 2018).

Colour is the commonest criterion used for the classification of tigernut, of which four varieties exist: yellow, yellowish-brown, red and black (Ejoh et al., 2006; Abano & Amoah, 2011). Among these, the yellowish-brown and black varieties are common in the Ghanaian markets (Ayeh-Kumi et al., 2014). The yellowish-brown type is more preferred to the black variety due to its innate characteristics, such as its eye-catching colour, fuller body and larger size. The

yellowish-brown variety also contains more protein, fewer anti-nutritional elements, mainly, polyphenols and produces more milk with less fat. (Okafor et al., 2003).

There are two tigernut planting periods in Ghana; minor planting period (September to November) and major planting period (April to July) (Tetteh & Ofori, 1998). Harvest time ranges from 90 to 120 days. In Ghana, tigernut farmers dry the harvested tubers under the sun for about 2 to 3 months until the tubers are dried, shrivelled and wrinkled. Dried tubers are then sold immediately after harvesting or stored in a well-ventilated area until sold or consumed.

1.1.3 Nutritive and Nutraceutical Value of Tigernuts

According to Suleiman et al., (2018), a fresh tigernut tuber weighs between 70mg to 900mg whilst that of a dried tuber is between 30mg to 350mg. The major components of tigernuts are carbohydrates (mainly starch and fibre) and fat. Carbohydrates represent 43.4g/100g, starch content is 29.9g/100g and fibre content is 8.81g/100g. The fat content is between 22% to 45% in dry matter. The key fatty acids present in tigernuts are oleic (56% to 85%), linoleic acid (8% to 12%), palmitic (10% to 20%), and stearic acid (0.3% to 5%). Tigernuts also contain high calcium and phosphorus. However, iron, zinc, magnesium, manganese and copper are present in small concentrations (Arafat et al., 2009).

Many researchers have reported on the numerous health benefits of tigernut including the lessened occurrences of cardiovascular disease, diabetes, cancer (specifically colon cancer), thrombosis and obesity, following significant consumption of the tubers. The tubers have been said to preserve the internal mechanisms, stimulate blood circulation and inhibit both constipation and diarrhoea and are ideal for children, older persons and sportsmen (Adejuyitan et al., 2009). According to Belewu and Abodunrin, (2008), allergic reactions from the consumption of tigernut have not been reported.

1.1.4 Food applications of Tigernuts

In Ghana, tigernut is mostly consumed in its raw state as a snack. The most popular processed form of the tuber is the tigernut porridge, locally known as "*Atadwe"* milk. This porridge is consumed as a complementary food by babies. It is also consumed by the elderly, weight-loss aspirants, adults and convalescents at health centres (Chukwuma et al., 2010). A few industries in the country have incorporated tigernut as an ingredient in their manufacturing process for alcoholic drinks and for yoghurt. However, tigernut is used in food applications in various other forms across the globe.

Tigernut has been applied in the ice-cream and baking industries as a flavouring agent and as flour respectively. It is combined with cereal and spices in the production of a popular local Nigerian beverage ("*Kunnu"*). Oil, soap and starch extracts have been successfully made from tigernut (Ezekiel et al., 2019). "*Horchata de chufa*", which is a sweetened water extract from tigernut, is the most globally recognised food application of the tuber (Sanchez-Zapata et al., 2012).

Researchers across the globe have established that, tigernut and its by-products encounter challenges with fungal growth as the tuber travels along the value chain. As a result of this, there has been reports of the presence of mycotoxins in varying concentrations, along the value chain of tigernuts in other parts of the world.

1.1.5 Occurrence of Mycotoxins in Tigernuts

Mycotoxins including aflatoxins, ochratoxin A and fusarium have been identified in tigernuts (Rubert et al., 2011) in various parts of the world. Fungal colonisation and subsequent mycotoxin production are promoted by physical (temperature, moisture, mechanical damage, and relative humidity), biological (spore load, stress, and insects) and chemical (fungicides, oxygen, substrate composition, pesticide, carbon dioxide) factors. The climatic conditions of

Ghana and most sub-Saharan African countries are ideal for the growth of mycotoxin-producing fungi, which require an optimal temperature of 25°C to 30°C; water activity of 0.83 aw to 0.99 aw and moisture greater than 9% (Ribeiro et al., 2006). The conditions surrounding the pre-harvest, harvest and post- harvest of the tubers, as well as other parts of the value chain, are conducive for mycotoxin contamination (Bhat & Vasanthi, 2003).

Global attention is given to mycotoxins due to the weighty economic loss it imposes on human health, animal productivity and trade (Cinar & Onbaşı 2019). Effects of ingestion, inhalation and absorption of mycotoxins in the skin cause animal and human mortality as well as general performance decrease. According to Campos-Mondragon et al. (2009), chronic and acute exposure to aflatoxins can lead to liver cancer (10% adults' death) and immunosuppression in adults; stunting (35%), mental impairments and acute poisoning in children. Nearly $670 million is lost by African countries for the non-compliance of African produce to aflatoxin levels outlined by European Union (Otsuki et al., 2001).

Fungi colonisation can occur at any stage of the supply chain of mycotoxin-susceptible crops. For this reason, prevention mechanisms can be applied throughout the supply chain, especially at stages where the risk of colonisation has been identified as most likely. This requires analysis of the players in each stage of the supply chain to identify the vantage points. Regulation of the mycotoxin levels in food helps to ensure control along the supply chain and avoid the detriments of its presence in unacceptable levels.

1.2 Problem Statement and Justification

As mentioned in section 1.1 above, the government of Ghana has called for the industrialization of tigernut under the 1D1F initiative, which would result in increase in local consumption and demand for the crop, as well as may become a revenue generating crop for the nation through exports. This venture has high prospects since the soil profile in many parts of the nation is

favorable for tigernut cultivation. As industrial application of tigernut tubers is underdeveloped in Ghana, an increase in the demand for the tuber and subsequent increase in its production may not be sustained unless the food applications of tigernuts are known. This study will enlighten the industry and general public on the functional and physicochemical properties of Ghanaian tigernuts and its derivatives in order to increase its use in Ghanaian diets. Eventually, the demand of the tubers will increase, in line with the Government of Ghana's agenda of increased production of food crops such as tigernuts.

It is well-known that tigernuts are susceptible to mycotoxin contamination. In many of such nations, where the presence of mycotoxins such as aflatoxins and Ochratoxin A have been established in tigernuts through numerous research studies, measures have been put in place to minimise fungal colonisation on the tubers. In Ghana, however, there is inadequate published data on the mycotoxin levels of tigernuts grown in the country. Furthermore, there is limited available information that shows that measures have been specifically put in place to reduce the contamination of the tigernuts along the value chain by mycotoxin-producing fungi. Lack of information on its levels of contamination across the value chain hampers remedial strategies to minimise contamination. Additionally, there is no standard on the maximum mycotoxin level in tigernut specifically in Ghana. Lack of regulatory measures pose a risk of increasing financial drain on health systems as well as reducing international trading in the form of exports.

1.3 Objectives

The main objective of the study is to characterize Ghanaian tigernut as an ingredient for possible food applications.

1.3.1 Specific Objectives

The specific objectives of the study are:

- To assess the level of mycotoxin knowledge of the stakeholders in the tigernut value chain.
- To determine the occurrence and level of mycotoxins (Aflatoxins and Ochratoxin A) in tigernut along the supply chain.
- To evaluate some compositional and functional properties as well as the shelf life of tigernut flour
- To determine the effect of roasting and addition of alpha amylase on the functional properties of tigernut milk.
- To evaluate the polyphenol content and stability of tigernut oil during heating at different temperatures.
- To determine the physical properties of tigernut tuber and some of the nutritional composition of tigernut tuber, milk and oil.

1.4 Significance of the Study

Data gathered in this study will offer baseline information on the level of mycotoxin contamination of Ghanaian tigernuts. It will also establish the level of mycotoxin information that players in the tigernut value chain have. Furthermore, it will highlight the focus areas for mycotoxin control programs along the value chain. The information on the mycotoxin levels will provide a basis for regulation of the mycotoxin level of the tigernuts. Published results of the study will create awareness on mycotoxins in tigernuts for the general public to minimise their effects on the health of tigernut consumers. Furthermore, data on the functional properties of tigernut tuber, tigernut flour, tigernut oil and tigernut milk would direct industry on the food application to industrialise. In the long term, information shared from this study will lead to increase in demand of the tuber and revenue for stakeholders of the tigernut value chain as well as serve as a cash crop for the country.

CHAPTER TWO

2. Literature

This section provides theoretical and empirical basis for the study. The chapter begins with an overview of the agricultural sector of Ghana and the Government of Ghana's One District One Factory (1D1F) initiative. It then delves into the identification of tigernut as an input to the 1D1F program. The chapter also provides an overview on tigernuts, its composition, horticulture and its uses. The safety of tigernut in terms of the presence of mycotoxins is introduced in this chapter. There is also a review of empirical works on the effect of fungal colonisation in the value chain. Finally, gaps in available literature pertaining to the topic are highlighted.

2.1 Overview of the Agricultural Sector of Ghana

The primary cash crops produced in Ghana include beans, cocoa, palm oil, pineapple, cotton, tomato, banana, citrus fruits, coconut, cashew, tobacco, and fresh vegetables. The cocoa industry is known to be vital to the strength of the economy (GIPC, 2020). The export returns from the agricultural sector depend mainly on cocoa and a few other unprocessed commodities (Business and Financial Times, 2015). According to this report, the estimated non-traditional export earnings were valued at $2.4 billion as at 2019. The agricultural sector in Ghana is marked by low yields for both staple and cash crops (Ghana Agriculture Sector Policy Note, 2017).

It would be very beneficial to the country if a few more crops provide economic benefits to the scale of cocoa's contribution. The Government of Ghana has, over the years, implemented policies aimed at increasing the nation's raw agricultural products (GIPC, 2020). Additionally,

the Ghanaian Government has made intensive efforts to process most of the nation's raw agricultural products (GIPC, 2020).

2.2 One District One Factory (1D1F) Program

Industrially, measures and initiatives have been put in place by the Government of Ghana to revamp and increase the production of the nation's cash crops. A prominent initiative is the "One District One Factory" (1D1F) program. The 1D1F program is a vital element of the Industrial Transformation Agenda of the Government. The Government's role in this program is to provide support in the creation of District Enterprises through the creation of the right setting such as the provision of amenities including good roads, regular flow of water and undisrupted power.

The program aims at establishing at least one medium scale to large scale factory in all the 254 districts in the country. This means that, a district could have more than one factory depending on its resource base. The emphasis on the establishment of medium to large scale factories is because the enterprises to be established are expected to impact positively and significantly on the economies of the districts through the creation of jobs, stable income for the residents and improved livelihoods. The implementation of this program will restore the industrial sector of the economy through the implementation of targeted initiatives to make industrial production competitive and attractive to the private sector.

In this regard, the 1D1F program has become a vital component for the revolution and transformation of the industries and the Agricultural sector of the economy. With the implementation of this program, the economies of the Districts would be positively impacted and improved significantly through the creation of jobs, and provision of stable income for the resident as well as a source of livelihood.

2.3 Tigernut as a Potential for 1D1F in Kwahu-East District of Ghana

Despite the numerous health and economic benefits that the production of tigernut promises, its production and utilization have not received the due attention as compared to other cash crops by the Government of Ghana (Wongnaa et al., 2019). Efforts to popularise and commercialise this crop are left in the hands of rural indigenous farmers and small-scale traders (Wongnaa et al., 2019). Added to these, the high cost of land preparation and cultivation has been a major setback to tigernut production. It has been one of the many underutilized crops which is yet to attract the attention of relevant institutions and receive the needed support to uncover its full significance (Business and Financial Times, 2015).

The climatic and soil profile of most parts of Ghana have been found to be favourable for the cultivation of this food commodity. Nonetheless, *Aduamoah* is regarded as the tigernut hub of the nation. Under the 1D1F initiative, measures have been put in place to increase the production and cultivation of tigernuts. The development of tigernut industries may improve the livelihood of the rural populace in the districts and eventually create foreign exchange earnings for the country (Agoo FM, 2017).

2.4 Overview of Tigernuts

The use of tigernut (*Cyperus esculentus L.)* as food originated from ancient times. It was cherished food in ancient Egypt (Pascual et al., 2000). There have also been reports revealing that the edible tuber originated from Spain and Africa (Deatra, 1999). It is known to be among the first domesticated crops cultivated by ancient Egyptians at the Nile valley. According to Kaufman (2006), paintings of tigernut have been found in Egyptian tombs with inscriptions, giving instructions on how small loaves of a combination of tigernut and honey were made. In other reports, the paintings depicted the weighing of tigernuts by labourers whilst a scribe took

records of their work (Deatra, 1999). In Egypt and the Mediterranean, tigernuts were used for medicine and perfumes. They were also roasted and eaten by nursing mothers.

In places such as Southern Europe, tigernut has been grown for hundreds of years (Sánchez-Zapata et al., 2012). It has been reported to have been introduced by the Arabs into Europe in the Middle ages. In the 13th century, beverages made from tigernuts were consumed in the Southeast of Spain (Pascual et al., 2000). The use of tigernut in industry is not prominent and as such, has been poorly studied (Shikhov et al. 2011).

2.4.1 Description

Cyperus esculentus (tigernut) is a plant from the family *Cyperaceae* (Omoniyi et al., 2014), which is a perennial grass-like plant that produces somewhat spherical rhizomes and tubers from its base (Cortes et al., 2005). Daniel and Maria (2000) argued the plant to be a tuber rather than a nut. According to Deatra, (1999), the tigernut plant grows to a height of about 90 cm and bears slender leaves of 3-10 mm wide. It has very rough and fibrous plant foliage, which is often mistaken for a grass and the edible brown spike-like flowers mostly grow to a height of 1cm to 1.5 cm long.

There are different varieties of tigernut tubers. However, the prominent ones are the yellow, brown, and black, which come in various shapes and sizes (Barminas et al., 2001). The most ubiquitous varieties are mostly long and round. These varieties are: *Cyperus esculentus var. leptostachyus, Cyperus esculentus var. sativus, Cyperus esculentus var. hermannii., Cyperus esculentus var. esculentus, Cyperus esculentus var. rotundus, Cyperus esculentus var. macrostachyus.* Each variety has a distinct morphological feature. For example, the leaves *of Cyperus esculentus var. leptostachyus* are narrow, shiny and long and are arranged in rows of 3 with a triangular stem (FAO, 1988). *Cyperus eculentus var. rotundus* is non-flowering and dark brown in colour with blunt tipped leaves with no shoulders.

Cyperus esculentus is the common name used for both the useful and weedy sedge in most literature. The difference however is that, the weedy *esculentus* produces a lot of seeds whereas the cultivated variety *sativus* produces few seeds (Lapham & Drennan, 1990). The two varieties which are of most interest to many are the *Cyperus esculentus var. sativus* (cultivated) and *Cyperus esculentus var. esculentus* (weedy). According to Ayeh-Kumi et al. (2014), the light brown or yellowish-brown and the black tigernut are the two types sold in the Ghanaian market (Figure 2.1).

Figure 2.1: Varieties of tigernut on the Ghanaian market
(Source: Ayeh-Kumi et al.,2014)

2.4.2 Horticulture

Tigernuts are prevalent in damp grasslands and periodic wet grassland. Fluvial soils with moderately high concentration of manganese (Mn), sulphur (S), calcium (Ca) and boron (Bo) are specifically suitable for the cultivation of tigernut. Sandy soil and a mild climate have been proven by research to be the most suitable for the cultivation of tigernut. *Cyperus esculentus* is prevalent in temperate and tropical zones (Holm et al., 1997; Larridon et al., 2011). It is a plant

that tolerates cold temperatures but is mostly found in warmer zones (Holm et al., 1977). According to Mulligan and Junkins (1976), it is found in moderately dry environments such as fields. *Cyperus esculentus* var. *esculentus* is prevalent in Asia, Africa and South Europe (Schippers et al., 1995). *Cyperus esculentus* var. *hermannii* have been reported only to be found in Southern USA (Schippers et al., 1995). *Cyperus esculentus* var. *eptostachyus* was reported by Guillerm (1987) to be widespread in Europe because of its ability to adapt to the cold climate. Lastly, *Cyperus esculentus* var. *macrostachyus* was found mostly in Central America and Southern USA (Schippers et al., 1995).

In Ghana, cultivation of tigernut for commercial purposes are prevalent in areas such as *Aduamoah* and *Esereso* in the *Kwahu* province of the Eastern Region of Ghana (Obeng-Koranteng et al., 2017; Tetteh & Ofori, 1998). It is also cultivated in other areas such *Bewjiase* and the surrounding villages in the *Awutu Senya* East District, *Ampenyi* and its environs in the *Komenda Edna Eguafo* District (Obeng-Koranteng et al., 2017). In the Ashanti Region, the cultivation of tigernut is found in areas like *Adansi Danyameso* in the *Adansi* South District and *Tanoso* and its peripheral villages. Asante (2015), also reported cultivation of tigernut in areas of the Western Region (*Adowa*), and in some parts of the Northern Region (*Tampong* in the *Savelugu Nantom* District). The survey conducted by Tetteh and Ofori (1998) on the cultivation of tigernut in *Kwahu-Aduamoah* showed that, men constituted approximately 30% of the farmers whiles women were about 70%.

Donkor et al. (2019) studied the variation in the local accessions of tigernut in Ghana. The results proved that the variations were wide, therefore buttressing the point that breeding of improved variations of tigernuts was possible. They also highlighted the fact that the tigernuts from *Twifo Praso, Kasoa, Bawku, Bodwiase, Asukese Donkokrom, Krachi, Kwanyako, Wa* and *Bewjiase* produce high yield. This means that tigernuts from these areas could be further exploited for future large-scale farming.

So far, tigernut cultivation has contributed slightly to Ghana's economy in terms of foreign exchange from its exportation (Donkor et al., 2019). In 1998, Ghana's revenue for the export of tigernut was $8, 687.78 (Tetteh & Ofori, 1998). However, this trend increased in 2010, when Ghana exported 63,462 tonnes of tigernut, which was valued at $25,130.82 (GEPC, 2010).

2.4.3 Composition of Tigernuts

Carbohydrates have been found to be the principal component in tigernuts. Starch and dietary fibre are the main composition of the carbohydrate content in tigernut. The starch content in tigernut is reported to decrease when reducing sugar levels elevate during storage (Sánchez-Zapata et al., 2012; Coşkuner et al., 2002). According to Ros (2010), the fibre composition of tigernut were found to be like that of nuts. However, the moisture and carbohydrate levels were found to be higher. The lipid and protein levels were also found to be lower than in tree nuts (Ros, 2010; Sánchez-Zapata et al., 2012).

Researchers such as Yeboah et al. (2011) found some levels of phytosterols and vitamin E in tigernut oil. The appreciable content of phytosterol have been found to enrich the quality and commodity value of tigernut as a source of food (Sánchez-Zapata et al., 2012). The fatty acid composition in tigernut have also been studied. Linssen et al. (1988) have analysed and compared the composition of fatty acids and triglycerides in tigernuts and olive oil. They found out that, both showed great resemblance in composition even though the tigernut is a tuber and the olive is a fruit. This showed that tigernut oil could be a suitable alternative for imported olive oil (Deatra, 1999). Table 2-1 shows the proximate composition of tigernut tuber and a few of its beverages.

Table 2-1: Nutritional composition of tigernut tubers and beverage

Nutrient	Tigernut Tuber (g/100g)	Horchata De Chufa (g/100g)	Tigernut Beverage (g/100g)	
Total fat	24.49	3.09	1.26-1.59	1.88-2.27
SFA (% total fatty acid)	17.5			
MUFA (% total fatty acid)	72.9			
PUFA (% total fatty acid)	9.3			
Ratio *n*-6/*n*-3	22			
Proteins	5.04	0.91	2.34-2.51	0.47-0.54
Ash	1.7	0.25	0.31-0.39	0.16-0.18
Carbohydrates	43.3	*Not detected*	1.93-2.34	2.31-2.74
Total dietary fibre	8.91	1.03	0.23-0.31	0.53-0.65
Sucrose	13.03	>10		
Total energy (kcal/100g)	413.8	>71.45	28.42-33.71	28.04-33.55

(Source: Sanchez-Zapata et al., 2012)

According to Sanchez-Zapata et al. (2012), tigernut contains essential vitamins and minerals such as calcium, potassium, Vitamin C and E (Belewu & Belewu, 2007). Besides the main composition of tigernuts, the phenolic and anti-nutrient compounds have also been analysed and studied. Ekeanyanwu et al. (2010) showed that, the main phenolic compounds found in tigernut oils are the trans-ferulic, vanillic acid, vanillin and trans-cinnamic acid (Roselló-Soto et al., 2018). The concentration of quercetin in tigernut has also been studied and found to be 3.76×10^{-3} -60.63 mg GAE/100 g (Oladele et al., 2017). Additionally, tigernut contains gallic acid and catechin in concentrations of $3.95 \times 10^{-3} - 1.74$ mg GAE/100 g and $8.83 \times 10^{-4} -$ 6.58 mg GAE/100 g respectively (Oladele et al., 2017).

2.4.3.1 Health benefits of tigernuts

Studies conducted by Salem et al. (2005) revealed that, tigernuts exhibit anti-inflammatory properties. It has also been confirmed that arginine, the prevalent amino acid in tigernut, significantly lowers blood and peripheral vascular resistance in healthy adults and in patients with vascular diseases (Moore, 2004).

The milk of tigernut has been found to be good for the prevention of arteriosclerosis (Sánchez-Zapata et al., 2012). According to Chukwuma et al. (2010), its consumption, leads to the prevention of heart related problems and the facilitation of blood circulation. Tigernut milk has also been found to be a suitable beverage for celiac patients who are lactose intolerant (Sánchez-Zapata et al., 2012). It has also been found to be beneficial to those who have problems with digestion and diarrhoea, as it aids in the production of the essential digestive enzymes such as amylase and catalase (Adejuyitan, 2011).

The nutritional analysis conducted by Bixquert (2003), concluded that tigernut contained high levels of oleic acid, which lowers the cholesterol levels, owing to its high vitamin E content. It has also been found to prevent colon cancer (Zimmerman, 1987). In Ghana, tigernuts have attracted numerous unproven or unsubstantiated health claims. The tubers are mostly consumed for its nutritional and health benefits as well as for its aphrodisiac properties (Ayeh-Kumi, 2014). For this reason, it has been labelled as a substitute to Viagra. It is also believed to improve fertility in women.

2.4.4 Food applications of tigernuts

2.4.4.1 Tigernut milk

The use of tigernut as milk had its origin from Spain, which according to literature, may have been introduced by the Arabs. Many names have been ascribed to it; the Spaniards call it "*chufa de horchata"* whilst Northern Nigerians call their variant '*kunnu aya'* (Bamishaiye &

Bamishaiye, 2011). It has been proven to be a rich source of magnesium, potassium, calcium, carbohydrates, protein and enzymes which aid in digestion (TTSL, 2005). The tigernut milk has also been found to be a rich source of vitamin C and E (Bamishaiye & Bamishaiye, 2011). Tigernut milk is not readily available on the Ghanaian market as compared to its pudding ("*Atadwe"* milk), which is sold in markets, by street sellers, a few supermarkets, few restaurants and some eateries.

According to Tapsoba (2015), there are six kinds of tigernut milk in Spain. These include the untreated or fresh, pasteurized, sterilized, Ultra High Temperature (UHT), condensed pasteurized and the powdered tigernut milk. TTSL (2005) however, acknowledged the concentrate tigernut milk as the 7th type in addition to the six mentioned above. These different types of tigernut milk have different modes of preparation and characteristics.

According to TTSL (2005), "the natural or fresh tigernut milk is prepared with the right quantity of tigernuts, water and sugar to get a product with not less than 12% of soluble solids, 2.2% of starch, 2.5% of fats, a pH of 6.3 and not more than 10% of sugar in form of sucrose." The pasteurized tigernut milk is prepared by subjecting the milk to a pasteurization treatment below 72ºC, without the addition of additives or technologic fertilizers while the sterilized tigernut milk is obtained by submitting the tigernut milk into a technological process, which transforms or removes its contents of starch totally or partially (TTSL, 2005).

The sterilized tigernut milk must be thermally treated after being packaged, in order to destroy microorganisms and inactivate its forms of resistance. The Ultrahigh temperature tigernut milk is the type of tigernut milk submitted to a process that eliminates the starch and is processed by a thermal treatment (UHT) which ensures that after its aseptic packaging, microbes especially pathogens are destroyed (TTSL, 2005).

The concentrate tigernut milk is the type of milk with the right proportions of tigernut, water and sugar to obtain a product that has at least a concentration of dissolved solids of 42% and a pH of 6 (TTSL, 2005). It is the milk which when dissolved in water, results in a product which has the same characteristics as the natural tigernut milk. According to TTSL, (2005), the condensed tigernut milk can be pasteurized (with about 60% or more of dissolved solids, 4% of starch and 5% of fats) or frozen (at least 50% of dissolved solids, 5% of starch and 7% of fats).

The powdered tigernut milk is subjected to a technological process which can eliminate or partially or totally transform its starch components into solid granulates or particles. It is obtained through a drying process with not more than 5% content of water (TTSL, 2005). As compared to the other forms of milk, it is required to consume the natural tigernut milk within 2 to 3 days after opening or keep in the fridge to prevent spoilage.

2.4.4.2 Tigernut flour

As a result of its gluten free content, tigernut flour has been found to be a suitable substitute to several other flours. It is utilized in the confectionery industry as a component or main flour (Bamishaiye & Bamishaiye, 2011). According to Ade-Omowaye et al. (2009), tigernut flour is considered a good substitute to cassava flour in the baking industry and has been used to flavour biscuits and ice creams (Osagie & Eka, 1998). Additionally, tigernut flour has been regarded as a potential ingredient for the bakery industry due to its moderate natural sugar content (Anderson et al., 1994). In *Keta* (Ghana), sugar is added to the sun-dried tigernut flour to be eaten raw or as a beverage when water is added (Adejuyitan et al., 2009). It is used to supplement flavour to ice creams and biscuits (Osagie & Eka, 1998). Tigernut has been confirmed to have more essential amino acids than the ones purported in the protein standard by the FAO/WHO (FAO/WHO, 1985). It is also a rich source of calcium and iron, which are

necessary for body development and growth (Oladele & Aina, 2007). Tigernut flour maintains its nutritional properties in the milling process. Due to the therapeutic and nutritional benefits, tigernut flour could work as a suitable substitute to cassava flour in the baking industry (Ade-Omowaye et al., 2008).

The evaluation of the nutritional application and sensory properties of tigernut flour products have been reported in literature. Akajiaku et al. (2018) evaluated the sensory and proximate properties of cookies made from tigernut flour. The results obtained showed that, cookies made from tigernut have good nutritional profile with carbohydrates and proteins relative to that of cookies made from wheat flour. In their analysis, Eke-Ejiofor and Deedam (2015) stated an increase in proximate composition of cakes and biscuits made from a composite flour of wheat and tigernut. Twum et al. (2015) investigated the physicochemical properties of a composite flour mixture of tigernut, maize and soybeans flour in different percentage compositions. The results reported an increase in physicochemical properties (ash content, moisture content, pH and protein content) of the composite flour relative to that of the individual flours. The increase in weight of cakes prepared from a mix of tigernut and wheat flour has been ascribed to the bulkiness of tigernut flour in wheat-based cakes (Chinma et al., 2010). Oladele and Aina (2007) concluded that, tigernut flour could be used as an ingredient in food applications owing to its little to no retrogradation effect, low bulk density as well as its peculiar setback and breakdown viscosities.

The term oil is generally used to define greasy or oily materials that are fluid at room temperature (Buba, 2005). Lipid is a collective name for fats and oils that are naturally occurring. Lipids are of massive commercial use in terms of frying operations since they are a suitable way of transferring heat rapidly (Andrew et al., 2012). They occur naturally along with carbohydrates and protein in main crops and are extensively present in nature. In chemical terms, esterification is used to describe the reaction between three molecules of fatty acids and

a triol (glycerol). Fats and oils are mainly acquired from two sources: vegetable sources and animal sources (O'brien, 2008). Fats and oils from animals are obtained from both land-dwelling and aquatic animals. Vegetable oils, that is, oils obtained from plant sources, occur most abundantly in seeds and fruits. These include tigernut oil, groundnut oil, cotton seed oil, sunflower oil, palm oil etc. Plant-source oils suitable for human consumption have gained much interest in several food processes and factories as they give distinctive flavour and texture qualities to foods as vital dietary elements (Odoemelam, 2005), while serving as a derivative of oleo compounds (Morrison et al., 1995). Vegetable oils contribute positively to the diet in various nations, providing a rich basis of lipid and fatty acids as well as protein for human nutrition by restoring worn-out tissues, developing new cells and functioning as a useful source of energy (Gaydon et al., 1983; Grosso & Guzman, 1995; Grosso et al., 1997; Aremu et al., 2015). The core nutrients essential to the human body include carbohydrates, proteins, fats and oil, together with vitamins and minerals. The principal significance of the vegetable oils is in their food value.

Tigernut oil has been found to be a good source of oleic acid and low polyunsaturated fatty acid (Okladnikov et al., 1977). According to Bamishaiye and Bamishaiye, (2011), tigernut oil is suitable for salads because of its ability to form a uniform liquid at cold temperature. They also noted that it is classified as a high-quality oil since it is extracted without the application of heat. Furthermore, the oil serves as a suitable substitute for other oils such as soybeans, olive, cotton seed oil and corn oil. He et al. (1996) reported that tigernut oil could be the future source of biodiesel.

2.4.4.3 Economic importance of tigernuts

There is no denying the fact that, the production and export of tigernuts has brought considerable economic returns to many countries. Reports from Rubert et al. (2011) indicated

that, the '*horchata*' industry has been of a great economic benefit to Spain. Approximately, 40 to 50 million litres of '*horchata*' are produced annually in Spain (Maduka & Ire, 2019). This is valued at about $60 million according to Sánchez-Zapata et al. (2012). The use of tigernut to produce lactose-free products have led to an increase in tigernut cultivation globally (Maduka & Ire, 2019). According to Decker & Kurnik (2018), there has been a considerable increase in the value of gluten-free market from $ 1.7 billion in 2011 to $ 3.5 billion in 2016. This was expected to be valued at $ 4.7 billion in 2020.

Tigernut is cultivated and used as a side dish in countries like Ghana, Northern-Nigeria, Mali, Senegal and Togo (Omode et al., 1995). Some of the tigernuts are exported from these countries to Spain (Sánchez-Zapata et al., 2012), where the tubers are used to- process ''*horchata de chufa*" (Beneyto et al., 2000). Countries such as Chile, Brazil, Mexico, and Missouri cultivate tigernuts mainly for animal feed (Sánchez-Zapata et al., 2012).

Tigernut oil can be used as a waterproofing agent in textile fibres (Hleba et al., 2020). Presently, tigernuts are planted for medicinal and food purposes in Africa, Asia and Europe (Pascual-Seva et al., 2013; Bamishaiye & Bamishaiye, 2011).

2.5 The other side of the coin: Food Safety of Tigernuts

Though there is scarce literature on mycotoxin contamination in tigernuts, a few researchers have evaluated the levels of mycotoxins in tigernut tubers in different countries to assess the danger to the consumer. Mycotoxins are lethal chemical compounds produced naturally by mycotoxigenic fungi like *Claviceps, Stachybotrys, Aspergillus, Fusarium, Penicillium* and *Alternaria* (Bankole & Adebanjo, 2003; Custódio et al., 2019). These fungi sprout on many agricultural commodities such as cereals, dried fruits, tubers, nuts and spices (WHO, 2018). Mycotoxins can occur either before, after or during storage and are mostly detected in humid

and moist environments. Research has showed that, most mycotoxins are chemically stable and can survive the rigorous operation of food processes (WHO, 2018).

2.5.1 Mycotoxins in Tigernuts

Aflatoxins are harmful secondary ancillary generated by *Aspergillus parasiticus* and *Aspergillus flavus* (Omoniyi et al., 2014). These fungi, which primarily contaminate cereal (corn, wheat, sorghum and rice), spices, tubers and tree nuts survive in soil, hay, decaying vegetation and grains. Four main types of aflatoxins have been identified in tigernuts; Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1) and Aflatoxin G2 (AFG2) (Moss, 1998). Of all the identified mycotoxins in tigernuts, Aflatoxin B1 is considered the most dangerous since it is placed in the Class 1A human carcinogen category (Rothschild, 1992). Omoniyi et al. (2014) studied the aflatoxin contamination of tigernut sampled randomly from various markets in Kano, Kaduna, and Gombe states of Nigeria. The report indicated that, 90% of the tigernuts were above the 10µg/kg limit while 17% exceeded the alarm limit of 20 µg/kg established by United States Food Regulation and the Codex Alimentarius. Bankole and Adebanjo (2003) also reported aflatoxin concentrations extending from 10-20 µg/kg in 35% of tigernut samples from various marketplaces in Nigeria.

Ochratoxin A has also been identified in tigernuts in other countries in the world. Ochratoxin A (OTA) is a chemical substance yielded by *Aspergillus ochraceus* and various species of *Penicillium* and *Aspergillus*. It is the predominant of the Ochratoxin family (Mitchell et al., 2014) and is known to be the common mycotoxin that contaminates food commodities such as spice, juice, coffee beans, cereals and cereal products (WHO, 2018). It is produced during crop storage and is known to cause harmful effects in animal species. OTA has been connected to a few diseases in both animals and humans including hepatotoxic and immunotoxic effect

(Mitchell et al., 2014). It has been confirmed to cause adverse influence on cocoa beans exported from West Africa (Bankole & Adebanjo, 2003).

In certain parts of Africa, OTA have been identified in tigernuts (Rubert et al., 2011). In Nigeria, a study conducted by Adebajo (1993) to determine how moisture content and pH levels affected mycotoxin levels in tigernut tubers as storage time increases, observed that, contamination of samples with aflatoxin B1, B2 and Ochratoxin A increased with increasing storage time as moisture content increased and pH levels declined. From the analysis conducted by Bankole and Eseigbe (1996), 35% of tigernuts from Nigeria contained aflatoxin levels extending from 10 to 20 µg/kg and therefore labelled tigernut as a food commodity susceptible to aflatoxin concentration.

In Europe, Sebastia et al. (2012) identified Fusarium-emerging mycotoxins in Spanish tigernuts in concentrations between 32.1 and 4400 µg/kg. In West Africa, Adebajo (1993) and Bankole and Eseigbe (1996) detected mycotoxins in Nigerian tigernuts in concentrations between 10 and 120 µg/kg. Couvillion et al. (1991) did not identify mycotoxin in all samples of tigernuts in Mississippi.

Tigernut tubers sampled from different marketplaces in Ghana were reported to have significant levels of Aflatoxins B1, and G1 and Ochratoxin A (Rubert et al., 2011). Agyeman (2011) also isolated fungi of the genera *Aspergillus, Fusarium, Cladosproium, Mucor, Neosartorya, Monoascus, Neurospora, Penicillium, Paecilomyces, Rhizopu, Syncephalastrum, Torula, Rhodotorula,* and *Saccharomyces* in tigernuts from Ghana.

Studies have also been organized to ascertain the levels of mycotoxins in tigernut-related products. Rubert et al. (2011) investigated the concentrations of mycotoxins in tigernut and tigernut beverages sampled from different markets in Spain. Out of a total of 238 analysed samples, 32 were detected to be contaminated with OTA, AFB1, AFB2 and AFG2.

Mycotoxin contaminated tigernut tubers have been rejected by importing nations as experienced in 2002, when on three occasions, mycotoxin-contaminated tigernuts were refused entry into the European Union. Added to this, Malian tigernuts imported to Europe in April 2004, which contained mycotoxin concentration of 300 µg/kg AfB1, were also refused entry (European Commission, 2019).

2.5.2 Conditions for Fungal growth

Mycotoxins can develop on food commodities at any stage of the supply chain: processing, transport, and storage (Ferrão et al., 2017). There are numerous factors which are responsible for the growth of mycotoxins. These include temperature, relative humidity, fertilizers, geographical location and insect infestation (Ferrão et al., 2017). Table 2-2 shows the conditions for growth of common fungi.

Table 2-2: Common Mycotoxin causing fungi and their conditions for growth

Fungi	Mycotoxins	Growth Temperature	Optimal Toxin Production Temperature	Optimal Growth pH	Water Activity
A. flavus	AFB1, AFB2	25-30°C	28-35°C	5-6	0.94-0.97
A. parasiticus	AFB1, AFB2, AFG1, AFG2	15-33°C	28-35°C	5	0.95-0.99
A. niger	FB2	24-37°C	25-30°C	5	0.97-0.99
A. ochraceus	OTA	24-37°C	31°C	3-10	Min. 0.8
A. fumigatus	GTX	Under 42°C	37°C	7.35-7.45	0.92-0.97

(Source: Ráduly et al., 2020)

The incidence of fungal growth and infection have been connected predominantly to the presence of moisture (Kortei et al., 2019). Improper drying of stored food commodities such as cereals, tubers and legumes increases their susceptibility to the increase in mycotoxigenic fungi like *Aspergillus* species (Kortei et al., 2019). The susceptibility is speculated to increase with storage time (Pinotti et al., 2016). Kortei et al. (2019) established the fact that, contamination of food crops with the metabolites of *Aspergillus flavus* could increase at maturity and harvest periods at the terminus of the raining season.

Methods used for traditional drying of the food crops entails bare ground and field drying as well as improper handling. These methods significantly impact fungal infectivity. According to Mitchell et al. (2014), crops prone to mycotoxin contamination are those harvested and stored in tropical and subtropical regions susceptible to humid and dry environment (Mitchell et al., 2014). Aflatoxin production has been declared to be facilitated by tropical and Mediterranean climates (Ráduly et al., 2020). This was backed by the fact that, the production of toxins secreted by *A. flavus* and *A. parasiticus* occurs between 28 and 35 ℃ (Ryu et al., 2008). This buttresses the point that food commodities produced from these regions are susceptible to aflatoxin contamination.

2.5.3 Effects of mycotoxins on the economy

According to the World Health Organization's report on mycotoxins, mycotoxins have caused a lot of agricultural loss and pose a serious threat to both human and livestock, when consumed in high concentration.

2.5.3.1 Health effects of mycotoxins on the economy

The extent of biological damage elicited by mycotoxins differs based on many factors including the class and source, exposure route and dosage, species vulnerability and contributory

subclinical circumstances of the consumer (Mitchell et al., 2014). When consumed in high concentrations, mycotoxins can elicit diseases called mycotoxicosis. Hepatic disease and alimentary toxic aleukia are the most common mycotoxicosis (Armenda´riz et al., 2014). Intake of large amounts of food commodities containing mycotoxins within a short period of time will initiate severe toxicity leading to death (Darwish et al., 2014). Aflatoxins have been marked to cause human ailments including *Kwashiorkor*, occupational respiratory diseases, liver cancer, Indian childhood cirrhosis and Reye's syndrome (Darwish et al., 2014). Humans are most sensitive to acute AFB1 toxicity (Mitchell et al., 2014). Symptoms of acute aflatoxicosis include; anorexia, depression, ataxia, dyspnoea, anaemia and haemorrhage (Mitchell et al., 2014). Aflatoxins also have deleterious effects when exposed to high concentrations which includes immune suppression, gastroenteritis and hematomas (Mitchell et al., 2014). Aflatoxins can cause acute hepatitis by causing damage to the hepatocytes which can lead to death (Ráduly et al., 2020).

Protracted aflatoxin contamination causes flawed DNA multiplication in the bone marrow which lowers leucocyte concentrations (Ráduly et al., 2020; Benedict et al., 2016). Chronic toxicity occurs from long periods of exposure to moderate levels of aflatoxin concentrations. (Wagacha & Muthomi, 2008). Symptoms includes decreased egg or milk production in animals, immuno-suppression, and decline in rate of development (Wagacha & Muthomi, 2008). Kenya recorded the largest outburst of Aflatoxicosis to date, which resulted in 215 deaths out of 317 cases recorded (Darwish et al., 2014). In Nigeria, a study conducted by Uriah et al. (2001) reported an increase in aflatoxin levels in infertile men as compared to fertile men. Also, the posthumous autopsy of children who suffered from *Kwashiorkor* indicated high levels of Aflatoxins in their brains due to exposure to contaminated maize (Gbashi et al., 2018). In Gambia, children who suffered lowered salivary secretory Immunoglobulin A were connected to high exposure to aflatoxins (Turner et al., 2003; Gbashi et al., 2018). In Eastern Kenya,

aflatoxin poisoning had been documented with a fatal rate of 40% between the years of 2005 and 2006 (Daniel et al., 2011).

Ochratoxin A has been connected to several diseases in both humans and animals including hepatotoxic and immunotoxic effect (Mitchell et al., 2014). Biological consequences of Patulin, which results when exposed to high concentration Ochratoxin A in animals include; spleen, liver and kidney damage and impairment to the immune system (WHO, 2018). Fumonisin B1 has been found to cause oesophageal cancer in humans (Mitchell et al., 2014).

Not all mycotoxins are malignant and some of them have been used for their curative effect. The utilization of the antibiotic property of mycotoxins for commercial purposes begun in 1928 with the detection of penicillin (Mitchell et. al., 2014). Ergostamine has been exploited for its therapeutic properties in the treatment of vascular headaches and uterine contraction (Stein, 2017; Armenda´riz et al., 2014).

Approximately 35% of food and feed globally produced have been contaminated by mycotoxins (Gbashi et al., 2018). Due to this, 1 billion metric tons of various food commodities are wasted annually (Rahman et al., 2008; Gbashi, 2018). In Zimbabwe, fumonisins (FUMs) were detected in retail cereal products and soyabeans (Darwish et al., 2014). Ochratoxin A levels were detected in maize, millet, rice and peanut samples in Cote d'Ivoire (Sangare-Tigori et al., 2006). In Nigeria, significant levels of AFs were found in approximately 33% of maize sampled from various agro-ecological regions (Darwish et al., 2014).

The presence of mycotoxins in food products reduces the quality and hence its consumption. Certain Central African countries like Tanzania and Zambia have had their share in mycotoxin contamination. In Tanzania for example, significant levels of AFs were discovered in maize samples of which 12% of the samples exceeded the Tanzanian maximum limit of 10 µg/kg (Kimanya et al., 2008; Darwish et al., 2014). A survey organized in Zambia by Mukanga et

al. (2010) reported significant concentrations of AFs and FUMs in maize samples selected from six different districts in Lusaka, which were more than the tolerable limit of 2 µg/kg endorsed by FAO/WHO. Two hundred and sixty samples of various cereal and cereal products analysed for mycotoxin contamination in Tunisia and Morocco reported 53% contamination (Serrano et al., 2012). Significant levels of OTA up to 4 mg/kg higher than EU regulatory levels, have been discovered in cocoa powder in countries such as Ghana, Nigeria, Ivory Coast and Cameroon (Aroyeun & Adegoke, 2007). Figure 2.2 shows the distribution of mycotoxins in food commodities.

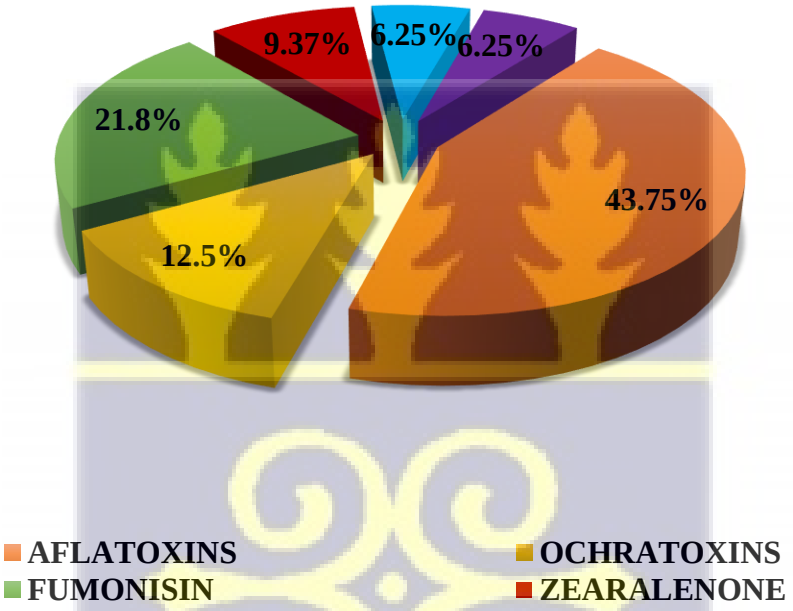


Figure 2.2: Distribution of mycotoxins in African countries

(Source: Darwish et al., 2014)

2.5.3.2 Effect on trade and Agricultural export

The impact of mycotoxins on trade has been a major global concern. Its effect is seen by the reduction in the value of commodities sold. This is evident at different levels via the reimbursement in case of claims, reduction in prices, and charge of sampling and analysis

(Gbashi et al., 2018). It costs Africa over USD 750 million yearly due to aflatoxin contamination of agricultural produce and an estimated cost of USD 670 million for food exporters due to European regulation of Aflatoxin (Gbashi et al., 2018).

According to Marechera and Ndwiga (2015), 2.3 million bags of maize were rendered unsafe for consumption and marketing during the aflatoxicosis crisis in Kenya from 2004 to 2006. Several border rejections of products from Africa have been recorded. 130 exports were rejected due to mycotoxin contamination from Egypt, 91 from Ghana, 60 from Nigeria, 5 from Morocco and 1 from Tunisia (Kareem, 2014). In Nigeria, 13 shipments of groundnut and associated products were refused by the European Union (EU) between 2007 and 2017 (Atanda et al., 2013). The rejection of consignments was due to failure to meet EU legislative limits in terms of mycotoxin levels. This rejection led to losses incurred by the affected countries. Not only has mycotoxin contamination of products affected trade export, it has also caused damage to the African food and agricultural reputation in the export market (Gbashi et al., 2018). Also, in the year 2008, consignments of food commodities which had been exported from Rwanda to the United Kingdom were ejected due to Aflatoxin contamination (Matsiko et al., 2017).

2.5.3.3 Impact on National budget

The allocation of resources by African countries to combat or mitigate the widespread of mycotoxin contamination through the establishment of interventions, has had a toll on their national budget. These preventive measures have significant cost implications with respect to their design and implementation (Gbashi et al., 2018). In 2004, a policy introduced by the Economic Community for West African State (ECOWAS) required its member states to increase budgetary allocation to at least 1% of national GDP, for strengthening of aflatoxins control strategies (WCOWAP, 2014). A yearly cost of 7.5 million USD was agreed by the

member state of the African Groundnut Council (Sudan, Senegal, Mali, Niger and Gambia) for the execution of the aflatoxin reduction program (Atanda et al., 2013).

2.6 Conclusion

The Government of Ghana is seeking to establish at least one tigernut factory for the industrial production of tigernut under the 1D1F initiative. Very limited information exists on the mycotoxin levels of Ghanaian tigernut. Yet the occurrence of mycotoxins in the food has detrimental effects on consumers and economies. Additionally, the effect that the mycotoxin knowledge of stakeholders of the tigernut supply chain have on their practices has not yet been established. Furthermore, the public as well as the Ghanaian industry need to be well informed about the functional properties of tigernut tuber and its products. Such information would increase its food applications and inclusion in the Ghanaian diet and subsequently increase demand of the tuber.

Tigernut milk, tigernut oil and tigernut flour have so far proven to be the most preferred food application of the tubers globally. Existing information on the food applications of tigernuts from other countries have so far centred on the local context, with the aim of increasing the consumption of the tubers and benefiting from its nutritional and health benefits. Researchers have reported on the sensory properties and proximate composition of tigernut flour products (Obinna-Echem & Robinson, 2019; Eke-Ejiofor & Deedam, 2015; Twum et al., 2015; Oladele & Aina, 2007). However, the current literature lacks enough information on the functional properties of Ghanaian tigernut flour, its adaptability in the Ghanaian diet as well as its shelf life. Other researchers have explored the use of tigernut in the manufacture of plant-based milk using different technologies and recipes. However, there is insufficient published data on the use of alpha amylase in the making of tigernut milk, application of roasting in the process of

producing the milk as well as their effect on the functional and physicochemical properties of the resulting milk.

In view of the above, the concentration of mycotoxin contamination at different stages of the supply chain needs to be analysed to determine if the country has a problem with mycotoxin in its tigernut tubers, along the value chain. The level of mycotoxin knowledge of key players of the supply chain should be known so that the focal points for mitigation programs can be established. Additionally, functional properties and the effect of processing of Ghanaian tigernut tubers, tigernut oil, tigernut milk extracts and tigernut flour need to be determined to provide information on their possible food applications.

CHAPTER THREE

3. OBJECTIVE 1: To assess the level of mycotoxin knowledge of the stakeholders in the tigernut value chain

3.1 Introduction

The prevalence in mycotoxin contamination of farm commodities and its consequent detrimental effect on both humans and livestock poses a key concern to food security and safety. Regardless of the efforts made to control fungal contamination, a considerable number of mycotoxin contamination incidents have been reported, in literature, to occur in both food and feed. An estimated value of 25% of the global food and feed output has been found to be contaminated by mycotoxins, which denotes a threat to food supply with an effect on international trade and economies (Gbashi et al., 2018; Pinotti et al., 2016). Furthermore, between 30% to 100% of feed and food samples are co-contaminated on a global level with mycotoxins (Binder et al., 2007).

It has been reported that, the prevalence of mycotoxin contamination could increase in the future. This foretelling is based on the eminent reality of climatic change that may influence agriculture and intensify the risk of fungal attack on agricultural crops (Gbashi et al., 2018; Pinotti et al., 2016). Mycotoxin contamination of agricultural commodities is an accumulative procedure which starts from the pre-harvest stage through to the post-harvest stage and storage, and spreads throughout the whole food production chain (Choudhary et al., 2010; Milićević et al., 2010; Ferrão et al., 2017). Immediately a crop is infected at farm level, mycotoxin contamination persists intensively to post-harvest and storage conditions. According to Cole et al. (1995), the possibility of mycotoxin contamination during pre-harvest depends on the type of soil, plant genotype, and ecological situations such as drought, fertilizers, temperature,

geographical location, relative humidity and insect infestation. The incidence of fungal growth and infection has been connected predominantly to the presence of moisture (Kortei et al., 2019). Certain practices such as poor handling of agricultural commodities have also been stated to be a principal source of mycotoxin contamination on the farm (Gbashi et al., 2018). Lack of regulatory policies that control debasement of foods with infected agricultural products and discarding food, are other parameters that promote mycotoxin infection along the African food and feed chain (Gbashi et al., 2018). Improper drying of stored food commodities such as cereals and legumes makes them susceptible to the growth of mycotoxigenic fungi like *Aspergillus* species (Kortei et al., 2019), which are speculated to increase with storage time (Pinotti et al., 2016). Industrially, food processing affects the concentration and distribution of mycotoxins (Pinotti et al., 2016). According to Cheli et al. (2013), mycotoxin-contaminated foods are usually employed as animal feed. They translate into remnants in animal products and find their way into the food supply chain (Pinotti et al., 2016).

The population of the world is increasing rapidly and with it comes the increase in competition for food commodities. It is therefore of great importance that, a good understanding of how mycotoxins are being distributed along the distribution chain and during processing is attained to control such occurrences. The purpose of this study was to evaluate the level of mycotoxin knowledge of the stakeholders in the tigernut value chain.

3.2 Methodology

The study examined the mycotoxin knowledge and prevention practices of various stakeholders in the value chain of tigernut tubers in Ghana, through the administration of questionnaires. Participation in the survey was completely voluntary and anonymity of participants was guaranteed. Black and yellowish-brown tigernuts were traced from farmers in *Adwoa* in the *Ahanta* West District of the Western Region and *Aduamoah* in the *Kwahu* East District of the

Eastern Ghana. These areas were randomly selected from the list of known tigernut farming areas in Ghana. Traders (wholesalers/retailers) and consumers of tigernuts were interviewed in the 16 districts of Greater Accra Region of Ghana.

To trace the tigernut value chain, the farmers were the first point of contact. They were asked questions to assess the post-harvest practices on their farms. Their knowledge on mycotoxin contamination was also assessed. The trail was followed to identify the next group of people in the value chain before the tigernuts reached the final consumer, which were the traders (wholesalers/retailers). All the major markets where tigernuts were either retailed or wholesaled, were also identified in the process. Consumers were recruited on the basis that, they had purchased tigernuts as at the time the survey was conducted.

Three semi-structured questionnaires were used to interview 1277 stakeholders in the value chain. These included 79 farmers, 487 traders (wholesalers/retailers) and 711 consumers. All questions on the questionnaires were asked in the local dialect or English and responses given were entered by the interviewer. All questionnaires administered were pre-tested before they were used. The questionnaire designed for farmers (Appendix 1) and traders (wholesalers/retailers) (Appendix 2) assessed mycotoxin contamination awareness based on 9 questions on the questionnaire, whilst the questionnaire designed for consumers (Appendix 3) assessed mycotoxin contamination awareness using 10 items. Storage of tigernuts was also assessed based on 8 items on the questionnaire for traders (wholesalers/retailers) and 10 items on the questionnaire for farmers.

3.3 The Study Area

Figure 3.1 highlights the study area used in this study. Three regions were used in this study:

- Greater Accra Region: The region has a land space of 3,245 sq. km and had a population of 4,943,075 in 2019. It lies 5.8143° N, 0.0747° E.

- Western Region: The region has a land space that covers an area of 23,921 sq. km and had a population of 2,165,241 in 2019. It lies 5.3902° N, 2.1450° W.
- Eastern Region: The region covers an area of 19,323 sq. km and had a population of 3,244,834 in 2019. It lies 6.2374° N, 0.4502° W.

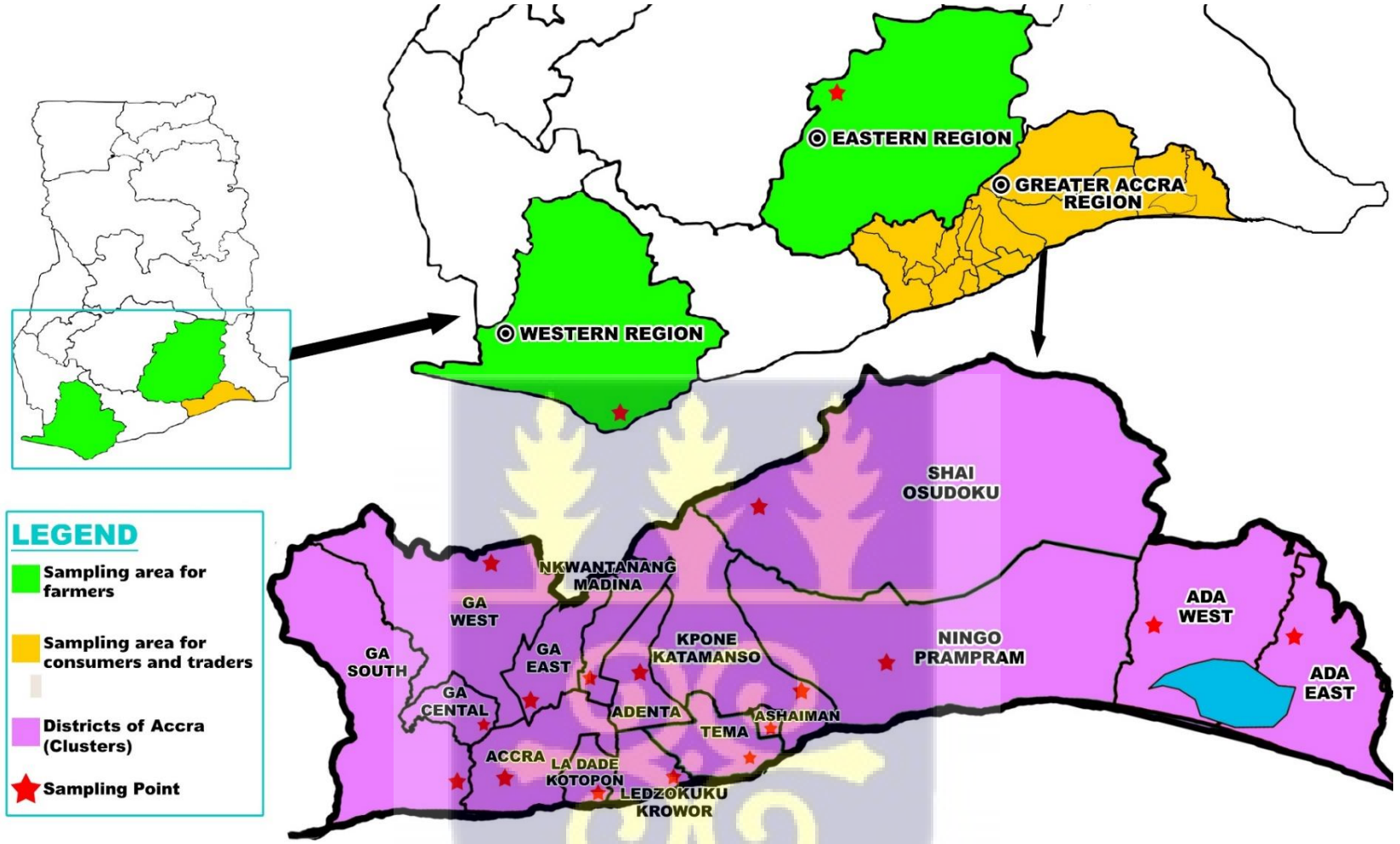


Figure 3.1: Map of Ghana indicating study area

3.4 Sampling and sample size determination for farmers

Sampling of farmers was done using cluster sampling method. From existing literature and online research, a sampling frame consisting of major tigernut cultivating towns were obtained. There were ten towns on the sampling frame namely *Adowa, Bawjiase, Aduamoah, Ampenyi, Twifo Praso, New Ebu, Adansi, Danyameso, Tanoso* and *Savelugu Nantom*. The towns were considered as clusters since farmers within each town had varying methods for cultivating tigernuts. A simple random sampling method was used to choose two towns (*Adwoa* and *Aduamoah*) from the sampling frame. All farmers present at the time of the survey in the two selected towns were interviewed. 19 farmers were sampled from *Adwoa* in the Western region and 60 farmers were sampled from *Aduamoah* in the Eastern region.

3.5 Sampling and sample size determination for traders

Traders (wholesalers/retailers) were sampled using a multi-staged stratified sampling procedure. The stratification variable that was used was districts, hence traders (wholesalers/retailers) within each district (stratum) were considered to share similar characteristics. A list of the major markets and streets were obtained for each district and a simple random sampling procedure was used to obtain one market and one street each from a district (Table 3-1and Table 3-2). A systematic random sampling method was then used to obtain the traders (wholesalers/retailers) involved in the study. Retailers, available on each street, were interviewed until the allocated number of respondents were obtained. The entrance to each market served as the starting point and at each second street after the entrance, traders (wholesalers/retailers) of tigernuts were enumerated.

Table 3-1: List of Selected Markets in the Districts in Greater Accra Region

District	Market	District	Markets
Accra Metropolis	Makola Market	Ga West Municipal	Achimota Market
Ada East District	Kassah Market	Kpone Katamanso	Kpone Market
Ada West District	Sege station	La Dade Kotopon Municipal	La Market
Adenta Municipal	Adenta Market	La Nkwantanang Madina Municpal	Madina Market
Ashaiman Municipal	Ashaiman Market	Ledzokuku/Krowor Municipal	Nungua Market
Ga Central Municipal	Anyah Market	Ningo Prapram	Dawhwenya Market
Ga East Municipal	Dome Market	Shai Osudoku (Dangme West)	Dodowa Market
Ga South Municipal	Mallam Market	Tema Metropolis	Community 1 Market

Table 3-2: List of selected streets in the Districts in Greater Accra Region

District	Streets	District	Streets
Accra Metropolis	CMB	Ga West Municipal	Achimota Main Road
Ada East District	Ada Foh Road	Kpone Katamanso	Kakasunanka
Ada West District	Sege Road	La Dade Kotopon Municipal	La Park Road
Adenta Municipal	Adjiriganor Traffic Light	La Nkwantanang Madina Municpal	Madina Trafic Light
Ashaiman Municipal	Ashaiman Road	Ledzokuku/Krowor Municipal	Nungua Main Road
Ga Central Municipal	Awoshie Traffic light	Ningo Prapram	Dawhwenya-Afienya Junction
Ga East Municipal	Dome Pillar 2	Shai Osudoku (Dangme West)	Dodowa Main Road
Ga South Municipal	Gbawe Junction	Tema Metropolis	Community 1 Road

A proportionate sample size determination criterion was used to obtain the number of traders (wholesalers/retailers) to be enumerated in this study. The reason for using proportionate sample size determination criterion was that, the number of traders (wholesalers/retailers) of tigernuts varied greatly across the various markets, hence for each market, the sample size obtained for the traders (wholesalers/retailers) was based on the Equation 3-1:

$$\frac{\text{Intended Sample}}{\text{Population size of Accra}} \text{ X District population size} \qquad \textbf{Equation 3-1}$$

The 2020 population projection estimate was used as a baseline for the determination of sample sizes for traders (wholesalers/retailers) in the respective markets. The intended sample size for the traders (wholesalers/retailers) was 500 but as a result of non-response from some of the retailers, a total response of 487 were gathered. Additionally, it was observed that the number of tigernut traders (retailers) on the streets (street hawkers) were fewer than market traders

(wholesalers/retailers) in the streets and markets selected. Therefore, in this study, a third of the sample was allocated to the various street traders (wholesalers/retailers) and the remaining two -thirds to the market retailers.

Table 3-3 represents the sample size allocation of retailer per district and per location in Greater Accra

Table 3-3: Sample size allocation of traders (wholesalers/retailers) in each district

District	Accra Population size (> 20 years)	District Population size (> 20 years)	Sample size allocation	Street sample size allocation	Market sample size allocation
Ada East	3049427	44686	7	2	5
Tema Metropolitan	3049427	225246	36	12	24
Ada West	3049427	34142	6	2	4
Ga East Municipality	3049427	111354	18	6	12
Ga South Municipality	3049427	433213	71	24	47
Ga West Municipality	3049427	149322	24	8	16
Ga Central Municipality	3049427	83175	14	5	9
Ashaiman Municipality	3049427	140437	23	8	15
Accra Metropolitan	3049427	1290774	212	71	141
Kpone Katamanso Municipality	3049427	77755	13	4	9
La Dade-Kotopon Municipality	3049427	141483	23	8	15
Adenta Municipality	3049427	59139	10	3	7
Ningo-Prampram Municipality	3049427	45449	7	2	5

Ledzokuku-Krowor Municipality	3049427	92864	15	5	10
Shai-Osudoku	3049427	33814	6	2	4
La Nkwantanang Municipality	3049427	86574	14	5	9

3.5.1.1 Inclusion Criteria for Traders (wholesalers/retailers)

Traders (wholesalers/retailers) were recruited for the study based on the following criteria:

- they sell or retail tigernuts in the various selected market centres and streets (Table 3-1 and Table 3-2),
- they have been in the business of retailing/wholesaling tigernuts for a year or more and
- they were 20 years or older.

3.5.2 Sampling and sample size determination for consumers

Consumers were sampled using a multi-staged stratified sampling procedure. The stratification variable that was used was districts, hence consumers within each district (stratum) were considered to have similar characteristics in terms of tigernut consumption. The same streets and markets selected for the retail survey were used for the consumer survey (Table 3-1 and Table 3-2).

A convenience sampling method was then used to obtain consumers for each street and market selected per district. A convenience sampling method was used because, there was no existing list of tigernut consumers (sampling frame) and consumers were mainly recruited on the basis that they were observed buying the tigernuts and they were 20 years or older. Consumers of tigernuts were classified into two main groups namely market shoppers and street shoppers. Market shoppers were consumers that bought tigernuts from the market sellers or traders

(wholesalers/retailers) directly, whiles the street shoppers were the consumers that purchased tigernuts from street hawkers.

A proportionate sample size determination criterion was used to obtain the number of consumers to be enumerated from the selected streets and markets in the respective districts. The reason for using the proportionate sample size determination criterion is that, from the pilot survey, it was observed that, the number of tigernut shoppers or consumers varied greatly across the streets and markets selected in the various districts. This could be attributed to the uneven distribution of the region's population across the 16 districts, therefore for each district, the sample size obtained for consumers or shoppers was based on the Equation 3-1.

The 2020 population projection estimate was used as a baseline for the determination of sample sizes for consumers or shoppers in the respective streets. The intended sample size for the consumers was 750 but as a result of non-response from some of the consumers, a total response of 711 responses were gathered. Additionally, it was observed that it was difficult obtaining the street shoppers for some of the streets selected, therefore the researcher attributed a third of the sample allocation for the various street shoppers and the remaining two -thirds to the market shoppers.

Table 3-4 represents the sample size allocation of consumers for each street and market per district in Greater Accra.

Table 3-4: Sample size allocation for each street and selected per district

District	Accra Population size (> 20years)	District Population size (> 20years)	Sample size allocation	Street sample size allocation	Market sample size allocation
Ada East	3049427	44686	11	4	7
Tema Metropolitan	3049427	225246	55	18	37
Ada West	3049427	34142	8	3	5
Ga East Municipality	3049427	111354	27	9	18
Ga South Municipality	3049427	433213	107	35	72
Ga West Municipality	3049427	149322	37	12	25
Ga Central Municipality	3049427	83175	20	7	13
Ashaiman Municipality	3049427	140437	35	10	25
Accra Metropolitan	3049427	1290774	317	46	271
Kpone Katamanso Municipality	3049427	77755	19	6	13
La Dade-Kotopon Municipality	3049427	141483	35	10	25
Adentan Municipality	3049427	59139	15	5	10
Ningo-Prampram Municipality	3049427	45449	11	4	7
Ledzokuku-Krowor Municipality	3049427	92864	23	7	16
Shai-Osudoku	3049427	33814	11	4	7
La Nkwantanang Municipality	3049427	86574	21	7	14

3.5.2.1 Inclusion Criteria for consumers

Consumers were recruited for the study based on the following criteria:

- they were observed buying the tigernuts in the selected streets and markets for each district,
- they confirmed being consumers of tigernut tuber and
- they were 20 years or older.

3.6 Ethical Clearance

Ethical clearance (ECBAS 062/19-20) was obtained from Ethics Committee of the College of Basic and Applied Sciences (CBAS) in University of Ghana. The provisions relating to ethics have been observed and respected.

3.7 Statistical analysis of survey data

All statistical analyses were done using IBM SPSS version 21 and Microsoft Excel (2016).

Descriptive statistics such as frequencies and cross tabulations were used to assess the level of mycotoxin knowledge of stakeholders of the tigernut value chain. Additionally, a logistic regression model was fitted to ascertain the correlation between the demographic characteristics and degree of stakeholders' knowledge of mycotoxin levels in the tigernuts.

3.8 Results and Discussion

3.8.1 Mapping out the Artisanal Tigernut Value chain

Figure 3.2 shows the value chain of tigernut as traced from the farmers in the Eastern and Western regions of Ghana.

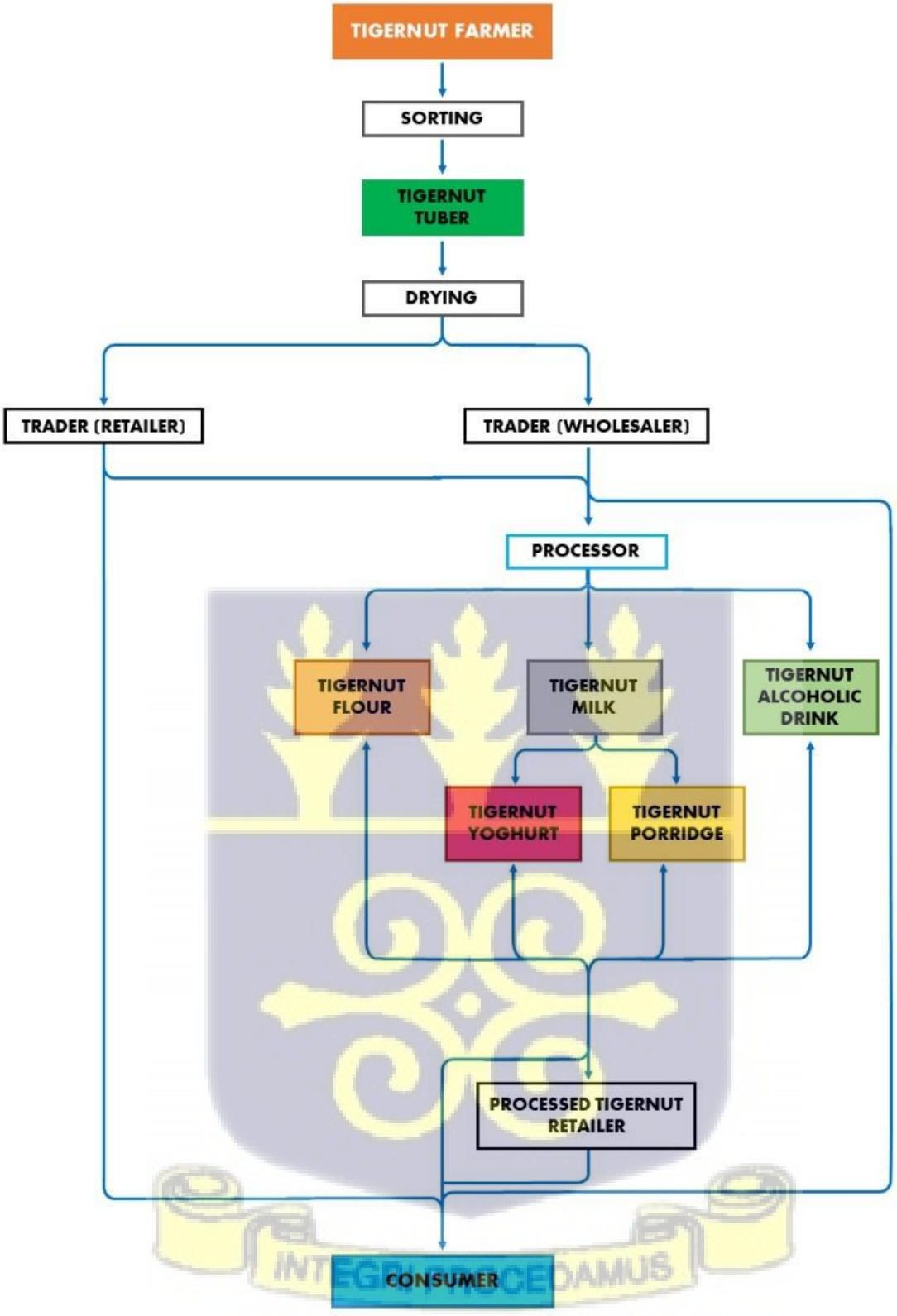


Figure 3.2: Artisanal Tigernut Value Chain

The value chain of the tigernut tubers began with the farmers, who own farms of sizes ranging from a quarter of an acre to half an acre. An acre of a tigernut farm yields 360 kg of tigernut tubers on a good harvest and 240kg of tigernut tubers on a poor harvest season. Tigernuts are sorted after harvest to separate the tubers from foreign bodies such as stones, sand and weeds. Drying of tigernut takes place on a raised platform and storage is done in jute bags in between drying times. Dried tigernuts are sold to traders who may either be wholesalers or retailers. Some retailers also purchase tigernuts from other wholesalers.

Consumers purchase tigernut tubers directly from wholesalers or retailers. Purchased tigernut tubers are either consumed raw or processed further on a small scale into tigernut flour or tigernut milk. The tigernut milk may further be processed into tigernut porridge/pudding or tigernut yoghurt. Processed tigernut products may be sold directly to consumers or to traders (wholesalers/retailers) who further sell the products to the final consumers.

This study was however limited to the artisanal value chain of the tigernut tuber only and excluded the processing stage of the value chain.

3.8.2 Demographic Characteristics of Tigernut stakeholders

Table 3-5 shows the demographic characteristics of respondents in the surveys conducted in this study. Farmers and traders (wholesalers/retailers) of tigernuts were predominantly females (65% being farmers and 95.9% being traders (retailers/wholesalers)). Similar reports were found by Tetteh (1998), who highlighted that 70% of tigernut farmers in the Kwahu South in Ghana were female due to the care and time required during harvesting.

Table 3-5: Demographic characteristics of tigernut stakeholders

Biodata	Categories	N STAKEHOLDER (%)		
		FARMERS (N=79)	TRADERS (N=487)	CONSUMERS (N=711)
Gender	Male	14 (17.7)	20 (4.1)	424(59.6)
	Female	65 (82.3)	467 (95.9)	287(40.4)
Marital status	Married	59(74.7)	237(48.7)	226(31.8)
	Single	1(1.3)	208 (42.7)	472(66.4)
	Divorced	7 (8.9)	19 (3.9)	9(1.3)
	Widowed	12 (15.2)	23 (4.7)	4(0.6)
Age categories	20 – 30	0	181(37.2)	164(23.1)
	31 – 50	35(44.3)	248(50.8)	335(47.1)
	51 – 60	31(39.2)	47(9.7)	112(15.8)
	>60	13(16.5)	11(2.3)	100(14.1)
Religion	Christianity	62(78.5)	456 (93.6)	648(91.1)
	Islam	12(15.2)	22 (4.5)	30(4.2)
	African Tradition	5(6.3)	9 (1.8)	33(4.6)
Educational Status	Elementary/Primary	44(55.7)	274 (56.3)	67(9.4)
	Junior High School	0	58 (11.9)	0
	None	32(40.5)	87 (17.9)	87(12.2)
	Secondary/Technical School/vocational	2(2.5)	68 (13.9)	450(63.3)
	Tertiary	1(1.3)	0	107(15.0)
How long have you been selling/farming/buying tigernuts	>10yrs	62(78.5)	29 (6.0)	703(98.9)
	1-5yrs	0	336 (69.0)	0
	6-10yrs	17(21.5)	122 (25.1)	8(1.1)
Do you belong to any tigernut trader's association	Yes	52(65.8)	0 (0)	NA
	No	27(34.2)	487 (100)	NA
Is farming of tigernuts your major occupation	No	0(0)	NA	NA
	Yes	79(100)	NA	NA
Is retailing of tigernut your only/major business	No	NA	103 (21.1)	NA
	Yes	NA	384 (78.9)	NA
Who are your major customers	Caterers	0	22 (4.5)	NA
	Other retailers	0	228 (46.8)	NA
	Individual Home Users	0	237 (48.7)	NA
	Wholesalers	79(100)	20 (4.1)	NA

It was however discovered in this study that majority of consumers (59.6%) of tigernuts were males. This outcome was expected as most of the media awareness on the benefits of tigernut has centred on its positive effect on the fertility of men (Yeboah, 2014), thereby attracting more male consumers. Majority of farmers and traders (wholesalers/retailers); 74.7% and 48.7% respectively, were married whilst majority of the consumers (66.4%) were single. Most of the stakeholders were between the ages of 31 and 50 (44.3% for farmers, 50.8% for traders and 47.1% for consumers). Tetteh and Ofori (1998), attributed the high percentage of the youth in tigernut production to its associated quick money-making benefit. Additionally, almost all the farmers, traders (wholesalers/retailers) and consumers were Christians and 6.3% of farmers, 1.8% of traders (wholesalers/retailers) and 4.6% of consumers were African Traditional believers. These religious affiliations of the stakeholders of the tigernut value chain are in line with the Office of International Religious Freedom, (2019) that noted that 71%, 18% and 5% of Ghanaians are Christians, Muslims and African traditional believers respectively. As many as 40.5% of farmers had no formal education, whilst 17.9% of traders (wholesalers/retailers) and 12.2% of consumers had no formal education. The illiteracy rate of farmers as highlighted in this study may suggest that the trend of illiteracy among Ghanaian farmers may not have improved drastically over the last decade because the Ghana Statistical Service, (2013) revealed that 44% of Ghanaian farmers were illiterates. Iftikhar et al. (2015) posited that the growth of the agricultural sector is highly dependent on the literacy rate of the farmers since most modern and more effective and efficient methods require reading and comprehension in order to apply.

In total, 8.5% of farmers had been farming tigernut for more than 10 years, 6.0% of traders (wholesalers/retailers) had been trading in tigernuts for more than 10 years whilst almost all the consumers (98.6%) had been consuming tigernuts for more than 10 years. More than half (65.8%) of farmers belonged to a tigernut related association (*Ensonyameye* Farmers

Association), whilst all the tigernut traders (wholesalers/retailers) did not belong to any tigernut-related association. According to Acheampong et al. (2017), associations are one of the most reliable sources of information for farmers and other stakeholders in the agricultural sector. In the absence of associations/groups, these stakeholders rely on each other, which may not result in the rapid rate of improvement in the sector as desired. The government of Ghana may need to organise tigernut farmers into groups and educate them on the right practices, considering that some of them are illiterates.

All the farmers (100%) indicated that farming tigernuts was their only occupation. According to Dittoh (2010), farming is the main source of livelihood for most small-scale farmers in Ghana. Also, 78.9% of traders (wholesalers/retailers) indicated that retailing tigernuts was their major occupation. All the farmers (100%) indicated that wholesalers of tigernuts were their major customers whilst the major customers of the traders (wholesalers/retailers) were other traders (wholesalers/retailers) and individual home users with corresponding percentages of 46.8 and 48.7 respectively.

3.8.3 Raw Material Supply for Tigernut Farmers

All the farmers indicated that they sourced tigernuts from other farmers. Additionally, 65.8% of the farmers sourced their tigernuts from *Atakora* and *Offinso*, whereas 34.2% sourced their tigernuts from *Bewjiase* (Figure 3.3). When the farmers were asked the reason why they sourced their tigernut seeds from these towns, they all (100%) indicated that these towns were reliable sources of tigernuts. Poku et al. (2018) posited that, lack of access to quality seeds has been a hinderance to the growth of the agricultural sector in sub-Saharan Africa as most farmers depend on informal access to quality seeds.

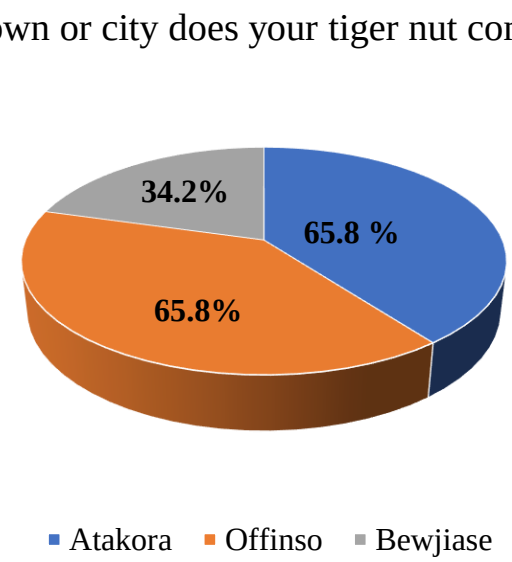


Figure 3.3: Source of tigernut seeds for tigernut cultivation

3.8.4 Raw Material Supply of Traders (retailers/wholesalers)

Table 3-6 shows the responses to questions related to the tigernut supply of traders (retailers/wholesalers). Approximately half of the traders (wholesalers/retailers) (46.0%) indicated that they sourced their tigernuts directly from farmers, 37.8% sourced theirs from wholesalers whiles 16.2% sourced their tigernuts from other retailers. Most traders (65.7%) purchased their tigernuts monthly, 11.1% purchased tigernuts weekly whilst 23.2% purchased their tigernuts every fortnight.

All the traders (wholesalers/retailers) assessed the quality of their tigernuts by using colour, absence of insect's activity, texture and buying tigernuts from the same supplier. However, 74.1% also indicated that they check for the presence of moulds to ascertain the quality of their tigernuts. This suggests that the traders may know that mouldy tigernuts are unhealthy. However, none of the traders (wholesalers/retailers) have ever rejected tigernuts supplied to

them. Reasons for this observation may be that, either all the tigernuts supplied to traders have been mould free or that the farmers practiced a no-return policy.

Table 3-6: Responses to questions on raw material supply by traders (retailers/wholesalers)

Question	Categories	Frequency	Percentage
Where do you source your tigernuts from	Another retailer	79	16.2
	Directly from farmer	224	46.0
	Wholesaler	184	37.8
How often do you purchase tigernuts	Every fortnight	113	23.2
	Monthly	320	65.7
	Weekly	54	11.1
How do you assess raw material quality	Colour	487	100.0
	Buying from same supplier	487	100.0
	Absence of insect's activity	487	100.0
	Texture	487	100.0
	Absence of moulds	361	74.1
Have you rejected supplied tigernuts	No	487	100.0
	Yes	0	0.0
What is the approximate quantity you buy	>20kg	305	62.6
	11-15kg	10	2.1
	1-5kg	31	6.4
	16-20kg	41	8.4
	6-10kg	100	20.5
In which form do you purchase your tigernuts	Dried	238	48.9
	Semi-dried	249	51.1
Which variety of tigernut do you sell	Both (Yellowish Brown and black)	351	72.1
	Yellowish brown	136	27.9
Why do you sell this/these variety	Most demanded	106	21.8
	Ease of supply	5	1.0

A total of 72.1% of the traders (wholesalers/retailers) indicated that they sell both black and yellowish-brown varieties of tigernuts whiles 27.9% of the traders (wholesalers/retailers) sell only the yellowish-brown variety, mainly due to demand. Majority of the traders (wholesalers/retailers) (62.6%) purchase more than 20kg per batch of supply, 2.1% purchase between 11-15kg, 6.4% purchase between 1-5kg and 20.5% purchase tigernuts quantity between 6-10kg. About half (51.15%) of the traders (wholesalers/retailers) preferred to purchase semi-dried tigernuts whiles 48.9% preferred dried tigernuts. These quantities are small and may be related to the small yield of the farmers and/or low demand from consumers. In any case, both possible reasons may point towards the fact that the production of tigernut is underdeveloped in Ghana and its use in Ghanaian food applications is limited.

3.8.5 Raw material supply and Knowledge on tigernut products by consumers

Table 3-7 shows the responses to questions related to tigernut supply to consumers.

Most (63.4%) of the consumers enumerated mentioned that, they purchase tigernuts from street hawkers and 36.6% indicated that they purchase tigernuts from open markets. Limited industrialisation of tigernut tubers may be a contributory factor to the absence of tigernuts in modern trade (supermarkets). Furthermore, approximately half of the consumers eat raw tigernuts and/or tigernut products occasionally, 24.65% eat raw tigernut and/or tigernut products once a week whiles 23.6% eat raw tigernuts and/or tigernut products more than once a week. Almost all the consumers (90.0%) enumerated preferred to purchase dried tigernuts whiles 9.6% preferred overly dried tigernuts. All the consumers knew of raw tigernut tuber, tigernut porridge, tigernut alcoholic drink and tigernut flour. However, only 55.9% of consumers had knowledge about the existence of tigernut oil.

Table 3-7: Responses to questions on raw material supply by consumers

Question	Categories	Frequency	Percentage
Where do you purchase your tigernuts from?	Open market	260	36.6
	Street hawkers	451	63.4
How often do you eat tigernut/ tiger product	More than once a week	168	23.6
	Occasionally	368	51.8
	Once a week	175	24.6
In which form do you purchase your tigernuts	Dried	640	90.0
	Overly dried	68	9.6
Check from the list below all the tigernut products you know	Raw tigernut tuber	711	100.0
	Tigernut porridge	711	100.0
	Tigernut oil	398	55.9
	Tigernut flour	648	91.1
	Tigernut alcoholic drink	711	100.0
Are the tigernut products easily accessible	Yes	0	0
	No	711	100.0
Which product would you want to see more on the market	Tigernut flour	245	34.5
	Tigernut milk	159	22.4
	Tigernut oil	278	39.1
	Tigernut porridge	29	4.1
Are you willing to try new tigernut products?	No	14	2.0
	Yes	697	98.0

All the consumers responded in the negative when they were asked if tigernut products are easily accessible. More than a third (39.1%) of the consumers would like to see tigernut oil more often in the market centres, 22.4% would like to see tigernut milk more often in the market centres and 34.5% of consumers prefer seeing tigernut flour in the market centres more often. Almost all the consumers (98.0%) were willing to try new tigernut products. This may suggest that there is a ready market for industrialised tigernut products.

3.8.6 Food safety practices of tigernut farmers

Table 3-8 shows tigernut safety practices by farmers.

Table 3-8: Tigernut safety practices of farmers

Question	Categories	N (%)
Do you intercrop tigernuts with other crops	Yes	79(100.0)
Which crops do you intercrop with tigernut	Maize	79(100.0)
	Cassava	79(100.0)
	Plantain	79(100.0)
When is the major harvest period	July - August	79(100.0)
Is this period considered early harvest/ late harvest?	Early harvest	79(100.0)
Why is this harvest time chosen to harvest tigernuts?	Dry season period	79(100.0)
Is there any form of sorting/grading done after harvesting?	Yes	79(100.0)
What criteria is used for sorting/grading (what do you look out for to certify good/bad tigernuts)?	Presence or absence of moulds	79(100.0)
	Presence/absence of insect/disease activity	79(100.0)
	Broken/Whole tubers	79(100.0)
What do you do with bad tigernuts	Throw away on farm	28(35)
	Throw away in rubbish dump	34(43)
	Use at home	7(9)
	Feed to farm animals	10(13)
	Sell at reduced price	0 (0)
What drying method do you use	Sun drying	79(100.0)
Do you have knowledge of any other drying method?	No	79(100.0)
How do you store harvested tigernuts?	Jute bags	79(100.0)
In what form do you prefer to store tigernuts?	Semi - dried	79(100.0)
Do you encounter any losses during storage?	Yes	79(100.0)
What method is used to protect the tigernuts during storage?	Storage in cool, dry area	79(100.0)
How long do you store them after drying?	1 week	79(100.0)
In what packaging do you prefer to store tigernuts?	Jute sacks	79(100.0)

All farmers (100%) indicated that tigernut was usually intercropped with other crops such as cassava, maize and plantain. These crops have been identified as mycotoxin susceptible crops (Abass, 2017) and therefore intercropping them with tigernut may further increase the risk of

fungal colonisation of tigernut farms. All the farmers indicated that the major harvest period was between July and August. This period is considered by all the farmers as early harvest and the basic reason for harvesting during these months is because it is the end of the wet season and ideal for the next stage after harvesting, which is the drying of tigernut. Nakole and Adebanjo (2002), posited that early harvest is an effective way of minimising the risk of the tubers' exposure to fungal infection. He however noted that harvesting at inappropriate times is normally due to lack of resources (labour, money) and unpredictable weather.

Additionally, all the farmers indicated that sorting of the tigernuts is done after harvesting with presence or absence of moulds; presence or absence of insect or disease activity; and broken or whole tubers as the sorting criteria. About a third (35%) of farmers dispose of their bad (mouldy, insect infested, diseased, broken) tigernuts on the farm as (manure) whilst 43% dispose of the bad tigernuts in the rubbish dump. About 9% further process the bad tigernuts for home consumption whilst 13% of farmers feed their farm animals with bad tigernuts. However, bad tigernuts are not sold. Improper disposal of bad/unwholesome tigernut on the farm introduces the fungi back onto the fields and predisposes the crops on the farm to fungal colonisation. Secondly, feeding farm animals with unwholesome tigernut including mouldy tigernut exposes the animals as well as human to the harmful health effects of mycotoxins. Additionally, nutritional value of meat from farm animals fed on mouldy tigernut have less nutritive value (Zain, 2011).

Sun drying is the only drying method used by the farmers in drying their tigernuts because it is the only drying method they know. Unavailability of other drying methods could be a problem because in situations when sun drying is difficult (rainy season), harvested tigernuts would not be adequately dried and subsequently would be expose to fungal infection (Bankole and Adebanjo, 2003). Drying is done for a week by majority of the farmer (79%). All the farmers were not familiar with any other alternative method of getting their tigernuts dried. Harvested

tigernuts are kept in jute bags as indicated by all the farmers interviewed. All the farmers preferred to store tigernuts in a semi-dried form in a cool, dry area to protect the tigernuts from spoilage. This notwithstanding, they encountered losses during storage due to insect infestation and mould growth. This outcome is in line with a report by Kumari et al. (2017), in which after storing grains in different packaging, observed that most spoilage (higher water activity, most insect infested, variation in grain content and highest aflatoxin content) was experienced in the bags made up of jute.

Most farmers (86%) grew both tigernut varieties whilst 6% and 8% grew only the black and yellowish-brown varieties respectively (Figure 3.4).

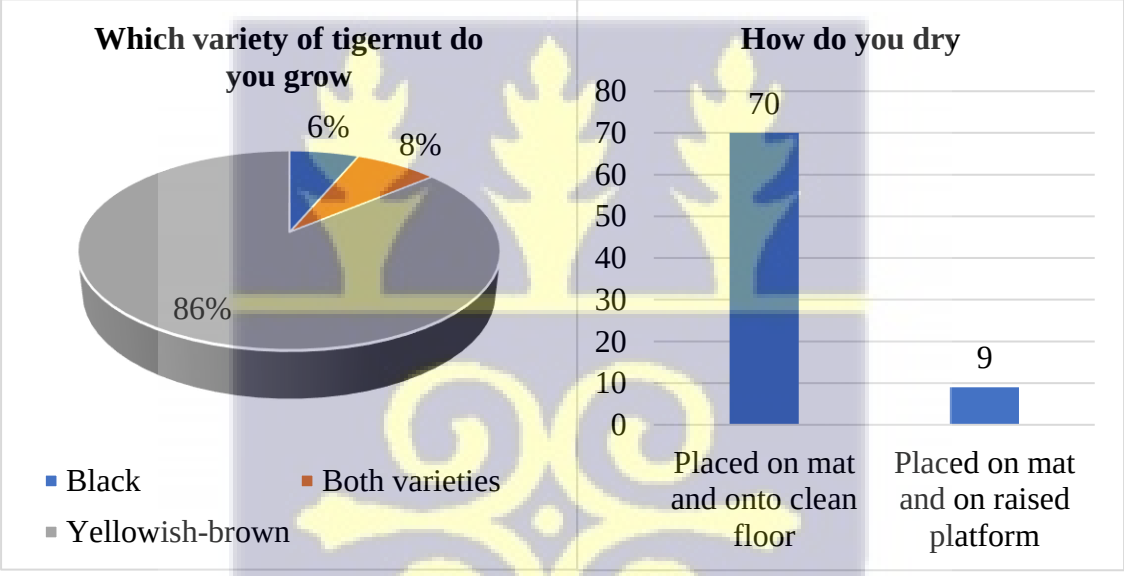


Figure 3.4: Cultivated tigernut varieties and methods of tigernut storage by farmers

In all, 70 of the sampled farmers dried tigernuts by placing the tigernuts on a mat only whilst the remainder of the farmers placed the mats on a raised surface (Figure 3.4). Drying of tigernut on the mat close to the bare ground exposes the tigernuts to insect, rodents and other foreign body which would subsequently lead to fungal infestation.

It was also observed that whilst 56% of the sampled farmers used chemicals in preserving harvested tigernuts, 43% of farmers did not use preservatives and only 1% of farmers used a liquid called poison to preserve tigernuts (Figure 3.5). The official name of the chemical termed poison could not be verified due to the unavailability of the chemical at the time of the survey.

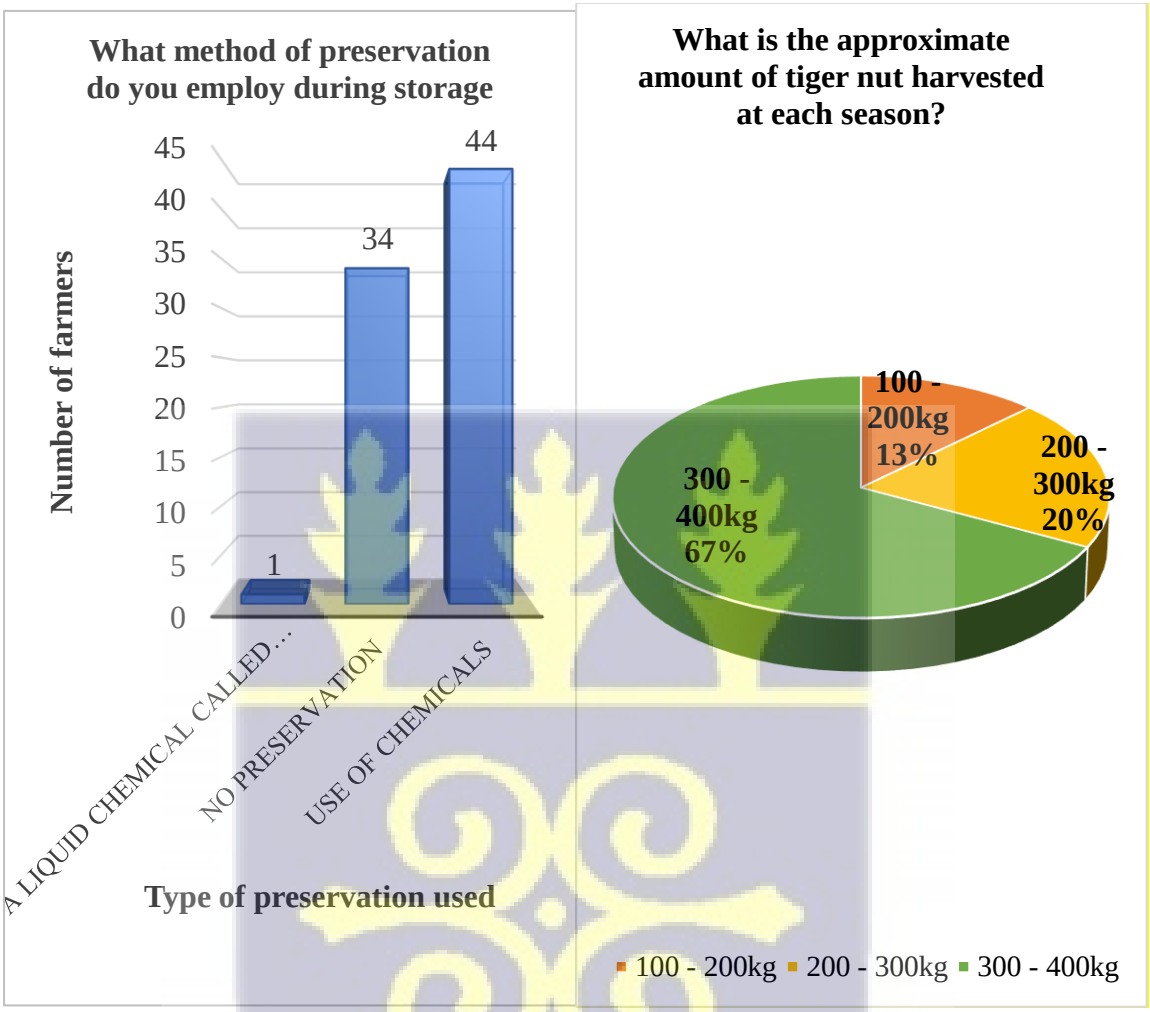


Figure 3.5: Methods of tigernut preservation and yield of tigernut

The chemical, used as an insect or rodent repellent may also alter the composition of the tigernut tuber and in some cases expose consumers to health risks. More than half of the farmers (67%) indicated that, they harvest between 300kg and 400kg of tigernuts per season, 20% harvest between 200kg and 300kg whiles 13% of the farmers harvest between 100kg and 200kg

of tigernuts per season (Figure 3.5). Nin-Pratt and Mcbride (2014) posited that in Ghana most of the increase in agricultural yield is due to increase in number of small farms and not improved processes. The low yield of tigernut farms may be attributed to agronomic reasons such as soil quality, rainfall patterns, planting seed quality and viability.

3.8.7 Food safety practices by Tigernut Traders

Table 3-9 presents the questions asked to tigernut traders, to assess the safety practices they employ, and the responses received.

One-third (33.90%) of tigernut traders (wholesalers/retailers) displayed their tiger nuts in tied polythene bags, 36.60% displayed them in big open polythene bags, 29.0% displayed tigernuts in open baskets and 0.6% displayed them in closed baskets. Packaging and other handling practices of the tigernut prior to sale also affects the susceptibility of the tubers to the fungal growth. Polythene for instance increases the humidity within the packaging, which would be conducive for fungal growth. Displaying heaps of tigernut in basins or baskets without occasionally turning the heap to improve aeration of the tubers would also increase the humidity at the bottom of the basin, thereby increasing its susceptibility to fungal growth (Maduka & Ire, 2019). Majority of the traders (wholesalers/retailers) (81.10%) sun dried tigernuts in baskets in between sales, 14.0% sun dried whilst spread out and 4.90% sun dried their tigernuts on the bare floor. Crops which are not subjected to field and ground drying are less prone to mould growth and insect damages (Omari et al., 2020). More than half of the traders (wholesalers/retailers) (65.7%) determined the end of the drying period by the hard texture of the tigernuts, 24.6% by its sweet taste and 9.7% by its shrivelled tubers.

Table 3-9: Tigernut safety practices of tigernut traders

Question	Categories	N (%)
How do you display your tigernut	In a tied polythene	165(33.90)
	In a big open polythene bag	178(36.60)
	In an open basket	141(29.0)
	In a closed basket	3(0.6)
Method of drying tigernuts in between sales	Sun drying in basket	395(81.10)
	Sun drying on bare floor	24(4.90)
	Sun drying whilst spread out	68(14.0)
If you dry your tigernuts, how do you determine the end of the drying period	Hard texture	320(65.7)
	Shrivelled tubers	47(9.7)
	Sweet taste	120(24.6)
How long does it take to completely sell out a batch	< 1 month	135(27.7)
	>1 month	319(65.5)
	<2 weeks	4(0.8)
	2-3 weeks	29(6.0)
Where do you store tiger nuts during this period	At home	168(34.5)
	Container in the market	151(31.0)
	Market store	168(34.5)
What method of preservation do you employ during storage	No preservation	484(99.4)
	Use of chemicals	3(0.6)
Is there any form of sorting/grading done after harvesting?	Yes	487(100.0)
What criteria is used for sorting/grading (what do you look out for to certify good/bad tigernuts)?	Presence or absence of moulds	487(100.0)
	Presence/absence of insect/disease activity	487(100.0)
	Broken/Whole tubers	487(100.0)
What do you mostly do to bad tigernuts?	Throw away in rubbish dump	73(15)
	Use at home	19(4)
	Feed to animals	248(51)
	Sell at reduced price	147 (30)
What do shoppers say they use bad tigernuts for?	Use as animal feed	60(41)
	Process into other products	87(59)
Which deterioration observation do you encounter most?	Breakages	1(0.2)
	Fungal growth	419(86.0)
	Rodent / insect infestation	67(13.8)
How many of your tigernuts get deteriorated per each batch of tiger nut purchased	Less than ¼	319(65.5)
	¼ - ½	140 (28.8)
	>½	28(5.7)

Approximately 65% of traders (wholesalers/retailers) took more than one month to completely sell out a batch of tigernut, 27.7% took less than a month, 6.0% took between 2-3 weeks and

0.8% took less than 2 weeks. 34.5% of the traders (wholesalers/retailers) indicated that they store their tigernuts at home and in market stores whiles 31.0% store tigernuts in a container in the market. All traders indicated that they sort their tigernuts before sale using the presence/absence of moulds, presence/absence of insect of disease activity and presence/absence of broken tubers as the sorting criteria. In dealing with unwholesome tigernuts, 15% of traders threw tigernuts in the rubbish dump, which failed the wholesome tigernut criteria, whilst 4% used the unwholesome/bad tigernut at home. Approximately, 51% fed the bad tigernuts to their animals and a further 30% of traders sold the bad tigernuts to willing customers. Traders indicated that 41% of the customers who buy the bad tigernuts use them as animal feed whilst 59% process the bad tigernuts together with wholesome tigernuts into other products such as alcoholic drinks, flour, pudding, and yoghurt. They further indicated that these products normally have added sugar which masks any unwanted taste caused by the unwholesome tigernuts. Lack of regularisation is one major causes for the practice of selling the unwholesome tigernuts. Regulatory bodies would need to intensify their surveillance to deter traders from indulging in such practice.

Almost all the traders (wholesalers/retailers) (99.4%) do not add any preservatives to tigernuts during storage. However, 0.6% use chemicals in preserving their tigernuts during storage. Most of the traders (wholesalers/retailers) (86.0%) indicated that they observe fungal growth on their tigernuts most often, 13.8% indicated that they experience rodent and insect infestation on their tigernuts during storage whiles 0.2% experience breakages. Majority (65.5%) of the traders (wholesalers/retailers) indicated that less than a quarter of their tigernuts gets deteriorated per batch of purchase, whilst 28.8% experience deterioration of between a quarter and half of a batch of tigernuts. However, 5.7% of traders experienced deterioration of more than half of their tigernuts. Inappropriate storage conditions (high moisture content, unclean storage areas

and exposure to pests) is a major cause of the fungal growth and deterioration rate of the tigernut tubers (Maduka & Ire, 2019).

3.8.8 Food safety practices by tigernut consumers

Table 3-10 represents tigernut safety practices employed by the consumers that were enumerated.

Table 3-10: Tigernut safety practices of consumers

Questions	Categories	Frequencies	Percentage
Do you buy unwholesome tigernuts on purpose (presence of mould, insect infestation, diseased)?	No	711	100
Do you do anything to tigernuts before and in between consumption	Wash with salty water	307	43.2
	Wash with water	323	45.4
Where do you store tigernuts before use	Fridge	262	36.8
	Kitchen	180	25.3
	Yard	227	31.9
In what do you prefer to store tigernuts	Aluminium basin	139	19.5
	Basket	118	16.6
	Polyethene bag	412	57.9
Which parameters are used in grading purchased tigernuts	Presence of moulds	212	29.8
	Presence of insect infestation	212	29.8
Which deterioration do you encounter most	Fungal growth	669	94.1
How many tigernuts gets deteriorated per each batch of tigernut supply	Less than 1/4	597	84.0
	About 1/2	72	10.1
What is the peak period of deterioration after purchase	Within 2 weeks	669	94.1

All consumers interviewed in this study indicated that they do not buy wholesome tigernuts on purpose. When the respondents were asked if they do anything to the tigernuts before and between consumption, 43.2% indicated that they wash with salty water whiles 45.4% indicated

that they wash with water only. According to Omoniyi et al (2014), washing tigernuts with distilled water and orange juice reduces Aflatoxin B2 and G1; and Aflatoxins B1 and G2 respectively. About a third (36.8%) of the consumers stored purchased tigernuts in the fridge, 25.3% stored it in the kitchen and 31.9% stored it in their yards. The cold storage of tigernut in a refrigerator helps to prevent spoilage and reduces mycotoxin contamination (Maduka & Ire, 2019).

More than half of the consumers (57.9%) preferred to store their tigernuts in polyethene bags, 19.5% of consumers stored them in aluminium basin whilst 16.6% of consumers stored tigernuts in baskets. Less than a third (29.8%) of consumers used presence of moulds and insect infestation as parameters for grading purchased tigernuts. Insect activities influence the extent of mycotoxin contamination (Wagacha & Muthomi, 2008). This is because insects are carriers of spores of mycotoxin-producing fungi and as such a proper management would be an effective control.

Almost all the consumers (94.1%) experienced fungal growth on their tigernuts most often. A great majority (84.0%) of the consumers indicated that less than 1/4 of the tigernuts get deteriorated per each batch of supply whiles 10.1% indicated that about 1/2 of the tigernuts get deteriorated per each batch of supply. In addition, approximately 94.1% of the consumers indicated that the peak period of deterioration after purchase was within 2 weeks of purchase.

3.8.9 Mycotoxin Awareness of tigernut stakeholders

To assess stakeholder's knowledge of mycotoxin, questions were asked, and responses were scored based on if the correct answer was received or not. Correct answers were awarded a mark and marks were not awarded to wrong responses.

Table 3-11 highlights the number of correct and wrong answers received for the questions asked.

Table 3-11: Mycotoxin Awareness of tigernut stakeholders

Question	% Stakeholders who gave wrong or correct responses					
	Farmers (79)		Traders (487)		Consumers (711)	
	Correct	Wrong	Correct	Wrong	Correct	Wrong
Do you have any idea on effect of mouldy tigernuts on human or animal health	0	79(100)	15 (3.1)	472 (96.9)	711 (100.0)	0
What is your opinion on what causes spoilage	79 (100)	0	487 (100)	0	711 (100.0)	0
Toxins in tigernuts due to moulds can completely be destroyed by cooking	60 (75.9)	19 (24.1)	0	487 (100)	321 (45.1)	390 (54.9)
Toxins in tigernuts due to moulds growth can be completely destroyed through washing	0	79 (100)	406 (83.4)	81 (16.6)	367 (51.6)	344 (48.4)
Do you have an idea on what measures to take to control moulds and fungi in storage	79 (100)	0	57 (11.7)	430 (88.3)	118 (16.6)	593 (83.4)
Do you sell Mouldy tigernuts	79 (100)	0			NA	NA
Would you buy mouldy tigernut	NA	NA	NA	NA	8 (1.1)	703 (98.9)
Average scores	3.2405 ± 0.43012		1.1478 ± 0.38317		2.9044±1.52975	

All the farmers enumerated responded in the negative when asked if they had an idea of the effect of mouldy tigernuts on human or animal health. However, 100% and 3.1% of consumers and traders (wholesalers/retailers) respectively responded in the affirmative.

This is not surprising since most of the farmers were illiterates and did not have any formal way of receiving such information. Also, all stakeholders (farmers, traders (wholesalers/retailers) and consumers) in the tigernut value chain responded correctly when asked their opinion on the causes of tigernut spoilage. In addition, 75.9% and 45.1% of the farmers and consumers were right to indicate that toxins in tigernuts due to moulds cannot be destroyed by cooking. According to Ryu et al., (2008), destruction of mycotoxins under normal cooking temperature (100° C to 210° C) and period (up to 1 hour) is not possible. However, all traders (wholesalers/retailers) (100%) had an opposite view to this assertion.

In contrast, 100% and 48.4% of farmers and traders (wholesalers/retailers) respectively were wrong to indicate that toxins in tigernuts due to moulds growth can be destroyed through washing whiles majority (83.4%) of the consumers indicated otherwise. All farmers (100%) had ideas on how to control moulds and fungi in storage. However, a substantial percentage of traders (wholesalers/retailers) and consumers, 88.3% and 83.4% respectively, had no such ideas on the measures to take. Almost all (98.9%) of the consumers indicated that they would purchase mouldy tigernuts at reduced price, which corresponds to a wrong answer whiles 1.1% indicated otherwise.

Generally, the average mean score of knowledge of mycotoxins for stakeholders in the tigernut value chain was not impressive, however, farmers had an overall mean score greater than that of traders (wholesalers/retailers) and consumers who scored 3.2405 ± 0.43012, 1.1478 ± 0.38317 and 2.9044±1.52975 for farmers, traders (wholesalers/retailers) and consumers respectively.

Figure 3.6 illustrates the percentage of farmers, traders (wholesalers/retailers) and consumers with sufficient (adequate) and insufficient (inadequate) knowledge of mycotoxins.

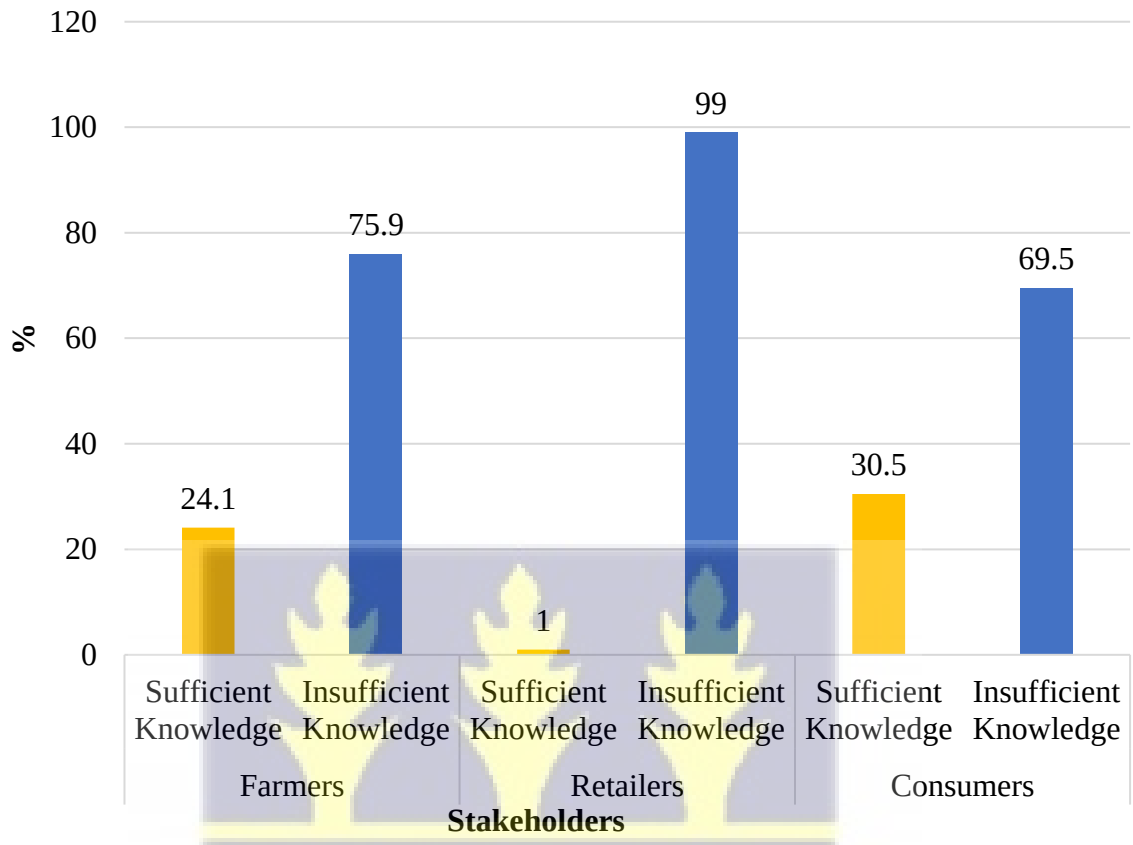


Figure 3.6: Level of Mycotoxin knowledge of farmers, traders (wholesalers/retailers) of consumers

After the scoring was done, farmers, traders (wholesalers/retailers) and consumers with a score less than 60% were considered to have insufficient (inadequate) knowledge whereas a score of 60% and above was considered as sufficient (adequate) knowledge. Amongst the stakeholders of the tigernut value chain, almost all the traders (99%) were considered as having inadequate knowledge on mycotoxins. Stakeholders with adequate knowledge on mycotoxins were the consumers. Since mycotoxin contamination is a cumulative process, the higher knowledge of

the consumers on mycotoxins may not necessarily prevent the contamination of the tuber prior to reaching their stage. However, consumers may refuse to consume mouldy tigernuts based on their knowledge on its detrimental health effects.

3.8.10 Association between demographic characteristics and knowledge categories

To assess the correlation between mycotoxin knowledge categories and demographic characteristics of stakeholders in the tigernut value chain, a Pearson Chi-square test was used (Table 3-12).

Table 3-12: Association between demographic characteristics and period of trading in tigernut

Biodata	Farmers		Traders		Consumers	
	Chi-square value	P-value	Chi-square value	P-value	Chi-square value	P-value
Sex	3.295	0.070	0.216	0.642	2.536	0.111
Educational status	2.170	0.538	20.260	0.000	65.781	0.000
Marital status	4.247	0.236	2.065	0.559	3.623	0.305
How long have you been selling tigernuts	1.499	0.221	0.811	0.667	3.554	0.059

From Table 3-12, there was no significant association between mycotoxin knowledge categories and demographic characteristics of farmers, traders (wholesalers/retailers) and consumers. However, it was observed that there was a significant association between educational status and knowledge categories of traders (wholesalers/retailers) (p-value <0.05) and a significant association was observed between educational status and mycotoxin knowledge categories for consumers. Hence it was concluded that educational status and knowledge categories are related with regards to traders (wholesalers/retailers) and consumers,

and religion and mycotoxin knowledge are also related with regards to consumers. Bankole and Adebanjo (2003) posited that information on mycotoxin is popular among researchers and the educated. They therefore suggested mass education of the general public on the harmful effects of mycotoxin as well as its prevention across the value chain.

3.8.11 Attitude of tigernut stakeholders

Table 3-13 represent stakeholders' responses to questions asked to assess their attitudes towards tigernuts handling and processing.

From the Table 3-13, all farmers (100%) responded correctly to all questions. However, 100% and 83.1% of traders (wholesalers/retailers) and consumers respectively indicated that tigernuts exposed to insects and rodents are not prone to contamination by fungi. Approximately half of traders (wholesalers/retailers) (49.9%) were wrong to support the view that tigernuts kept in cool dry place has a shorter lifespan than in hot humid condition. In addition, 36.3% of the traders (wholesalers/retailers) indicated that basins or baskets used to display tigernuts should not be washed and dried to avoid contamination, however this is a wrong option. Hell et al. (2000) postulated that cleaning of storage areas prior to storage of different batches is linked to decreasing mycotoxins concentrations.

Table 3-13: Summary of attitude of tigernut farmers, traders (wholesalers/retailers) and consumers

Question	% Stakeholders who gave wrong or correct responses					
	Farmers (79)		Traders (wholesalers/retailers) (487)		Consumers (711)	
	Correct	Wrong	Correct	Wrong	Correct	Wrong
I have a responsibility to ensure that tigernuts sold are safe and of good quality	79 (100)	0	487 (100)	0	711 (100)	0
Tigernuts exposed to insects and rodents are prone to contamination by fungi	79 (100)	0	0	487 (100)	120 (16.9)	591 (83.1)
Tigernuts kept in cool dry storage facilities keeps longer than hot humid areas.	79 (100)	0	244 (50.1)	243 (49.9)	711 (100)	0
Infected tigernuts must be sorted from wholesome ones frequently	79 (100)	0	487 (100)	0	711 (100)	0
Tigernuts stored in polythene bags deteriorates faster	79 (100)	0	487- (100)	0	711 (100)	0
Basins or baskets used to display tigernuts should be washed and dried to avoid contamination	79 (100)	0	310 (63.7)	177 (36.3)	711 (100)	0
Average scores	6.0 ± 0.0		4.1971 ± .83528		5.1688 ± 0.37482	

Figure 3.7 shows the percentage of stakeholders of tigernuts with appropriate (sufficient) and inappropriate (insufficient) attitude towards tigernuts handling and processing.

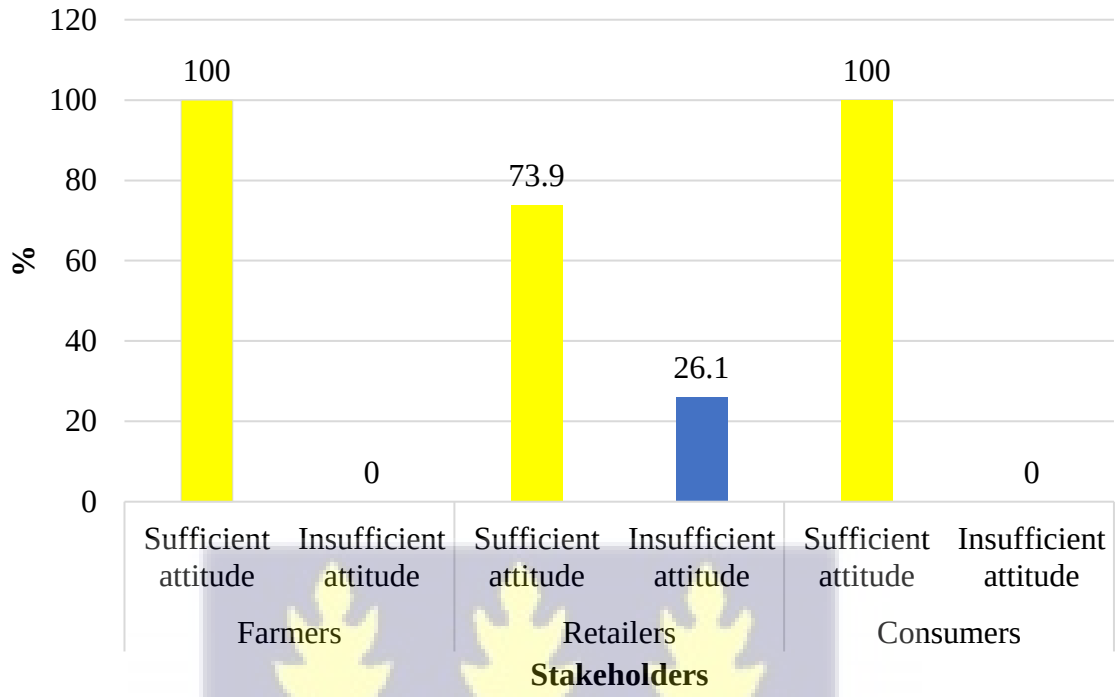


Figure 3.7: Attitude of Tigernut farmers, traders (wholesalers/retailers) and consumers in relation to mycotoxin contamination

After the scoring was done, farmers, traders (wholesalers/retailers) and consumers with a score less than 60% were considered to have inappropriate (insufficient) attitude whereas a score of 60% and above was considered as appropriate (sufficient) attitude. All stakeholders except traders demonstrated appropriate attitude towards the prevention of mycotoxins in tigernut. It may suggest that focus must be given to traders in mycotoxin prevention programs. This is because traders are the intermediaries between farmers and consumers and therefore their actions would influence the mycotoxin levels greatly before it reaches the final consumers.

3.8.12 Association between demographic characteristics and attitude categories

To determine the association between attitude categories and demographic characteristics of traders (wholesalers/retailers), a Pearson Chi-square test was used (Table 3-14).

Table 3-14: Association between demographic characteristic and period of trading in tigernuts with respect to attitude

Biodata	Traders	
	Chi-square value	P-value
Sex	0.166	0.683
Educational status	14.798	0.005
Marital status	4.374	0.224
How long have you been selling tigernuts	36.386	0.000

From Table 3-14, there was no significant association between attitude categories and sex and marital status of traders (wholesalers/retailers). However, it was observed that there was a significant association between educational status, religion and how long the respondents had been selling tigernuts as well as attitude categories of traders (wholesalers/retailers) (p-value <0.05). Hence it can be concluded that, educational status and how long the respondents have been selling tigernuts as well as attitude categories are related with regards to traders (wholesalers/retailers). From this trend, we can deduce that apart from education that may improve the knowledge of stakeholders as well as influence their actions, lessons learned during a longer time in a particular business (such as causes of losses incurred) may also influence the attitude of stakeholders positively.

3.8.13 Logistic Regression of Knowledge of tigernut farmers on mycotoxins

A binary logistic model was used to determine the knowledge categories of farmers using the demographic characteristics as covariates. The gender, educational levels, marital status, longevity in the tigernut cultivation and age of farmers were used to predict the probability that a farmer within the tigernut value chain would have sufficient (adequate) knowledge or not. The results of the binary logistic regression are indicated in Table 3-15.

Table 3-15: Logistic regression analysis of effects of demographics on the mycotoxin knowledge of farmers

Effect	df	Wald	Sig.
Sex (Male)	1	2.011	0.156
Education	3	0.078	0.994
Education (Elementary/Primary)	1	0.000	0.999
Education (Junior high)	1	0.000	0.999
Education (None)	1	0.000	1.000
Marital	3	2.016	0.569
Marital (Married)	1	0.007	0.936
Marital (Single)	1	1.374	0.241
Marital (Divorced)	1	0.000	1.000
How long selling tigernuts (More than 10 years)	1	2.332	0.127
Age	1	0.00	0.989

From Table 3-15, these demographic characteristics had no significant contribution in predicting the probabilities of the farmers having sufficient levels of knowledge, since p-value > 0.05 for all the covariates in the model.

3.8.14 Logistic Regression of Knowledge of Traders (wholesalers/retailers) on mycotoxins

A binary logistic model was used to determine the knowledge categories of traders (wholesalers/retailers) using the demographic characteristics as covariates (Table 3-16).

The gender, educational levels, marital status, longevity in the tigernut cultivation and age of traders (wholesalers/retailers) were used to predict the probability that a trader (wholesaler/retailer) within the tigernut value chain would have sufficient (adequate) knowledge or not.

Table 3-16: Logistic regression analysis of effects of demographics on the mycotoxin knowledge of Traders (wholesalers/retailers)

Effect	df	Wald	Sig.
Age	1	0.163	0.687
Sex (Male)	1	0.000	0.998
Marital status	3	0.651	0.885
Marital (Married)	1	0.000	0.998
Marital (Single)	1	0.000	0.998
Marital (Divorced)	1	0.000	1.000
Educational status	4	2.230	0.693
Education (Elementary/Primary)	1	0.000	0.994
Education (Junior high)	1	0.000	0.997
Education (None)	1	1.646	0.199
Educational status (Technical/Secondary School)	1	0.050	0.823
How long selling tigernuts	2	0.020	0.990
How long selling tigernuts (More than 10 years)	1	0.000	0.998
How long selling tigernuts (1 to 5 years)	1	0.020	0.888

The results of the binary logistic regression as indicated in Table 3-16 shows that these demographic characteristics had no significant contribution in predicting the probabilities of

the traders (wholesalers/retailers) having sufficient levels of knowledge. This is because p-value > 0.05 for all the covariates in the model.

3.9 Conclusion

From the results obtained, the farmers knew what to look out for after harvesting and during sorting. Majority of the farmers interviewed stated that, they practiced early harvesting during the dry season which enabled them to effectively dry the tigernuts. Also, the criteria farmers used during sorting indicated that, they knew what to look out for in an unwholesome tigernut tuber. These observations together with the low level of education of farmers suggests that government intervention may be required to introduce and train farmers to even better practices to reduce risk of fungal attacks of tigernut at farmer level.

Even though majority of the traders knew exactly what to look out for to segregate the unwholesome tubers, some looked at superficial parameters such as colour, texture, general appearance etc. All the traders confirmed that they had never rejected tigernuts supplied to them, irrespective of the degree to which improper sorting was done. They also indicated that they use unwholesome tigernut and sell to willing shoppers. This knowledge gap adversely impacts the implication and the severity of the damage that mycotoxin contamination has on human and animal health and to revenue generation during export. Also, more than half of the traders preferred semi-dried tigernuts to the dried and shrivelled ones and sourced more than 20 kilograms of tigernuts monthly, which takes more than a month to sell out. This gives enough time for increase in mycotoxin production, once storage conditions are poor. The method of display of tigernuts by majority of the traders was with the use of polythene bags, while sun drying them in between sales. These polythene bags may greatly influence the spread of mycotoxins by increasing the moisture content within the bags.

From the interviews conducted, 90% of consumers preferred just dried tigernuts to the overly dried ones and more than half of them purchased them from street hawkers. Majority admitted that they only wash the tubers with water and then store in polythene bags. This could create a conducive environment for the growth and multiplication of mycotoxins during storage/ before consumption. This practice by consumers nullifies any safety practices that may have been put in place from the farm and throughout the value chain to curb the presence and increase of mycotoxins.

Education, creation of awareness and surveillance on the impact and degree of damage caused by mycotoxins should be made available to all stakeholders in the value chain. This will help each sector to do their part in the campaign to curb the presence and increase of mycotoxins in tigernut and make tigernut survive in international business exchanges.

CHAPTER FOUR

4. OBJECTIVE 2: To determine the occurrence and level of mycotoxins (Aflatoxins and Ochratoxin A) in tigernut along the supply chain

4.1 Introduction

Two dominant mycotoxins that tigernuts are prone to are ochratoxin A (OTA) and aflatoxins (Weidenbörner, 2001). In Africa, food products that are most susceptible to OTA include maize, peanuts, beans as well as tigernut. Stoev, (1998) and IARC, (1993) described Ochratoxin A as carcinogenic, teratogenic, mutagenic and immuno-suppressive in several animals. According to IARC (1993), OTA is a probable group 2B carcinogen in humans. In the presence of Aflatoxin B1, OTA increases the ability of aflatoxin to mutate (Sedmikova et al., 2001). From his study, Moss, (1998), stipulated that Aflatoxin G1 (AFG1), Aflatoxin B1 (AFB1), Aflatoxin G2 (AFG2) and Aflatoxin B2 (AFB2) were the specific types of aflatoxins found in tigernut. Aflatoxins are not only generated by the accountable fungi at pre-harvest time but also continue through to post-harvest time and even during the storage period. This makes them one of the most difficult mycotoxins to handle.

High humidity and temperatures are common conditions that favour the growth of fungi throughout the entire supply chain, which results in contamination of food and feed (Thomson & Henke, 2000). These favourable conditions are caused by poor storage practices as well as lack of awareness. According to Benford et al. (2001), farmers choose to harvest crops at inappropriate times, due to lack of resources (labour, cash flow), inability to predict weather, pests and threat of burglary. Early harvesting has been promoted as a preventive measure to mitigate the risks of fungal growth. Rapid post-harvest drying (within 2 days of harvesting) has

also been found to reduce the risk of fungal colonisation of crops with its corresponding increase in mycotoxins.

Ochratoxin A has a lethal Dose- 50 (LD_{50}) of about 20mg kg^{-1} in humans (Chu, 2003) whilst aflatoxins in general have an LD_{50} of 0.3 to 17.9 mg kg^{-1} in humans and animals (Dhanasekaran et al., 2011). These LD_{50} values show that these mycotoxins are highly toxic. Furthermore, research has proven that toxicology of these mycotoxins is directly related to the duration and dose of exposure. For these reasons, regulatory limits have been established to help shield the consumer from the impacts of the mycotoxins. Most regulations are specific to foodstuffs. However, generally, aflatoxin limits range from 4 µg/kg (European Union regulations) to 17 µg/kg (United States of America) and maximum of 20 µg/kg (Latin America and Africa) (Van Egmond & Jonker., 2004). Globally, Ochratoxin A presence in food is limited to a range of 2 to 10 µg/kg for various foods (Jorgensen, 2005).

In this study, the presence and intensities of mycotoxins in tigernuts at the different stages along the supply chain (after harvesting, before distribution and retail levels) were analysed using an HPLC and the results were compared. Samples were collected at different stages of the supply chain:

- directly from the farm just after harvesting (regarded as farm samples)
- just before distribution (regarded as wholesale samples)
- markets and streets hawkers (referred to as retail samples)

4.2 Methods and Materials

4.3 Sources of Materials

Black and yellowish-brown varieties of tigernuts were each purchased at various points of the supply chain namely:

- Harvesting stage
- Post drying stage (prior to first sale)
- Traders (Wholesalers/retailers)

The sampling area is highlighted in Figure 4.1.

- **Samples collected at the harvesting stage**

Sixty (60) samples of freshly harvested tigernuts were purchased from different farmers in *Adowa* and *Aduamoah* towns by means of convenient sampling. One sample represents the tigernut purchased from one tigernut farmer. Therefore 20 tigernut samples were obtained from 20 individual farmers in Adowa and 40 tigernut samples from 40 individual farmers in Aduamoah. Each sample weighed 1kg.

- **Samples collected at the drying stage**

Sixty (60) other samples (1kg each) were purchased from the same selected farmers at the end of the drying stage. Each sample weighed 1kg. This is the stage prior to sale to wholesalers and retailers. At both stages, the intended sample size of both black and yellowish-brown varieties was 30 each variety, however, only 16 black variety samples were available at the time of purchase. Therefore, 42 yellowish-brown samples, (instead of the intended 30 yellowish-brown samples) were purchased to make up for the total sample size of 60.

- **Samples collected at the retail stage**

At the retail stage, 64 samples of each variety were purchased from one simple randomly selected market in all the districts in the Greater Accra Region (Table 4-1 & Table 4-2). One sample represents the tigernut purchased from one retailer. Each sample weighed 1kg.

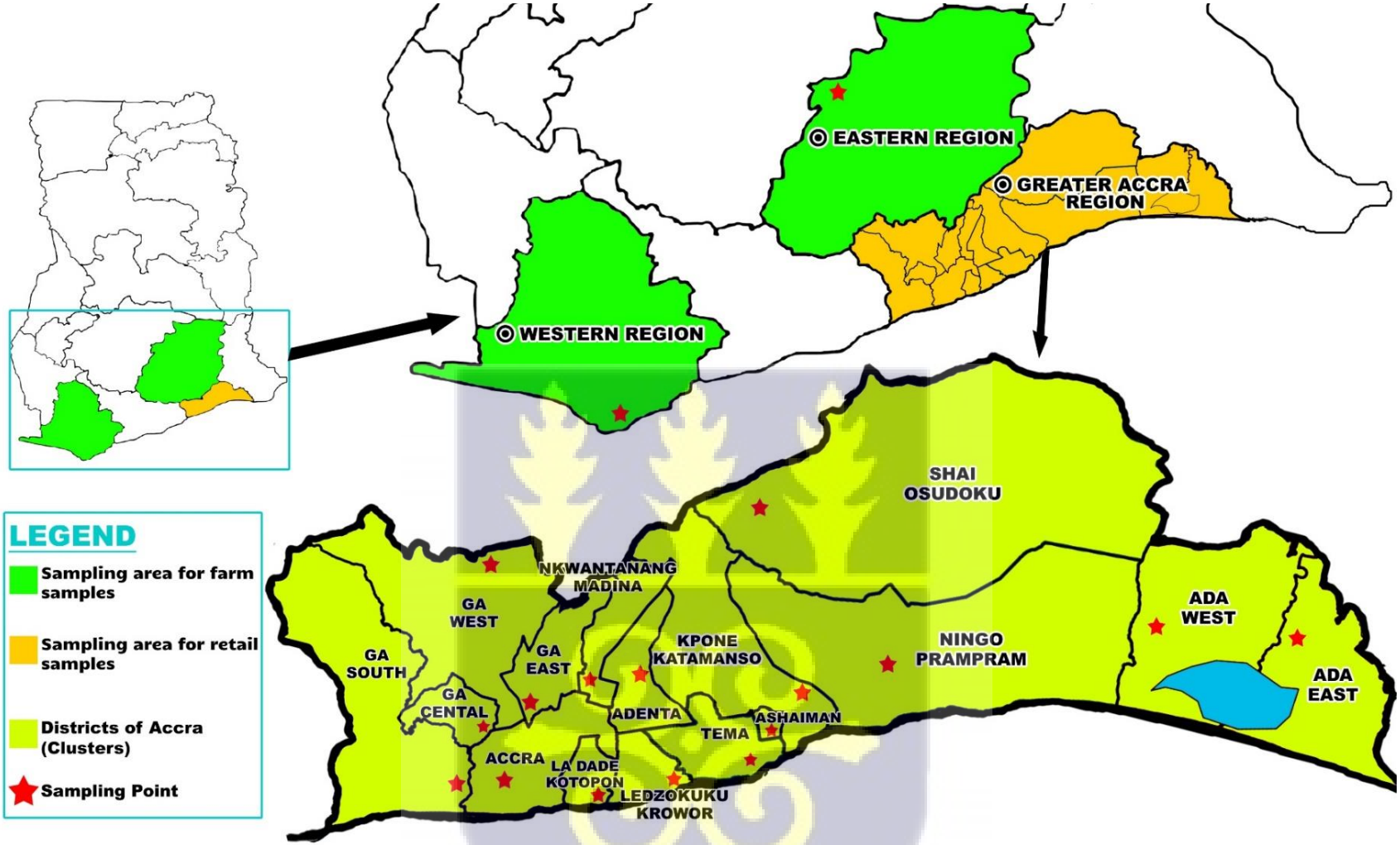


Figure 4.1: Map of Ghana indicating tigernut sampling area

Table 4-1: List of Selected Markets in the Districts in Greater Accra Region

District	Market	District	Markets
Accra Metropolis	Makola Market	Ga West Municipal	Achimota Market
Ada East District	Kassah Market	Kpone Katamanso	Kpone Market
Ada West District	Sege station	La Dade Kotopon Municipal	La Market
Adenta Municipal	Adenta Market	La Nkwantanang Madina Municpal	Madina Market
Ashaiman Municipal	Ashaiman Market	Ledzokuku/Krowor Municipal	Nungua Market
Ga Central Municipal	Anyah Market	Ningo Prapram	Dawhwenya Market
Ga East Municipal	Dome Market	Shai Osudoku (Dangme West)	Dodowa Market
Ga South Municipal	Mallam Market	Tema Metropolis	Community 1 Market

Table 4-2: List of Selected Streets in the Districts in Greater Accra Region

District	Streets	District	Streets
Accra Metropolis	CMB	Ga West Municipal	Achimota Main Road
Ada East District	Ada Foh Road	Kpone Katamanso	Kakasunanka
Ada West District	Sege Road	La Dade Kotopon Municipal	La Park Road
Adenta Municipal	Adjiriganor Traffic Light	La Nkwantanang Madina Municpal	Madina Trafic Light
Ashaiman Municipal	Ashaiman Road	Ledzokuku/Krowor Municipal	Nungua Main Road
Ga Central Municipal	Awoshie Traffic light	Ningo Prapram	Dawhwenya-Afienya Junction
Ga East Municipal	Dome Pillar 2	Shai Osudoku (Dangme West)	Dodowa Main Road
Ga South Municipal	Gbawe Junction	Tema Metropolis	Community 1 Road

Only tigernuts from traders (wholesalers/retailers) who confirmed sourcing their tigernuts from Ghanaian farmers were purchased. In all instances, purchased tigernuts were stored in nylon bags and refrigerated until they were ready for analysis.

4.4 Methods

Analysis to determine the mycotoxin levels of Ghanaian tigernut were performed at the Mycotoxins and Food Analysis Laboratories in the Department of Food Science and Technology, College of Science of the Kwame Nkrumah University of Science and Technology.

4.4.1 Mycotoxin (Aflatoxins and Ochratoxin A) Analysis

The mycotoxins that were analysed included the Aflatoxins AFG1, AFB1, AFG2 AFB2, as well as Ochratoxin A. The rationale was based on research studies which have outlined these mycotoxins as the most prevalent in tigernuts (Rubert et al., 2006; Rubert et al., 2012; Sebastia et al., 2010; Arranz et al., 2006).

4.4.2 Determination of Aflatoxin levels

All the solvents and reagents used for this analysis were of analytical and High-Performance Liquid Chromatography (HPLC) grade. Aflatoxin standards (B1, B2, G1, and G2) were obtained from Merck, USA. The concentrations of the Aflatoxins were analysed using a High-Performance Liquid Chromatography (HPLC). The methodology used was based on AOAC Official Method AOAC 2005.08-2005 with some alterations.

- **Preparation of Aflatoxin standard solutions**

Aflatoxin standard solutions for the analysis, using HPLC, were prepared from aflatoxin (AFB1, AFB2, AFG1, and AFG2) samples (mixed standard from Romer Labs, Austria). To prepare the stock solutions, appropriate amounts were weighed in a mixture of toluene-acetonitrile (9:1) (Omoniyi et.al, 2014). The concentration of each standard was determined using the Equation 4-1

$$\frac{\text{ng aflatoxin}}{\mu\text{l}} = \frac{(A \times MW \times 1000)}{\varepsilon} \qquad \textbf{Equation 4-1}$$

A = absorbance measured at 350 nm

MW = molecular weight

ε = molar absorptivity

To ensure precision and accuracy, the test was done in triplicates.

- **Extraction for HPLC analysis**

25g of finely ground test portions was weighed to the nearest 0.1g into a blender. Five (5) grams of sodium chloride and 125 ml of a mixture of methanol and water (7:3) were then added. The resulting mixture was blended for 2 minutes and filtered over a fluted filter paper. Fifteen (15) ml of the filtrate was pipetted into 100ml glass-stoppered Erlenmeyer flask. Water (30ml) was added after which it was stoppered and shaken to mix well. The diluted extract was filtered through a Whatman GF/B glass microfiber filter.

- **Immunoaffinity clean-up**

In this step, 15ml of the aliquot of the filtrate was applied to the column at a slow flow rate of 3 minutes, with the flow rate controlled by means of a vacuum system. The column was allowed to run dry by passing 3ml air through the packing material. The column was washed with 10ml of water at a maximum flow rate of 3ml/min. This was repeated with another portion of 10ml water and discarded. After disconnecting the assembly from the vacuum system, the aflatoxins were eluted with 2ml acetonitrile, with the flow rate controlled by means of a syringe plunger. To guarantee total removal of the bound aflatoxins, the solvent was left in contact with the column for 3 minutes. To achieve this, the flow rate of the acetonitrile was reversed three times. The eluate was collected in a 5ml reaction vial. It was then evaporated to dryness at a temperature of 40°C and the residue was taken up in 150µl of the HPLC mobile phase after which it was filtered through a 0.45µm membrane. 60µl was injected into the HPLC for analysis. Each aflatoxin peak in the resulting chromatograph was identified by comparing their retention time with the corresponding peak in the standard chromatograms. The quantity of each aflatoxin was determined from their respective calibration curves.

- **Operational Conditions**

The Aflatoxins (B1, B2, G1 and G2) were analysed in HPLC Agilent 1100 system equipped with a quaternary pump model G1311A, with a flow rate of 1ml/min and attached to a

fluorescence detector (Shimadzu 10AXL; Shimadzu Corporation, Tokyo, Japan) set at 365 excitation wavelength and 428 emission wavelength. The mobile phase used was methanol/acetonitrile/methanol (60:18:22). To 1 litre of the mobile phase, 119mg potassium bromide and 10µl nitric acid was added for post-column derivatization using the Kobra Cell (R-biopharm). As an internal standard, 5µg/kg, 10µg/kg and 20µg/kg AFB1, AFB2, AFG1 and AFG2 were used to spike the tigernut samples respectively.

4.4.3 Analysis of Ochratoxin A contamination in Tigernut samples.

The standard solution used for the analysis were made using analytical reagent of Ochratoxin A with a percentage purity of 99%. The solution was used to spike samples and diluted to achieve working solutions (100ng/ml) of the Ochratoxin A in methanol for the preparation of the calibration curves. The concentration range of the calibration curve (0.5 to 50ng/ ml) was prepared in a mixture of methanol: acetic acid: PBS (49:1:50, v/v/v) (Skarkova et al., 2013).

- **Operation Conditions for Ochratoxin A**

The analysis was done in a HPLC Agilent 1100 system equipped with a quaternary pump model G1311A, with a flow rate of 1 ml/min and attached to a fluorescence detector set at 365 excitation wavelength and 428 emission wavelengths. The retention time was from 4.8 to 5.2 minutes.

- **Extraction of Ochratoxin A**

Ochratoxin A concentration was evaluated using the procedures described by Teixeira et al. (2010) with minimal modification. 10g of ground sample was homogenized in 40ml of acetonitrile/water (120:80 v/v) solution for 3 minutes. The extract was filtered with a filter paper and transferred into a labelled flask. The extract was cleaned using the immunoaffinity chromatography. The extract (1.5ml) was applied on Ochratest ™ immunoaffinity columns with the aid of a syringe with a disposable needle. The column was subsequently washed with

6ml of pure water, after which 1.5ml methanol grade HPLC was used to elute the bound Ochratoxin A slowly through the column. The collected elute was evaporated to dryness under nitrogen flow (45-50°C) (Skarkova et al., 2013) and stored in a refrigerator. Before the HPLC analysis, the evaporated samples were dissolved in 300µl of methanol, after which they were diluted with 300µl water. With the aid of syringe pressure, the mixture was filtered through a filter paper into autosampler vials.

4.5 Statistical Analysis

All statistical analyses were done using IBM SPSS version 21 and Microsoft Excel (2016).

The Analysis of Variance test and independent samples t-test were used to determine the significant difference in the levels of occurrence and level of mycotoxins across the value chain and the varieties of tigernuts.

4.6 Results and Discussion

4.6.1 Occurrence of mycotoxins in Ghanaian Tigernuts

Figure 4.2 shows the occurrence of mycotoxin contamination in the samples analysed at the different sampling stages.

Out of 60 samples analysed, mycotoxins were detected in 58% and 77% of the samples at the farmer level (just immediately after harvest) and the wholesale level (just before distribution) respectively. All 64 samples analysed at the retail level showed some level of mycotoxin contamination. Figure 4.2 depicts an upward increase in mycotoxin contamination from the farmer level to the retail level. It was also observed that the mycotoxins were detected in more of the black tigernut samples at the farmer and wholesale stages, as compared to that of the yellowish-brown tigernut samples. This was attributed to the higher amount of moisture in the

black variety than the yellowish-brown which could have promoted more fungal activities as later found in this study (Nwaoguikpe, 2010).

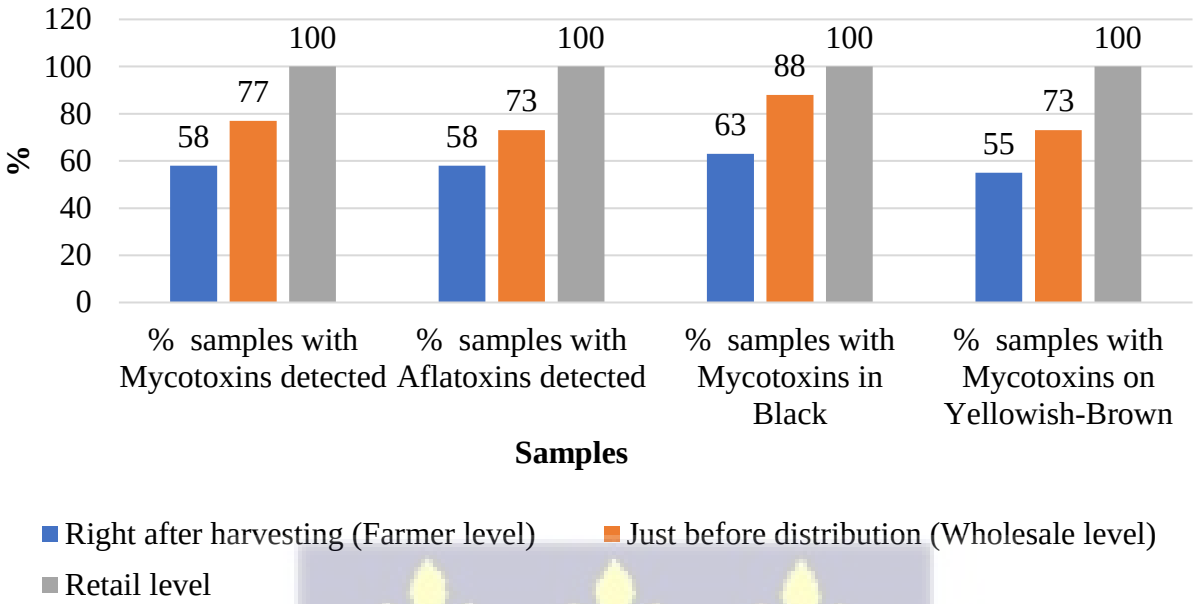


Figure 4.2: Occurrence of mycotoxins (aflatoxins and Ochratoxin A) in samples at the different stages of the supply chain

However, in both tigernut cultivars, an increase in mycotoxins levels along the supply chain was also observed. This is expected because once the mycotoxins were detected at the first stage, the quantity of mycotoxins would increase due to the continual activity of the mycotoxin producing fungi in the tubers.

Figure 4.3 shows the percentage of samples contaminated with different groups of mycotoxins.

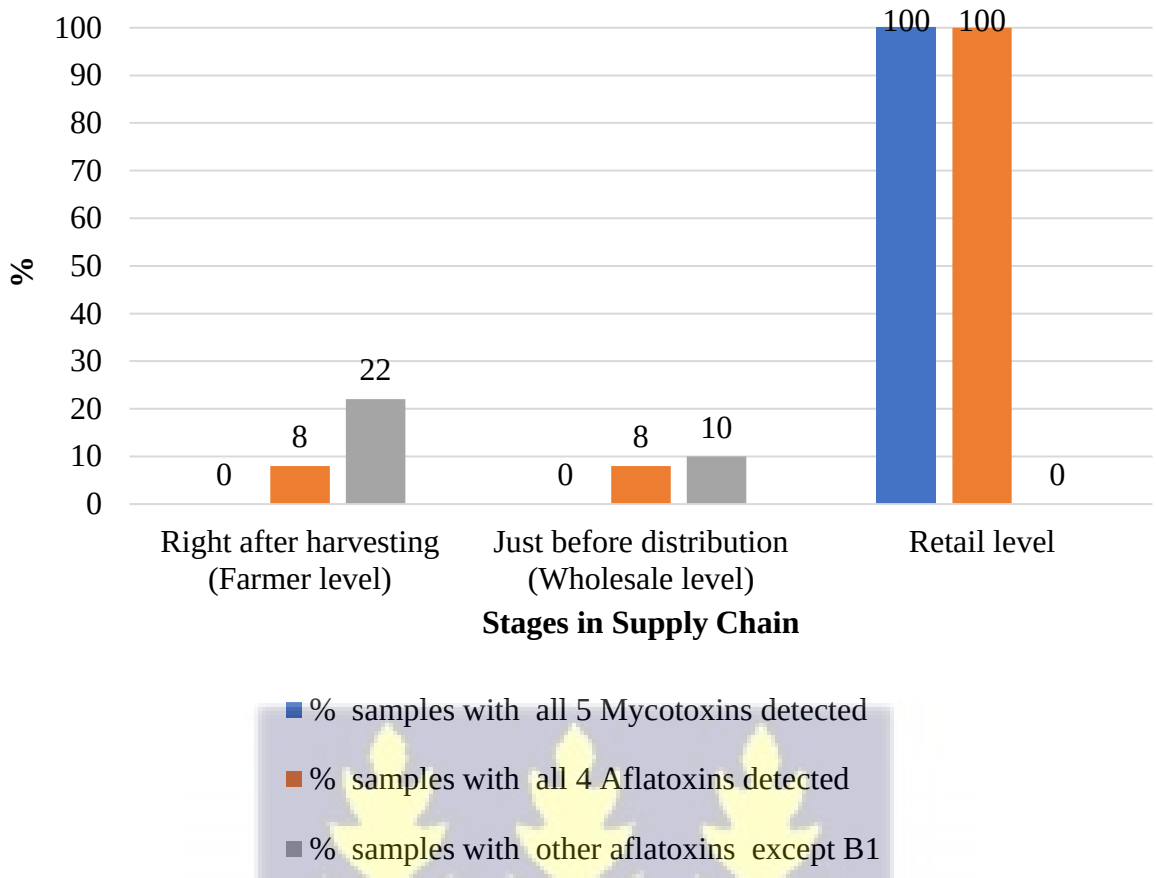


Figure 4.3: Percentage of samples contaminated with various types of mycotoxin at each stage of the supply chain

The results showed that, none of the samples were contaminated with all the five mycotoxins at the farmer and wholesale levels. It was also observed that some samples contained other mycotoxins other than AFB1 (22% for farmer level and 10% for wholesale level) respectively. However, samples at the retail levels contained all the mycotoxins analysed. This implies that, AFB1 alone should not be used as an indicator of mycotoxin contamination although it is the most carcinogenic mycotoxin (Benkerroum, 2020). At the retail level however, both black and yellowish-brown varieties of tigernut tubers were contaminated with some degree of mycotoxins.

Figure 4.4 shows the percentage of samples containing specific mycotoxins at the different stages of the value chain.

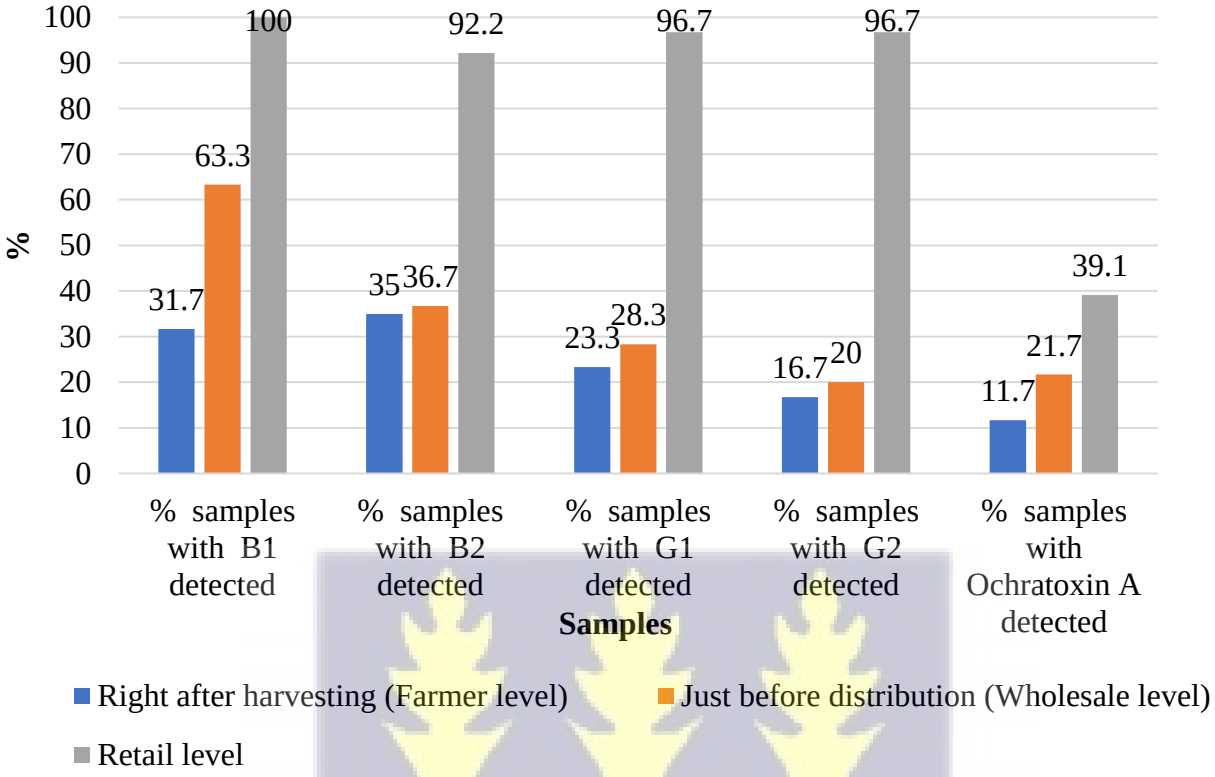


Figure 4.4: The variations in mycotoxin contamination at each stage of the value chain

AFB1 was detected in 31.7%, 63.3% and 100% of samples at farmer, wholesale and retail stages respectively whilst AFB2 was detected in 35%, 36.7 %, and 92.2% of samples at farmer, wholesale and retail stages respectively. AFG1 was detected in 23.3%, 28.3% and 96.7% of samples at farmer, wholesale and retail stages respectively whilst AFG2 was detected in 16.7%, 20% and 96.7% of samples at farmer, wholesale and retail stages respectively. OTA was detected in 11.7%, 21.7% and 39.1% of samples at the farmer, wholesale and retail stages respectively.

4.6.2 Concentration of mycotoxins in Ghanaian Tigernuts

The total mean mycotoxin and aflatoxin levels in the samples at the different stages were recorded in Table 4-3.

Table 4-3: The concentration of mycotoxins at each stage of distribution

	Right after harvesting (µg/kg)	Just before distribution (µg/kg)	Retail level (µg/kg)
Mean Mycotoxins	7.916 ± 11.523	8.375 ± 9.657	139.454 ± 228.661
Range of Mycotoxins	0 - 27.014	0 - 52.755	7.915 - 1115.480
Mean Aflatoxins	3.915 ± 5.748	8.013 ± 9.591	136.179 ± 224.347
Range of Aflatoxins	0 - 26.472	0 - 51.613	7.915 - 1106.848
Mean Mycotoxins in Black	1.297 ± 1.783	10.203 ± 8.579	165.373 ± 270.526
Mean Mycotoxins in Yellowish-Brown	3.752 ± 6.109	7.710 ± 10.137	125.812 ± 150.137

The mean mycotoxin levels were recorded to be: 7.916 ± 11.523 µg/kg for farm stage (after harvesting), 8.375 ± 9.657 µg/kg for the wholesale level (just before distribution) and139.454 ± 228.661 µg/kg for the retail level. From Table 4-3, it was observed that at the farm level, the mycotoxin levels were higher in the yellowish-brown variety (3.752 ± 6.109 µg/kg) than in the black variety (1.297 ± 1.783 µg/kg). However, this trend was reversed as it progressed through the value chain (10.203 ± 8.579 µg/kg at wholesale level and 165.373 ± 270.526 µg/kg at retail level in the black versus 7.710 ± 10.13 µg/kg at wholesale level and 125.812 ± 150.137 µg/kg

retail level in the yellowish-brown variety). Mycotoxin levels ranged from 0 µg/kg to 1115 µg/kg as tigernuts move along the value chain and aflatoxin ranged from 0 µg/kg to 1106 µg/kg.

The values attained in this study were higher than the 10-20 µg/kg reported by Bankole and Adebanjo (2003) in Nigerian samples. Research has shown that microbial contamination of crops can occur before and after harvesting (Ferrão et al., 2017). On the field, mycotoxin-producing moulds can be introduced from contaminated soil, irrigation water and faecal materials (Maduka & Ire, 2019). This accounts for the detected levels of mycotoxin contamination at the farm stage.

Post-harvest practices such as poor handling of tigernut samples, improper drying and storage are some of the ways through which crops become contaminated with mycotoxins at the whole sale level. The analysis reported the highest contamination of mycotoxin in the tigernut samples at the retail/wholesale level. Poor handling and treatment of tigernuts and poor storage at this level by vendors normally result in a significant increase in mycotoxins.

Table 4-4 shows the range of concentration of the individual mycotoxins at the different stages. From Table 4-4, the level of mycotoxins were observed to be in the order: AFB1> AFB2> AFG1> AFG1> OTA for "After harvesting" and "Before distribution" stages. However, at the retail level, the concentration of AFG2 was found higher than that of AFB2. The concentrations for the individual mycotoxins analysed in the tigernut tubers were observed highest at the retail level as compared to the other stages along the supply chain. The values were: AFB1 (87.429 ± 167.490 µg/kg), AFB2 (13.674 ±19.458 µg/kg), AFG1 (27.295 ± 60.200 µg/kg), AFG2 (7.780 ± 9.101 µg/kg) and OTA (3.275 ± 6.224 µg/kg). It was also observed that the standard deviations were relatively big indicating the irregular spread of mycotoxins amongst the samples.

Table 4-4: The range of the levels of individual mycotoxins detected at different stages

	Right after harvesting (Farm level) (µg/kg)	Just before distribution (Wholesale Level) (µg/kg)	Retail level (µg/kg)
Levels of B1	2.364 ± 4.84	4.879 ± 7.795	87.429 ± 167.490
Range of B1	0 - 23.231	0 - 45.360	1.110- 989.835
Levels of B2	0.805 ± 2.219	1.483 ± 3.117	13.674 ± 19.458
Range of B2	0 - 16.230	0 - 21.326	0 - 88.700
Levels of G1	0.531 ± 1.741	1.071 ± 2.600	27.295 ± 60.200
Range of G1	0 - 12.450	0 - 17.241	0 - 311.80
Levels of G2	0.215 ± 0.509	0.58 ± 1.294	7.780 ± 9.101
Range of G2	0 - 1.565	0 - 6151	0 - 36.320
Levels of Ochratoxin A	0.086 ± 0.341	0.362 ± 0.972	3.275 ± 6.224
Range of Ochratoxin A	0 - 220	0 - 6.235	0 - 26.819

The concentration of AFB1 was found predominantly amongst the aflatoxin species analysed in the samples. This supports the results of Lalah et al. (2019) who stated that, AFB1 is the most potent and prevalent of all the four major aflatoxin types. Alam et al. (2010) also reported that, the B1 type is the most common in food and feed products.

The percentage of samples containing total aflatoxin levels above the regulatory limits of 15 µg/kg (Codex Alimentarius (WHO, 2018)) and 20 µg/kg (United State Food Safety Regulation (Omoniyi et al., 2014)) at each stage of the supply chain, was computed and results are presented in Table 4-5.

Table 4-5: The percentage of samples exceeding regulatory limits for total aflatoxin

Regulatory limit	% of samples at Farm level	% of samples at Wholesale Level	% of samples at Retail level
Aflatoxin levels above 15 µg/kg	8	18	91
Aflatoxin levels above 20 µg/kg	3	12	89
Ochratoxin A levels above 5 µg/kg	0	1	21

It was also observed that, 8%, 18 % and 91 % of the samples had aflatoxin concentrations greater than the 15 µg/kg limit, at the different stages of the supply chain respectively, whilst 3%, 12% and 89 % of the tigernut samples had aflatoxin levels greater than 20 µg/kg at the farm, wholesale and retail levels respectively above the maximum tolerable limit established by the United State Food Safety Regulation and the Codex Alimentarius (Omoniyi et al., 2014).

The percentage of retail samples (89%) in this study that exceeded the total aflatoxin limit of 20 µg/kg was higher than that which was stated by Omoniyi et al. (2014) (17%). It was also observed from the analysis that, the concentration levels of Ochratoxin A (OTA) for some of the tigernut samples surpassed the 5 µg/kg limit set by the Codex Alimentarius; 1% and 21% at the wholesale level and retail level respectively. No sample obtained at the farmer level had OTA levels greater than the 5 µg/kg limit.

Total mycotoxin concentration determined in samples collected from the marketplaces and streets were compared and results are highlighted in Table 4-6.

Table 4-6: The total mycotoxin concentration at the street vs. marketplaces

	STREETS	MARKETS
Total Mycotoxins (µg/kg)	23.61 ± 73.839	27.891 ± 85.721

The mycotoxin contamination of the samples obtained from the streets were found to be higher than that of the marketplace. The difference may be accounted for by the fact that, at the market (retail level), tigernuts are usually packaged into polythene bags before being sold to consumers. Polythene bags as a packaging material has been reported to prevent reinfection of mycotoxins (Akomolafe & Awe, 2017).

4.7 Conclusion

In this study, it was observed that mycotoxin (aflatoxins and Ochratoxin A) contamination progressed as tigernuts travelled further along the value chain. This implies that once contamination occurs at the initial planting stages, progression continues throughout the lifecycle of the tuber. The worst level of mycotoxin contamination along the value chain was found at the retail level as all 5 mycotoxins analysed (AFB1, AFB2, AFG1, AFG1, OTA) were present. This is quite alarming as these are samples that are being sold directly to consumers. The lowest contamination of mycotoxins was found at the farm level, immediately after harvest.

All the samples analysed at each stage of the value chain showed a substantial degree of contamination and the black variety had significantly higher mycotoxins concentration than the yellowish-brown variety. Even though all 5 analysed mycotoxins were not found at the farmer and wholesale level, at least 4 of the analysed mycotoxins were found and this makes these stages as susceptible as samples at the retail level. The aflatoxin that was detected in the highest concentration was AFB1 (the most carcinogenic of all mycotoxins analysed). This was followed by a considerable quantity of AFB2, AFG1, AFG1 and finally OTA. These results suggest that no one mycotoxin can be used as an indicator of contamination as proposed by some researchers.

Results of analysis of samples from retailers on the streets and those at the market were compared. Although mycotoxins at this stage were highest irrespective of the locations of these retailers, a significant difference was observed in the levels at marketplaces and those from the streets, due to the various packaging techniques employed.

Due to the detrimental effects of mycotoxins in food crops including tigernuts, it is of primary importance to ensure that, the contamination of tigernut and its related products are, if not completely eradicated, reduced to the barest minimum. Pre-harvest strategies such as the use of biological methods of control and the employment of hygienic agricultural practices can be used in the control of mycotoxin contamination.

CHAPTER FIVE

5. OBJECTIVE 3: To evaluate some compositional and functional properties as well as the shelf life of tigernut flour

5.1 Introduction

The flour industry in Ghana is controlled by small groups as well as very few major flour mills. Most flours consumed by the nation are derived from maize and wheat. However, the flour industry competes for raw material because these grains form an integral part of the Ghanaian staples (Maur, & Shepherd, 2015). Maize is estimated to contribute more than one-quarter of calories consumed, approximately twice that of cassava (GSS, 2018). Wheat per capita consumption in Ghana has been estimated according to USDA to be about 20 kg/year. The consumption of wheat has been reported to experience a slight upward increase, owing to urbanization and changing dietary habits (Mottaleb et al., 2018).

As a result of this, Ghana imports about 80% of the flour it consumes. Approximately 80% of wheat flour used is for the preparation of bread and the other 20% is used in the preparation of cakes and pastries (Hughes et al., 2020). Studies have been organised to establish the feasibility of making tigernut composite flour. Twum et al. (2015) investigated the physicochemical properties of a composite flour mixture of tigernut, maize and soybean flour in different percentage compositions. The results reported an increase in physicochemical properties (protein content, pH, moisture content, and ash content) of the composite flour relative to that of the individual flours. A weighty motivation to produce composite foods is to enhance nutritional quality (Twum et al., 2015).

The starch of tigernut contributes greatly to its potential as a substitute flour in Ghanaian diet. According to Akonor et al. (2019), tigernut is an appropriate source of starch. It contains about

twice the amount of starch present in sweet potato (Coşkuner et al., 2002). Tigernut starch is odourless, appears to have an off-white colour and has flow properties like starch present in maize and potato (Akonor et al., 2019). Tigernut is noted to be high in resistant starch which has a similar physiologic effect as dietary fibre and can function as a milk laxative (Werner-Gray, 2019).

Research conducted by Akonor et al. (2019) showed that starch granules of the yellow tigernut cultivar were larger than that of the granules of the black tigernut cultivar. Both varieties had an amylose content ranging from 19% to 21% (Akonor et al., 2019). According to Akonor et al. (2019), high amylose content helps in slow digestibility and enhances film forming ability of starches in products. The swelling and water solubility properties of the yellow cultivar were also noted to be significantly higher than that of the black cultivar (Akonor et al., 2019). Colour plays an important function when selecting raw materials for food processing and when seeking to satisfy the expectations of consumers and satisfaction in finished products. Akonor et al. (2019) states that starch from both the yellow or black cultivar had a similar hue but starch from the yellow cultivar was much whiter and brighter. This is among the many reasons why the yellow cultivar is preferred to the black cultivar by most consumers (Adejuyitan, 2011).

Due to the high consumption of maize and wheat by Ghanaians and the availability of tigernut in most parts of the country, the preparation of tiger nut composite flour from these two flours can be nutritionally beneficial. This will help in the long run to mitigate post-harvest losses and enhance the income revenue of tigernut farmers. It will also reduce the burden on the wheat and maize flours as well as imports of flours into the country. Eventually, tigernut flour stands the chance of generating revenue for the country. The aim of this study was to assess some compositional and functional properties as well as the shelf life of tigernut flour as a potential substitute for flour in Ghanaian diets.

5.2 Materials and Methods

5.3 Sample Preparation: Preparation of Tigernut flour

Tigernut samples purchased from traders (wholesalers/retailers) in the Greater Accra Region (Table 3-1 & Table 3-2) were used for the preparation and analysis of the tigernut flour. Tigernut flour was prepared in the Nutrition and Food Science Department of the University of Ghana. The method outlined by Adejuyitan et al. (2009) was followed for the tigernut flour preparation. Each variety of tigernut was sorted to remove foreign bodies and infected tubers. The wholesome tubers were mixed together and thoroughly washed in clean water (65 °C). The washed samples were then dried in an oven (Astell Scientific PBS 160A) for 5 hours at a temperature of 70 °C and left to cool. The cooled and dried samples were then milled in a Hammer mill (Christy and Norris MODEL VDE0660). Fine tigernut flour was obtained by sieving through a 200 µm sieve. The resulting tigernut flours were sealed into plastic bags (Figure 5.1) and kept at room temperature for further analysis.

Figure 5.1: Yellowish Brown and Black Tigernut tubers and their corresponding Flours

5.4 Methods

The functional properties of tigernut flour were examined and the shelf life of the tigernut flour was also estimated using Arrhenius method. Starch analyses of the flours were done at the Mycotoxins and Food Analysis Laboratories in the Department of Food Science and Technology, College of Science of the Kwame Nkrumah University of Science and Technology. Functional properties of the flour were conducted in the Department of Nutrition and Food Science Laboratory of the University of Ghana. Shelf-life studies were conducted in the Quality Assurance laboratory of Nestlé Ghana Ltd.

5.4.1 Determination of Compositional properties of tigernut flour

5.4.1.1 Sugar profiling of tigernut flour

High Performance Liquid Chromatography (HPLC) was used for this analysis. To prepare samples for analysis, 0.5g of flour from each sample (black and yellowish-brown) was homogenized together with 5ml of distilled water and 9ml of 95% alcohol in a centrifuge tube (Bado et al., 2015). The mixtures were shaken and centrifuged at 12298 g-force for 30 min. 20µl of the filtered supernatant solutions were taken and used for the analysis. A mixture of acetonitrile/water (80/20) was used for the mobile phase. The peaks generated were assigned to the various sugars by comparing their individual retention times with each standard fructose, maltose, sucrose and glucose with 99% purity. Standards for the fructose, maltose, sucrose and glucose were sourced from Merck Group of companies -Germany.

5.4.1.2 Total Starch determination

The Amyloglusidase/α-amylase method, which was adopted from AOAC Method 996.11., was used for analysing the total starch content in each sample. Each sample (5g) was ground to pass through a 0.50mm screen. 10 mg of the milled samples were weighed in duplicate portions into corning culture tubes, with one of the tubes of each sample used as a sample blank. The tubes were measured, and the exact weight recorded. The tubes were given a gentle tap to ensure that

samples settled at the base of the tubes. 10 ml of 100 mm sodium acetate buffer with a pH of 5.0, containing 5mm of $CaCl_2$ was delivered into the tubes using Bottle-top dispensator. They were stirred vigorously with vortex mixer for 5 seconds. Undiluted thermostable α-amylase (0.1ml) was delivered to the sample tubes using a HandyStep® dispenser.

To prepare the sample blanks, 0.1ml of 100 mm sodium acetate buffer (pH 5.0) was delivered into the designated tubes. The tubes were vortexed for 3 seconds after which they were capped, and immediately transferred into a water bath to boil for exactly 2 minutes. The caps of the tubes were tightened after they were removed and mixed vigorously on a vortex mixer. The procedure was repeated for 5 and 10 minutes respectively, and after each allotted time, the tubes were shaken on a vortex mixture for 5 sec. The tubes were removed 15 minutes after the addition of the α-amylase, heated in a water bath to a temperature of 50 °C and then cooled to an equilibrium temperature for 10 minutes. Undiluted AMG (0.1 ml) together with 0.1 ml of 100 mm sodium acetate buffer with pH 5.0 was added again to the sample tube and the sample blank respectively.

The tubes were incubated for 30 minutes at a temperature of 50°C. After the incubation period, the tubes were taken from the water bath and cooled to ambient temperature for 20 minutes. After cooling, the tubes were inverted a few of times to ensure that the condensed water on the inside of the lid was mixed well with the liquid in the tubes. 2.0 ml of each solution (sample and sample blank) was pipetted into microfuge tubes which were centrifuged at 20,784 g-force for 5 minutes. Using the pipette dispenser, 0.1ml aliquot of the supernatants was pipetted into 12 ×120mm tubes containing 4 ml of 100mm sodium acetate buffer (pH 5.0) after which the contents were mixed. 0.1ml duplicate portions of the samples and the sample blanks were transferred to the bottom of a glass test tube with dimension 16 ×120mm. GOPOD reagent (3.0 ml) was pipetted into test tubes and incubated at a temperature of 50°C for 20 minutes. The

absorbance against the reagent blank for each sample was measured at a wavelength of 510nm. The following were incubated, and their absorbance measured:

- Glucose controls: 0.1 ml of glucose standard solution (1.0 mg/ml) with 3.0 ml of GOPOD reagent, in quadruplicates.
- Reagent Blank: 0.1 ml of acetate buffer (100 mm, pH 5.0) with 3.0 ml of GOPOD reagent in duplicates.

The starch content was then calculated according to the Equation 5-1 (Ranathunga, et al, 2017):

$$\text{Starch } \% = \Delta A \times F \times \frac{EV}{0.1} \times D \times \frac{1}{100} \times \frac{100}{W} \times \frac{162}{180} \qquad \textbf{Equation 5-1}$$

ΔA = absorbance of sample solution read against reagent blank, less the absorbance of the sample blank read against the reagent blank (only where a sample blank is determined).

F = factor convert absorbance values to mg glucose (100 mg glucose divided by the GOPOD absorbance value obtained for 100 mg of glucose).

EV = sample extraction volume.

0.1 = volume of sample analysed.

D = further dilution of sample solution (either undiluted, or diluted 5 – fold or 11 – fold)

1/1000 = conversion from g to mg

100/W = conversion to 100 mg sample; W = sample weight in mg.

162/180 = factor to convert from free D-glucose, as determined to anhydro-D-glucose, as occurs in β-glucans

5.4.1.3 Determination of Polysaccharides

ISO 6647-1:2015 was the reference method used for the determination of amylose content in the tigernuts. The analysis was done for both varieties of the tigernut samples. The procedure consisted of a few steps namely: test sample preparation, test solution preparation, preparation of calibration curve and calculation of amylose and amylopectin content.

- **Test sample preparation**

Tigernut sample (5g) was milled to a fine powder to pass through a sieve of 80 mesh (0.177mm). The flour was refluxed with methanol for 2 hours in order to defat it. After the fat extraction process, the flour was dispersed in a thin layer on a dish and left for 3 days in order to get rid of any residual methanol and to equilibrate its moisture content.

- **Test solution preparation**

The test samples (1.0mg) was weighed and transferred into a cleaned and dried 100 ml beaker. Ethanol (2ml) and 18.0ml of 1M NaOH were pipetted and added to the test sample consecutively. The test sample was heated in a water bath at 100°C for 10 minutes which was later cooled rapidly to the ambient temperature. The cooled sample was then transferred into a 100ml volumetric flask, topped up with distilled water and mixed.

A blank test was carried out in parallel with the procedure above with 5.0ml of 0.09 mol/ l NaOH solution instead of the test solution. A calibration graph was prepared using a series of mixture of amylose and amylopectin standard suspension and 2.0ml of the 0.09 mol/NaOH solution.

- **Preparation of the calibration graph**

The aliquot of each calibration solution (2.5ml) was pipetted into a 50ml test tubes. 0.5ml of acetic acid was added, and the solution mixed thoroughly. Iodine solution (1.0 ml) was added, and the solution was topped up with water and mixed. The solution was left to stand for 30

minutes after which the absorbance was measured at 620nm against the blank with the spectrometer. A calibration curve was developed by plotting absorbance versus the amylose content. The amylose and amylopectin used for developing the standards for the calibration curve was sourced from Merck Germany.

- **Calculation of amylose and amylopectin content**

The aliquot of the test solution (2.5ml) was pipetted into 50ml test tube and proceeded according to the fore mentioned procedure. The amylose content was expressed as percentage by mass and was quantified by checking absorbance from the calibration curve.

To determine the amylopectin, the Amylose content was subtracted from the total starch.

5.4.1.4 Determination of Resistant starch content

The resistant starch content of the two varieties of tigernut flours was determined using the procedure outlined by Champ et al. (1996).

The following solutions were consecutively added to 100mg of the sample: 10 ml enzyme solution in buffer (pH 6.9); pancreatic α-amylase (500U) and 0.1M tris-maleate buffer solution (calcium chloride 4mM) (Champ et al., 1996). The resulting mixture was mixed for 16 hours at 37°C after which 40ml of ethanol was added. The solution was left to stand for an hour and later centrifuged and filtered. The residue was washed with 80% ethanoic acid and dried at a temperature of 60°C. After drying, 2ml of water and 1.5ml of a 4M KOH solution were added. The solution was mixed for 30 minutes at ambient temperature after which 12 ml of water was added again.

To obtain a pH of 4.5, 0.65 ml of a 2M acetic acid was added to the solution. After which 0.1ml amyloglucosidase (20/0.1 ml 0.1 M Na acetate buffer pH 4.5) was added. The resulting mixture was shaken for 90 minutes. The results of the resistant starch were obtained by determining the amount of glucose using the glucose oxidase assay.

5.4.2 Determination of the Functional properties of Tigernut Flour

5.4.2.1 Pasting Properties of Tigernut starch

The American Association for Clinical Chemistry (A.A.C.C) Method 76-21.02 (General Pasting Method for Wheat or Rye Flour or Starch Using the Rapid Visco Analyser) was used to prepare a complete pasting curve of the starches in the tigernut flour samples. The Rapid Visco Analyser ((RVA 4500) from PerkinElmer was used for the analysis of pasting property of tigernut flour. The analysis was performed for both black and yellow-brownish tigernut samples. 10 mg of the powdered sample was weighed to the nearest 1mg into a container using an analytical balance. 10ml of distilled water was added to the sample, mixed thoroughly and sampled into a canister. A paddle was placed to mix and push down any small lumps. The canister was then inserted to begin the test. The analysis results were automatically generated and recorded.

5.4.2.2 Determination of oil absorption of tigernut flour

The oil absorption capacity of the tigernut flours was also assessed using methodology of Onwuka, (2015) with slight modifications. One gram (1 g) of sample was blended with 10 mL soybean oil (Sp. Gravity: 0.9092). The resulting mixture was left to stand at ambient temperature (30 ± 2 °C) for 30 min, then centrifuged for 30 min at 1200 g-force. Oil absorption was examined as percent oil bound per gram flour (Equation 5-2)

$$\text{Oil Absorption Capacity}(\%) = 100 \times \text{Density of soyabean oil} \times \frac{Amount\ of\ oil\ added - Free\ oil}{\text{Weight of sample}} \qquad \textbf{Equation 5-2}$$

5.4.2.3 Determination of water absorption of tigernut flour

The water absorption capacity of the flours was analysed using the procedure as described by Onwuka, (2015) with slight modifications. 1 g of the sample was weighed and transferred into

a clean centrifuged tube. 100 ml of the distilled water was added, and the resulting mixture was shaken gently and allowed to stand at room temperature for 30 minutes. The mixture was thereafter centrifuged for 35 minutes at 1200 g-force. The clear supernatant solution was filtered off and the water absorption capacity determined as percentage water bound per gram flour (Equation 5-3)

$$\text{Water Absorption Capacity}(\%) = 100 \text{ x Density of water x } \frac{Amount\ of\ water\ added - Free\ water}{\text{Weight of sample}} \quad \textbf{Equation 5-3}$$

5.4.2.4 Determination of swelling capacity of tigernut flour

The method used for this analysis was adopted from the procedures explained by Olapade et al. (2011) with slight changes. 1 g of the sample was weighed and transferred carefully into a 50 ml centrifuged tube. 50 ml of distilled water was added to top up the volume to the 50 ml mark. The mixture was shaken vigorously to mix the solute with the top of the centrifuged tube tightly covered to avoid spillage. The resulting suspension mixture was heated in a temperature-controlled water bath at a temperature of 75 °C for 15 minutes. The mixture was carefully stirred during the heating process to prevent the clustering of the flour. After heating, the tubes were centrifuged at 1107 g-force for 15 minutes. The resulting clear supernatant solution was filtered off immediately. The weight of the sediment was measured and recorded. The dry matter content of the gel was determined using the moisture content of the sediment gel. Equation 5-4 was used to compute the swelling capacity of the flour.

$$\text{Swelling capacity} = \frac{\text{Weight of wet mass sediment}}{\text{Weight of dry matter in the gel}} \quad \textbf{Equation 5-4}$$

5.4.2.5 Determination of foam capacity and foam stability of tigernut flour

The foam capacity (FC) and foam stability (FS) of the flours were determined utilising methods as depicted by Narayana and Narsinga Rao, (1982) with slight alterations. The 1g of flour sample was weighed and added to 50 ml distilled water at a room temperature of 30 ± 2 °C in a graduated cylinder. The suspension was blended and shaken for 5 min to foam. The volume of foam at 30 seconds after whipping was expressed as foam capacity utilising Equation 5-5:

$$\text{Foam capacity}(\%) = \frac{\text{Volume of foam AW} - \text{Volume of foam BW x 100}}{\text{Volume of foam BW}} \quad \textbf{Equation 5-5}$$

Where AW = after whipping and BW = before whipping

The volume of foam was recorded 1 hour after whipping to determine foam stability as per percent of initial foam volume.

5.4.2.6 Determination of emulsion capacity and stability of tigernut flour

The emulsion capacity and stability of the tigernut flours were determined employing a strategy depicted by Yasumatsu et al. (1972) with slight adjustment.

Each of the samples (1g) was approximately weighed and transferred into calibrated centrifuged tubes. Distilled water (10mL) and soybean oil (10mL) were added after which the resulting mixture was homogenised using a blender. The emulsion was centrifuged at 492 g-force for 5 min. The percentage emulsion stability was calculated as the proportion of the height of emulsion layer to the overall height of the blend.

The emulsion stability was assessed after warming the emulsion contained in the calibrated centrifuged tube at 80 °C for 30 min in a water-bath, cooling for 15 minutes beneath running

tap water and centrifuging at 492 g-force for 15 min. The percentage emulsion stability was calculated as the ratio of the height of emulsified layer to the total height of the mixture (Equation 5-6).

$$\text{Emulsion stability} = \frac{\text{Height of emulsified layers x } 100}{\text{Height of whole solution}} \qquad \textbf{Equation 5-6}$$

5.4.3 Accelerated shelf life study of tigernut flour

The shelf life was done using the method described by Pulungan et al. (2018) with slight modifications. Each variety of tigernut flour (50g) was placed in clear glass containers with a metal cap and stored in ovens of 30°C, 50°C and 60°C (Thermo Scientific Heratherm Oven) for up to 28 days (Figure 5.2). In total, 30 containers of each variety of flour were stored under each temperature condition. A container was taken from the ovens at set times (7, 14, 21 and 28 days) and contents subjected to various analyses including moisture, pH and titratable acidity analysis.

Figure 5.2: Storage of tigernut flour and tigernut oil in oven for shelf life studies

The Arrhenius equation (Equation 5-7) was used to estimate the shelf life of the flour.

$$k = Ae^{\frac{-Ea}{RT}}$$ **Equation 5-7**

k = rate

A = experimental constant for a specific reaction

R = gas constant

T = in Kelvin

K ~doubles for every 10°C increase

5.5 Statistical Analysis

The independent samples t-test was used to determine the difference in the functional properties of tigernuts between the two varieties.

5.6 Results and Discussion

In the discussions below, results of tigernut flour were compared with other root tubers. Cereal flours were also compared with the results of the tigernut flour in order to ascertain the possibility of substituting the cereal flour with tigernut flour in certain food applications.

5.6.1 Sugar profile of tigernut flour

The sugar contents in both the yellowish-brown and black tigernut cultivars are presented in Table 5-1.

Table 5-1: Sugar profile of tigernut tubers

Nutrient Component	Variety	Concentration (% g/vol)
Sucrose	Black	14.04 ± 0.05[a]
	Yellowish-Brown	13.28 ± 1.281[a]
Glucose	Black	1.75 ± 0.10[a]
	Yellowish-Brown	1.58 ± 0.07[a]
Fructose	Black	4.20 ± 0.05[a]
	Yellowish-Brown	3.54 ± 0.4[a]

Values are means of triplicates and ± standard deviation. Since the means for both varieties of tigernut have the same, it means they were not statistically different at $p \leq 0.05$

Overall, the black variety contained more sucrose, glucose and fructose than the yellowish-brown variety. This explains its perceived sweeter taste. The sugars in food combine with water to keep food moist and thereby makes it soft and flexible. This high content of sugar in the tigernut flour makes it an appropriate source of flour for baking as one important attribute for baking friendly flour is the ability of the flour to form less brittle baked products. This quality also helps to maintain the water content of food and therefore delays staleness and enhances a longer shelf life. The high natural sugar content in both varieties of tigernut makes the root a suitable ingredient for the preparation of desserts and other foods which desire a sweet taste profile. Wheat has been found to contain 0.54 - 1.55% of disaccharide sucrose consisting of glucose and fructose (Shewry & Hey, 2014). The sucrose, fructose and glucose content for both varieties of tigernut flours were found to be higher than those reported for sucrose (0.70 ± 0.03%), glucose (0.80 ± 0.01%) and fructose (0.16 ± 0.03%) for maize (Bathla et al., 2019).

5.6.2 Starch profile of Tigernut Flour

Table 5-2 presents the total starch, amylose, amylopectin and resistant starch composition of tigernut flour.

Table 5-2: Starch Profile of tigernut tubers

Type of Starch	Variety	Concentration (%vol/vol)
Total starch	Black	30.14 ± 0.54[a]
	Yellowish-Brown	31.64 ± 0.51[a]
Amylose	Black	21.29 ± 0.33[a]
	Yellowish-Brown	19.22 ± 0.22[a]
Amylopectin	Black	10.35 ± 0.56[a]
	Yellowish-Brown	11.19 ± 0.6[a]
Resistant Starch	Black	14.19 ± 0.13[a]
	Yellowish-Brown	14.83 ± 0.05[a]

Values are means of triplicates and ± standard deviation. For each property, means for both varieties of tigernut with different superscripts were significantly different at $p \leq 0.05$

The mean total starch contents obtained for both black and yellowish-brown tigernut cultivars from the analysis were 30.41 ± 0.54% and 31.64 ± 0.51% respectively. The value for yellowish-brown variety was higher than that of the black. According to Coşkuner et al. (2002), the quantity of starch in tigernut tubers decrease as the levels of reducing sugars increases during storage. The quantity of starch in tigernuts were found to be twice as much as that which is found in sweet potato or potato tubers (Coşkuner et al., 2002; Sanchez-Zapata et al., 2012). This was also confirmed by Kuneret al. (2002). According to Murphy et al. (2008), the daily recommended intake of resistant starch is 15-20g. A diet containing tigernut, for instance, pastries made from composite flours which contains tigernut flour, can provide this daily recommended dose once incorporated into the baking industry, desserts and other products such as porridge and pudding.

Amylopectin and amylose are the two principal constituents of starch. The observed amylose content of the tigernut starches in this study were 21.29 ± 0.33% for black variety and 19.22 ± 0.22% for yellowish-brown variety. These results were within the range of 19 to 21%, observed by Akonor et al. (2019). According to Noda et al., (2004) and Wickramasinghe et al. (2005),

type of soil, time of harvest, difference in conditions of crop development etc. are possible causes of the differences in amylose content. Amylopectin composition of the black and yellowish-brown varieties of flour were 10.35 ± 0.56% and 11.19 ± 0.6% respectively. The differences in the amylose and amylopectin ratios of the tigernut flours indicate that, products made from these starches may differ because relative amounts of amylopectin and amylose are predominantly accountable for the functional behaviour and nutritional properties of starch-based food products (Akonor et al., 2019).

Amylose is crucial in the baking industry as it prevents crumbling during the cooling process in bread making (Lee et al., 2001). Research has proven that, a low amylose content decreases the elasticity and stickiness of dough which in turn leads to an improved crumb texture, higher water absorption of starch, higher gas formation, and reduced staling (Lee et al., 2001; Alcázar-Alay & Meireles, 2015). Comparing the amylose content obtained from this study to the average amylose content of wheat flour (25-28%) shows that, tigernut flour has a lower amylose content. This shows that though bread made from tigernut flour may reduce elasticity and stickiness in dough formation, it is most likely to improve crumb texture and take a longer time to go bad as compared to bread made from wheat flour. A high amylose content plays a vital role by improving film-forming ability and slows down digestibility (Akonor et al., 2019). The amylose content obtained from this study falls within the range of 15- 30%, as reported by Bertoft (2017), 17.9 – 23.6% as reported by Defloor et al. (1998) for cassava and 10.1-20.2% as reported by Tortoe et al. (2017) for sweet potatoes. As such, in the baking industry, tigernut flour is reported to result in less open breadcrumbs (Akonor et al., 2019; Lee et al., 2001) and this may be beneficial for bread baking.

The resistant starch of the two tigernut cultivars was recorded in Table 5-2. The values were 14.19 ± 0.13% and 14.83 ± 0.05% for both the black and yellowish- brown variety respectively.

Resistant starch (RS) is characterised by its resistance to digestion in the small intestine and therefore is categorized as a type of dietary fibre (Lockyer and Nugent, 2017). The moderate amount of resistant starch contained in both tigernut cultivars makes them suitable in diet for managing diabetes. This shows the importance of including tigernuts in meal formulation for diabetic patients as their resistant starch levels helps to contribute to reducing the glycaemic index of foods. Additionally, moderate intake of natural sugars has been found not to increase the risk of developing type 2 diabetes (Macdonald, 2016). Research has also shown that the resistant starch can be helpful in the treatment of chronic kidney failure as an ingredient in oral rehydration (Lockyer & Nugent, 2017).

5.6.3 Pasting Properties of starch from tigernut flour

The pasting properties of flour from both tigernut cultivars were analysed and shown in Table 5-3.

Table 5-3:Pasting properties of the tigernut cultivars

Property	Variety	Result
Peak viscosity (cP)	Black	2589 ± 9.89[a]
	Yellowish-Brown	3688 ±204.35[a]
Trough viscosity (cP)	Black	2445 ±10.61[a]
	Yellowish-Brown	2280 ± 115.96[a]
Set Back viscosity (cP)	Black	769 ± 32.53[a]
	Yellowish-Brown	638.5 ±111[a]
Breakdown viscosity (cP)	Black	144 ±0.71[a]
	Yellowish-Brown	1408.5 ±88.39[b]
Final Viscosity (cP)	Black	3214 ±43.14[a]
	Yellowish-Brown	2919 ±4.95[a]
Pasting Temperature (^{o}C)	Black	79.63 ±0.18[a]
	Yellowish-Brown	75.45 ±0.28[b]
Peak time (min)	Black	9.74 ±0.09[a]
	Yellowish-Brown	8.63 ±0.14[a]

Values are means of triplicates and ± standard deviation. Since the means for both varieties of tigernut have the same superscripts, it means their properties were not significantly different at $p \leq 0.05$

Initially, when heat is applied in the presence of excess water, starch granules swell up as water is taken up and amylose leaches out causing an increase in viscosity. At the highest point of swollen granule formation, when heat or shear is added, hydrogen bonds between polymer chains are destroyed/disrupted. This causes the scattering of fragments of amylose and amylopectin, resulting in a drop in the molecular weight of amylopectin which makes pastes less viscous (Lillford, 1997). Peak viscosity is the stage between the maximum swelling point and the scattering which leads to a low viscosity. It measures the capability of the starch to swell easily before the physical breakdown. The peak viscosity was found to be higher in the yellowish-brown variety (3688 ±204.35cP) than in the black variety (2589 ± 9.89 cP). This indicated that, the yellowish-brown varieties had better water retaining capacity and its maximum viscosity attained during heating and holding cycles were found to be higher than that of the black varieties. Studies have shown that, the peak viscosity can be affected by the size of the starch granules, starch water concentration (Fortuna et al., 2000), and structure of the amylopectin (Shibanuma et al., 1996). From their work on the "Correlation between grain nutritional content and pasting properties of pre-gelatinized red rice flour", Ascheri et al., (2012) noticed that rice samples with a low amylose content took a longer time to cook effectively and were watery, sticky and soft. This observation was also corroborated by Pereira, (2009) who concluded that rice genotypes with sticky grains usually have a low amylose content. A high peak viscosity usually connotes a low amylose content and vice versa. Juhász and Salgó (2008) also noticed that during their experiment, anytime amylose was reduced by the addition of amylopectin, the peak viscosity sharpened and went higher. This explains the reason for the high peak viscosity obtained for the yellowish brown variety and the lower peak viscosity obtained for the black variety, as the yellowish brown variety had a lower amylose content and a higher amylopectin content while the black variety had a rather higher amylose content and lower amylopectin content (Table 5-2). Lipid have been found to have a major

effect on the pasting properties of starch. In the presence of amylose, they inhibit swelling and preserve the wholesomeness of the swollen granules whereas the amylopectin imparts on the swelling of starch granules and pasting (Tester & Morrison, 1990). Another school of thought postulates however that, amylose inhibits swelling irrespective of the presence or absence of lipids (Hoover, 2001; Noranizan et al., 2010; Oke et al., 2013; Zhang et al., 2017).

Comparing the peak viscosity values obtained in the two tigernut varieties with other crops such as yam (2,064±48cP), ginger (936±36cP) (Peroni et al., 2006), rice (2402cP) (Ascheri et al., 2012), it can be confirmed that their relatively low amylose content results in a high peak viscosity as established by Sasaki et al., (2000). Other researchers like Oladele and Aina (2007), reported peak viscosities of 9.75 and 16.33 RVU for both the flour of the yellow and black cultivars respectively. Adejuyitan et al. (2009) also reported the highest peak viscosity of 18.75 and 18.18 RVU for the tigernut flours they analysed, after fermenting it for 24 and 48 hours respectively. The structure of the starch gel disrupts easily during heating at lower amylose content (Gupta et al., 2009). From his research on the "Thermal analysis of French bread dough during freezing and thawing: optimization of additive use", Matuda, (2004) posited that, low amylose content, as characterized by high peak viscosity, shows the existence of starch granules with expansion and subsequent eruption during hydrothermal treatment while a high amylose content, as characterized by a low peak viscosity shows the existence of starch granules with a low resistance to expansion/eruption during hydrothermal treatment.

Tziotis et al., (2005) established that trough viscosity shows a negative relationship with amylose content in maize starch. Hu et al., (2004) also concluded that a low trough viscosity is due to a high amylose content. In the starch structure, the amylose component preserves the structure of the swollen granules. A high amylose therefore results in a low trough viscosity because of the rearrangement and positioning of molecules. Results obtained in this study however refutes this claim by Matuda, (2004); Tziotis et al., (2005) and Hu et al., (2004), as a

direct proportion is observed between amylose content and trough viscosity in both varieties of tigernut flour (Black tigernut flour had 21.29 ± 0.33% amylose content and 2445 ±10.61cP trough viscosity; yellowish-brown tigernut flour had 19.22 ± 0.22% amylose content and 2280 ± 115.96cP trough viscosity).

The improvement in the thickness of the starch during cooling is induced by the formation of a thin gel layer of rearranged leached amylose content (Flipse et al., 1996). Since the tigernut flour has low amylose content, it will decrease the quantity of amylose that leaches out which will as a result, suppress viscosity during cooling (Gupta et al., 2009). During cooling, retrogradation occurs. This is gradual reassembling of the starch polymers that were broken apart back to a tightly bound structure. Retrogradation causes quality defects such as, precipitation of starch molecules which are insoluble, gel formations, resurfacing of water from pastes and high viscosity (Swinkles, 1985). This makes retrogradation causes water to be discharged from the bread structure. It also causes staling as the bread will therefore be drier, and its crumbs will be firm. The extent of retrogradation can be determined by the setback viscosity as its value signifies the tendency of retrogradation of the amylose component in starch since the gel structure is dictated by amylose gelation (Huang et al., 2010). According to many studies, starch with a high amylose content corresponds to a high setback viscosity value. (Zaidul et al., 2007; Kurasawa et al., 1972; Swinkles, 1998). The results from this study confirms this statement as tigernut flour obtained from the black variety had a higher amylose content (21.29 ± 0.33%) and a corresponding higher setback viscosity (769 ± 32.53cP) as compared to tigernut flour obtained from the yellowish-brown variety (19.22 ± 0.22% amylose of and set back viscosity of 638.5 ±111cP). While amylose takes a short time to retrograde, amylopectin takes a longer time, and this determines how long a flour product can last before staling occurs. Tigernut flour as compared to wheat flour, has high amylose content and low

amylopectin, this shows that pastries/baked products of tigernut flour products will last for a shorter time compared to products of wheat and other cereal flour.

A lower breakdown viscosity implies a higher stability by the ability to endure heating and shear stress during processing. The lower breakdown viscosity of the black variety indicates its ability to withstand decomposition during heating and shearing as compared to the brown type (Ocheme et al., 2018). It also shows how stable flour from the black variety is as compared to the yellowish-brown variety. Comparing the black tigernut flour to wheat flour which has a breakdown viscosity of 899 cP, also shows that it is even more stable compared to wheat flour even though not as stable as the yellowish-brown variety.

Final viscosity is used to determine the stability as well as the ability to form various paste or gel after cooling. The higher final viscosity of the flours of the two tigernut varieties may be due to the clustering of the amylose molecules (Miles et al., 1985; Makanjuola & Makanjuola, 2018).

The temperature at which viscosity starts to increase when heat is applied is known as the pasting or gelatinization temperature. The pasting temperature of black and yellowish-brown tigernut flours were 79.63 ± 0.18°C and 75.45 ± 0.28°C respectively. The pasting temperature for the yellowish-brown variety was found to be like that which was reported by Ocheme et al. (2018). In similar studies, Makanjuola and Makanjuola (2018) reported that the pasting temperature of corn starch is in the range of 75.08 ℃ to 78.25 ℃. This was lower than the pasting temperature of the black tigernut flour but consistent with that of the yellowish-brown variety. In other studies, values reported by Gupta et al. (2009) for corn starch (70.4°C) and wheat starch (66.4 °C) were found to be lower than those reported for this analysis. The high pasting temperature of the starch of both tigernut flours gives an indication of their resistance against swelling (Makanjuola & Makanjuola, 2018). The pasting temperature gives an idea of

the lowest temperature needed to cook and shows how stable other components in the starch are. Swelling assesses the degree of water absorption during the gelatinization process. The higher water absorption, the higher the swelling capacity. This phenomenon as explained by Oh et al. (2008) occurs during heating, when the amorphous and crystalline components of starch disrupt, and the water molecules bind to the free hydroxyl of amylopectin and amylose. Marta and Tensiska (2017) in their research also proposed that, a low swelling capacity could be credited to the robust relationship between amylopectin and amylose components of starch which forms inelastic starch granules after heat treatments.

5.6.4 Emulsion capacity and Emulsion stability of Tigernut flour

The emulsion capacity and emulsion stability were observed to be higher for the yellowish-brown tigernut flour (50 ± 0.0 %) and (50 ± 0.0%) than for the black tigernut flour (45.5 ± 0.0%) and (45 ± 0%) respectively although their difference was insignificant ($p>0.5$) (Table 5-4).

Table 5-4: Emulsion capacity and stability of tigernut flour

Property	Variety	(% ml/g)
Emulsion capacity	Black	45.5 ± 0.02[a]
	Yellowish-Brown	50 ± 0.04[a]
Emulsion stability	Black	45.5 ± 0.08[a]
	Yellowish-Brown	50 ± 0.10[a]

Values are means of triplicates and ± standard deviation. Since the means for both varieties of tigernut have the same superscripts, it means their properties were not significantly different at $p \leq 0.05$

The values reported for both tigernut cultivars in this analysis were higher than those reported for wheat flour (43.14 ± 2.25%) and (38.08 ± 0.87%) for emulsion capacity and emulsion stability respectively Chandra et al., (2015). Solubility, concentration and pH affect emulsion stability. Kaushal et al. (2012) have established that the key parameter that influences

emulsifying properties is the hydrophobicity of proteins. The ability of proteins to improve the formation and stabilization of emulsions is vital for numerous applications in nourishment items such as cake, solidified sweets and coffee whiteners. In these food products, changing emulsifying and stabilization capacity are needed due to their different compositions and processes (Adebowale et al., 2005). According to Suresh and Samsher (2013), the major functional properties processors look out for during processing include increasing emulsion capacity, stability and fat binding. The higher emulsion capacity and ability of both tigernut flours gives it an advantage over wheat flour and will thus produce a thicker consistency in baking as postulated by Prajapati et al. (2015) as well as an additive for the stabilization of emulsions in cakes baking and soup preparation.

5.6.5 Foam capacity and Foam stability of Tigernut flour

Foam capacity (FC) of the yellowish-brown tigernut flour (13%) was found lower than that of the black variety 14%) (Table 5-5).

Table 5-5: Foam capacity and stability of tigernut flour

Property	Variety	(% v/v)
Foam capacity	Black	14 ± 0.09^{a}
	Yellowish-Brown	13 ± 0.10^{a}
Foam stability	Black	14 ± 0.05^{a}
	Yellowish-Brown	13 ± 0.09^{a}

Values are means of triplicates and ± standard deviation. Since the means for both varieties of tigernut have the same superscripts, it means their properties were not significantly different at $p \leq 0.05$

Although the values reported in this study were higher than those reported by Oladele and Aina (2017), the trends were similar in that, these researchers reported a higher FC for the black variety (11.07%) and a lower value for the yellowish-brown tigernut flour (10.28%). The values reported for the foam capacity for both tigernut flours in this present study was found

to be higher than the values reported by Oshodi et al. (1999) for millet flour (11.30%) and quinoa flour (9%) and by Adegunwa et al. (2017) for pure plantain flour (3.25 ± 1.06/100g).

The values reported for the foam stability in this analysis were lower than those reported by Oshodi and Ekperigin, (1989) for soy flour (14.6%) and Akubor & Badifu (2004) for pigeon flour (20%). The presence of surface-active proteins is what gives flours the ability to foam (Adebowale & Lawal, 2003). Black and Yellowish-Brown tigernut tuber is reported by Emurotu, (2017) to have 7.50% and 7.70% protein content respectively. Narayana and Naransing Rao (1982) posited that, the low foam capacity may be as a result of the low amount of protein in the tigernut flour since foamability is proportional to the amount of solubilized proteins. Kaushal et al. (2012) explains foam stability as the dispersion of proteins which causes a less surface tension at the interface between water and air and hence, is as a result of proteins forming a continuous cohesive film around the air bubbles in the foam. Tigernut flours from both varieties, when compared to wheat flour has a higher foam capacity and stability, which is mainly due to the protein content of these flours or as a result of highly ordered globular proteins, which do not allow for denaturation (Graham and Philips, 1976). According to them, for flour to have good foamability, it needs to have flexible protein components. A study by Adebowale et al., (2005), mentioned that the ability to undergo fast structural changes and rearrangement at the air-water interface during bubbling as well as the ability to adsorb quickly are vital for good foamability. Additionally, the ability to form a cohesive viscoelastic film through the intermolecular interactions is required for foam stability. In their study, Suresh and Samsher (2013), stated the foam capacity and stability of wheat flour as 12.922 ± 5.027% and 1.94 ± 0.048% respectively. Comparing these results of wheat flour to that obtained for tigernut flour in this study shows a higher foam capacity and stability. Flours with high foaming properties are considered suitable for food products like breads, cakes and ice creams because they help maintain their form and structure throughout processing and storage while

contributing to smoothness, lightness and dispersion of flavour (Nawaz et al., 2015; Adebowale et al., 2005). Also, the high foam capacity and stability of the tigernut flours analysed in this current study indicates that, they can serve as suitable replacement of flours or be ideal for composite flours in the making of cakes, salads and breads where high foamability and stability is required (Adebowale et al., 2005).

5.6.6 Bulk density and swelling capacity of Tigernut flour

The bulk density and swelling capacity both varieties of tigernut flour is shown in Table 5-6.

Table 5-6: Bulk density and swelling capacity of tigernut flour

Property	Variety	Result
Bulk Density ($g/cm^{3)}$	Black	0.69 ± 0.00^{a}
	Yellowish-Brown	0.69 ± 0.01^{a}
Swelling capacity (% w/w)	Black	35.75 ± 0.35^{a}
	Yellowish-Brown	37.25 ± 0.00^{a}

Values are means of triplicates and ± standard deviation. Since the means for both varieties of tigernut have the same superscripts, it means their properties were not significantly different at $p \leq 0.05$

The bulk density of the yellowish-brown tigernut flour was like that of the black cultivar. The bulk density reported for both tigernut cultivars in this current study was lower than those reported by Nina et al. (2018) whose values were 0.77 ±0.01 g/cm^3 and 0.76 ±0.01 g/cm^3 reported for the two samples they analysed for the Black and Brown varieties respectively. Researchers like Ayo et al. (2016) reported lower values (0.463 and 0.439 g/ml^3) for the bulk density of the black and brown type respectively.

The values were however like those reported for wheat flour (0.69 g/cm^3) (Oladele and Aina, 2007). Also, the values reported for the bulk density for this analysis were relatively lower

than those reported by Suresh and Smashers et al. (2013) for wheat flour (0.7682 ± 0.006), rice flour (0.914 ± 0.012) and potato flour (0.720 ± 0.009). The density of flour that is measured with no influence of compression is referred to as the bulk density. A high bulk density is suitable for many food applications even though for complementary foods for infants, a low bulk density is required. Flour with high bulk density is required to serve as thickening agents in food products. A low bulk density flour on the other hand, helps to lower the thickening activity, which is much needed for invalids and infant food preparations. Olaitan et al. (2014), posited that, low bulk density has been found to enhance the digestibility of foods for infants with digestive system which have not properly developed whiles high bulk density results in improper growth of children due to the decrease in caloric and nutritional value of its effects. The low bulk density has associated economic and nutritional significances as its consumption leads to higher energy and nutritional density (Nnam, 2001).

The swelling capacity of the yellowish-brown tigernut variety (37.25 ± 0.0%) was found to be greater than the value reported for the black variety (35.75 ± 0.35%) as depicted in Table 5-6. The swelling capacity for both varieties obtained in this analysis was found to be higher than those reported by Ayo et al. (2016) for both black variety (2.86 g/g) and brown variety (2.14 g/g). Also, values reported by Oladele et al., (2007) for the yellow cultivar (2.40 g/cm^3) and the black cultivar (2.10 g/cm^3) was found to be higher than the values reported for both cultivars in this analysis respectively. In comparison to other flours, the values reported in this study, were relatively lower than the highest value (38.38 ± 0.27%) reported by Adegunwa et al. (2017) for the plantain-tigernut composite flour. The swelling capacity for wheat flour (17.80 ±1.85%) and rice flour (15.20 ± 0.84%) (Suresh & Samsher, 2013) were lower than the values reported for this analysis. However, that reported for maize flour (205.98 ± 6.53%) by Nawaz et al. (2015) was relatively higher. Swelling capacity shows the ability of the flour to absorb water and swell during processing. This is a property that is looked out for the most in biscuit

processing as it helps to improve consistency. Comparing the results obtained from this study to that of the popular baking flour (wheat flour), tigernut flour has a higher swelling capacity, indicating it improves consistency better and absorbs water and swells better during processing and as such is ideal as a substitute or to be an added flour to composite flours for the baking industry.

5.6.7 Water absorption capacity and Oil absorption capacity of Tigernut flour

The water and oil absorption capacities of the two varieties of tigernut flour are shown in Table 5-7.

Table 5-7: Water absorption and oil absorption capacity of tigernut flour

Property	Variety	(% ml/g)
Water absorption capacity	Black	219.64 ± 3.726[a]
	Yellowish-Brown	233.3 ± 1.478[a]
Oil absorption capacity	Black	165.85 ± 0.891[a]
	Yellowish-Brown	167.76 ± 0.622[a]

Values are means of triplicates and ± standard deviation. For each property, means for both varieties of tigernut with different superscripts were significantly different at $p \leq 0.05$

According to Osungbaro et al. (2010), the water absorption capacity shows how cohesive an item is. Olaitan et al., (2014) reported that, high water absorption of flours diminishes the retention of nutrients. As indicated by Oyarekua and Adeyeye (2009), water retention limit is needed for enhancing mouthfeel and decreasing the consistency of items. According to Menon et al. (2015), the difference in water assimilation capacity of the composite flour may be attributed to certain factors such as concentration of protein, degree of association with water and conformational qualities.

The water absorption capacity was found to be higher for the yellowish-brown variety (233.32 ± 1.478%) than the black variety (219.64 ± 3.73%). The values were found to be higher than

those detailed by Oladele and Aina (2007). Their reported values were 1.37 and 1.26 ml/g respectively for the yellow and black tigernut variety. The capability of flour to take up water and swell for an enhanced consistency is referred to as the water absorption capacity of the flour. It is of a great importance in food applications as it gives body to food (Osundahunsi et al., 2003). In their work, Olatunde et al., (2016) concluded from their results that, there is a significant correlation between total sugar and water absorption capacity and this correlation is reliable enough to serve as a quality indicator in predicting pasting properties as well. They noticed a significant positive correlation between sugars and the water absorption capacity. Chandra et al., 2015 also posited that, flours with high water absorption capacity have hydrophilic components such as sugars (polysaccharides), and as such, the strong positive correlation. This trend was however not replicated in this work. The tigernut flour from the black variety has a higher total sugar value but a lower water absorption capacity value as against the tigernut flour from the yellowish-brown variety. This can be due to concentration of protein, degree of association with water and conformational qualities as already mentioned.

Suresh and Samsher, (2013) also reported lower values of water absorption capacity (192 ± 10.95) for wheat flour and higher values for potato flour (752 ± 21.68) as compared to the values of tigernut flour obtained in this study. The lack of availability of polar amino acids is a factor that contributes to a low water absorption capacity. A high water absorption capacity is as a result of the loss of the crystalline starch structure and the increase in amylose leaching. Flours with high water absorption capacity are said to have more hydrophilic components like polysaccharides which interact with water in food. They are good components in the processing of foods such as sausages, baked products, processed cheese and dough and tigernut flour is applicable for these food applications due to its high water absorption capacity.

As indicated in Table 5-7, the oil absorption capacity of the yellowish-brown variety (167.76 ± 0.622%) was also greater than that of the black variety (165.85 ± 0.891%). The values were

higher than those reported by Adejuyitan et al. (2009). The highest value reported for the oil absorption capacity for the different tigernut samples analysed was 71.3 ± 0.02 g/ 100g. The oil absorption capacity is the capability of the proteins in the flour to coalesce fat via capillary attraction and is of great significance as it can retain flavours and elevates the mouth feel of foods respectively (Awuchi et al., 2019). Notable, tigernuts have protein levels of about 5g/100g (Sanchez-Zapata et al., 2012). Fat content adversely affects the oil absorption capacity of flours. Also, because protein has both hydrophobic and hydrophilic components, it also greatly affects this property. Jitngarmkusol et al. (2008) explained that hydrophobic interactions occur between the non-polar amino acid side chains and the hydrocarbon chains of lipids. The oil absorption capacity of tigernut flour as obtained from this study gives them relevance in food interactions that bring about flavour retention, extension of shelf life as well as palatability improvement.

5.6.8 Estimation of Shelf life of Tigernut flour using accelerative experimental shelf-life studies

In order to achieve a practical and sustainable shelf-life study, key indicators that are fast, reliable and useful must be used. In the present study, a laboratory experiment was designed and executed to assess the effect of the different temperatures (30 ℃ (ambient); 50℃ and 60 ℃ (elevated)) and storage periods (7 days, 14 days, 21 days and 28 days). pH and titratable acidity, which are indicators of rancidity, were examined throughout the study (Panseri et al., 2011). Moisture content of the flour was also analysed because it is a shelf-life and stability indicator of flour (Gichau et al., 2020).

To determine the shelf life of the tigernut flour of both varieties, the reaction kinetics during the deterioration of flour quality was determined against moisture content, pH and titratable acidity. The Arrhenius plot was constructed and the R^2 values of the linear regression of the different parameters of the flour of the two tigernut cultivars were compared to determine the

best shelf-life index. The activation energy value of the best shelf-life index was calculated to predict the shelf life of the flour at other temperatures.

5.6.8.1 Reaction kinetics of moisture against tigernut flour quality

Figure 5.3 show the zero and first plot for moisture against storage period for the different temperatures of the yellowish-brown and black tigernut varieties. The choice of the order of reaction for the variation of the moisture values at the different temperatures with increasing storage periods was obtained by comparing the R^2 values of the linear regression equation of both the zero and first order plots.

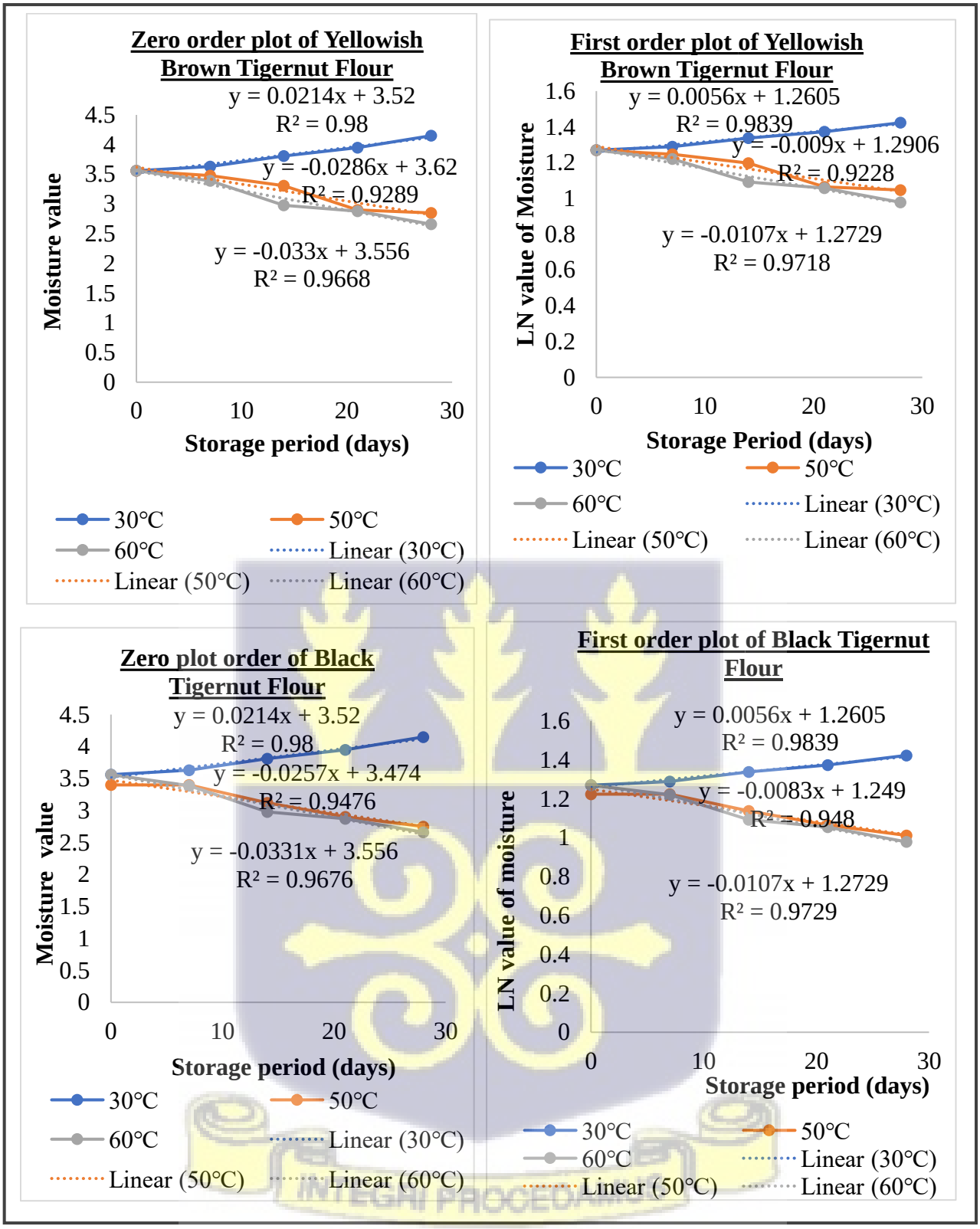


Figure 5.3: Zero and first plots for moisture value against storage period for the different temperatures of the yellowish-brown and black tigernut flours

From the plot, it was observed that, the R^2 value for the first order was greater than that of the zero order at both the black variety (at 60 ℃ and 50 ℃) and the yellowish-brown variety (at 30 ℃ and 60℃). Hence, the decreasing quality of the tigernut flours with respect to the moisture content was a first order reaction for both varieties.

It was observed that, storage temperature affected the moisture content of the tigernut flour of both tigernut varieties. Increasing the storage temperature (50ºC and 60℃) was found to decrease the moisture content of the tigernut flour. This can be explained on the basis that, evaporation of moisture from flour increased with increasing temperature (Ahmed, 2015). At an ambient temperature of 30℃, moisture content increased with increasing storage periods. This observation was consistent with the findings of Ahmed (2015) who reported a similar trend with wheat flour and attributed the phenomenon to the absorption of moisture from the atmosphere.

5.6.8.2 Reaction kinetics of pH against tigernut flour quality

Figure 5.4 show the zero and first plot for pH against storage period for the different temperatures of the yellowish-brown and black tigernut varieties respectively.

The choice of the order of reaction for the variation of the pH values at the different temperatures with increasing storage periods is obtained by comparing the R^2 values of the linear regression equation of both the zero and first order plots. From the plot of both varieties, it was observed that, the R^2 value for the first order was greater than that of the zero order for the black variety (at 50°C and 60°C) and for the yellowish-brown variety (at 50°C and 60°C). Hence, the decreasing quality of the tigernut flour with respect to decreasing pH quality of the tigernut flours was a first order reaction for both varieties.

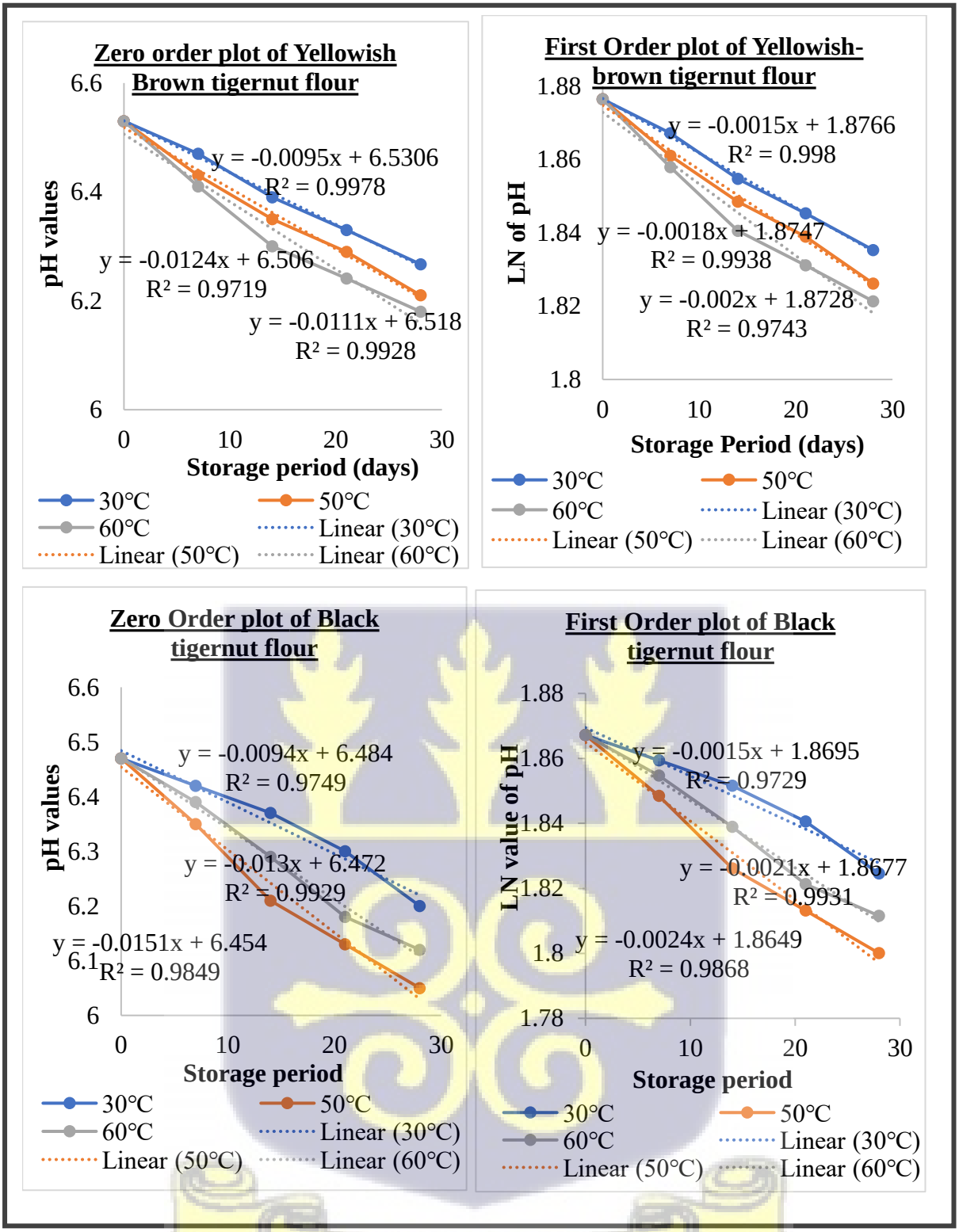


Figure 5.4: Zero and first plots for pH value against storage period for the different temperatures of the yellowish-brown and black tigernut flours

5.6.8.3 Reaction kinetics of titratable acidity against tigernut flour quality

Figure 5.5 shows the zero and first plot for titratable acidity against storage period for the different temperatures of the yellowish-brown and black tigernut varieties respectively.

There was an observed decrease in pH (Figure 5.4) and a corresponding increase in titratable acidity (Figure 5.5) with increasing storage time in all samples, irrespective of their storage conditions. According to Johnson and Decker, (2015), this phenomenon is due to oxidation of the product which is caused by the contact of oxygen with fat contained in the flour. However, pH and titratable acidity of the samples stored under elevated temperatures were significantly higher than that of those stored under ambient temperature. This is due to enhanced autoxidation of lipids, lipoxygenase and photooxidation caused by heat, which breaks down the linoleic and linolenic acids, resulting in a more pronounced increase in acidity (Johnson & Decker, 2015; Oliveira & Arce, 2004).

The choice of the order of reaction for the variation of the titratable acidity values at the different temperatures with increasing storage periods is obtained by comparing the R^2 values of the linear regression equation of both the zero and first order plots. From the plot, it was observed that, the R^2 value for the first order was greater than that of the zero order at 60℃, 30℃ and 50℃ for the black variety. The R^2 of the first order was, however, higher than that of the zero order at 50℃ and 60 ℃ for the yellowish-brown variety. Hence, the decreasing quality of the tiger nut flour of the yellowish and black variety with respect to decreasing titratable acidity quality of the flour was a first order reaction.

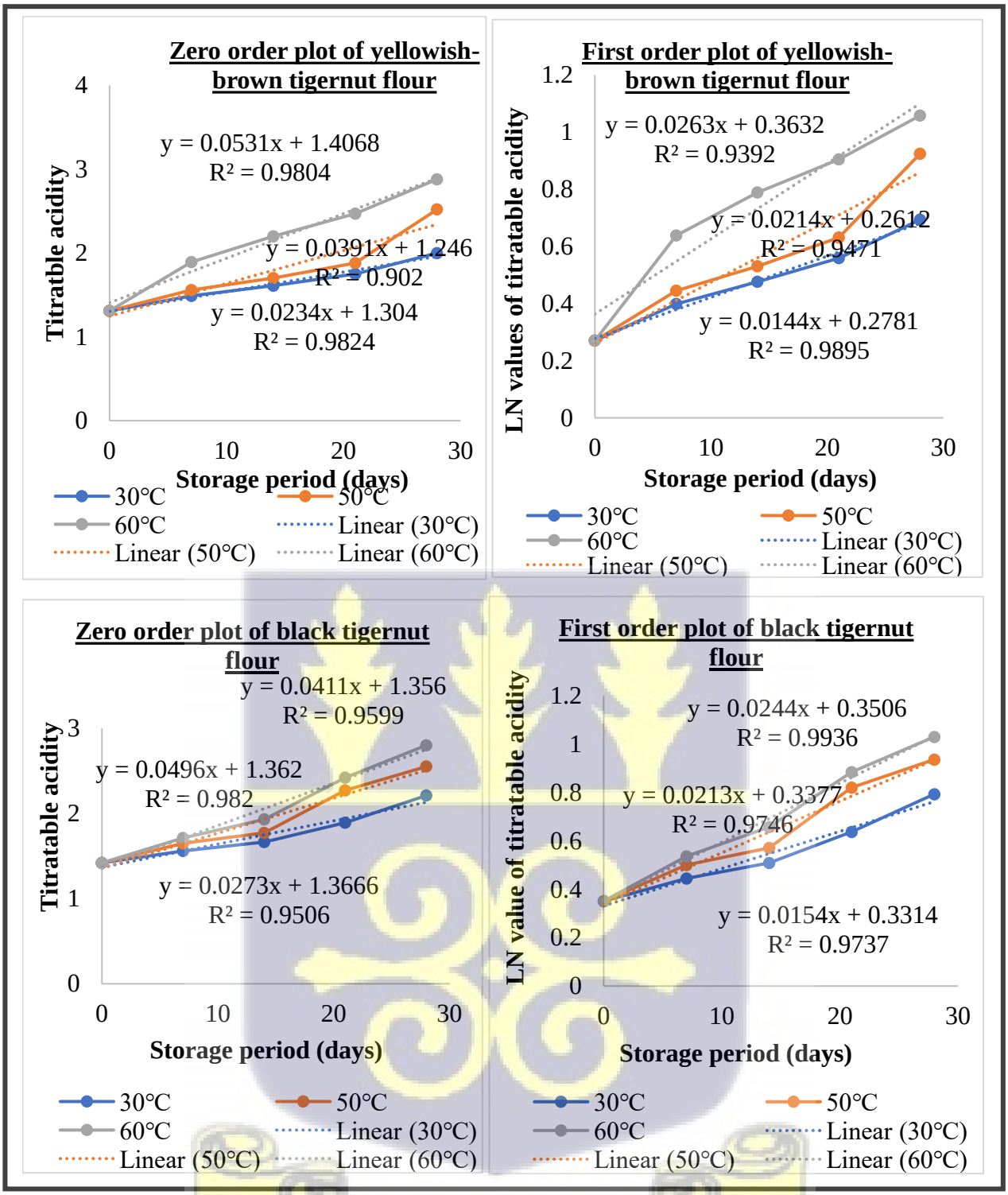


Figure 5.5: Zero and first plots for titratable value against storage period for the different temperatures of the black and yellowish-brown variety

5.6.8.4 Determination of shelf life of flour

The first order graphs (Figure 5.3, Figure 5.4, Figure 5.5) were used to construct the Arrhenius plots of moisture, pH and titratable acidity and results are shown in Figure 5.6.

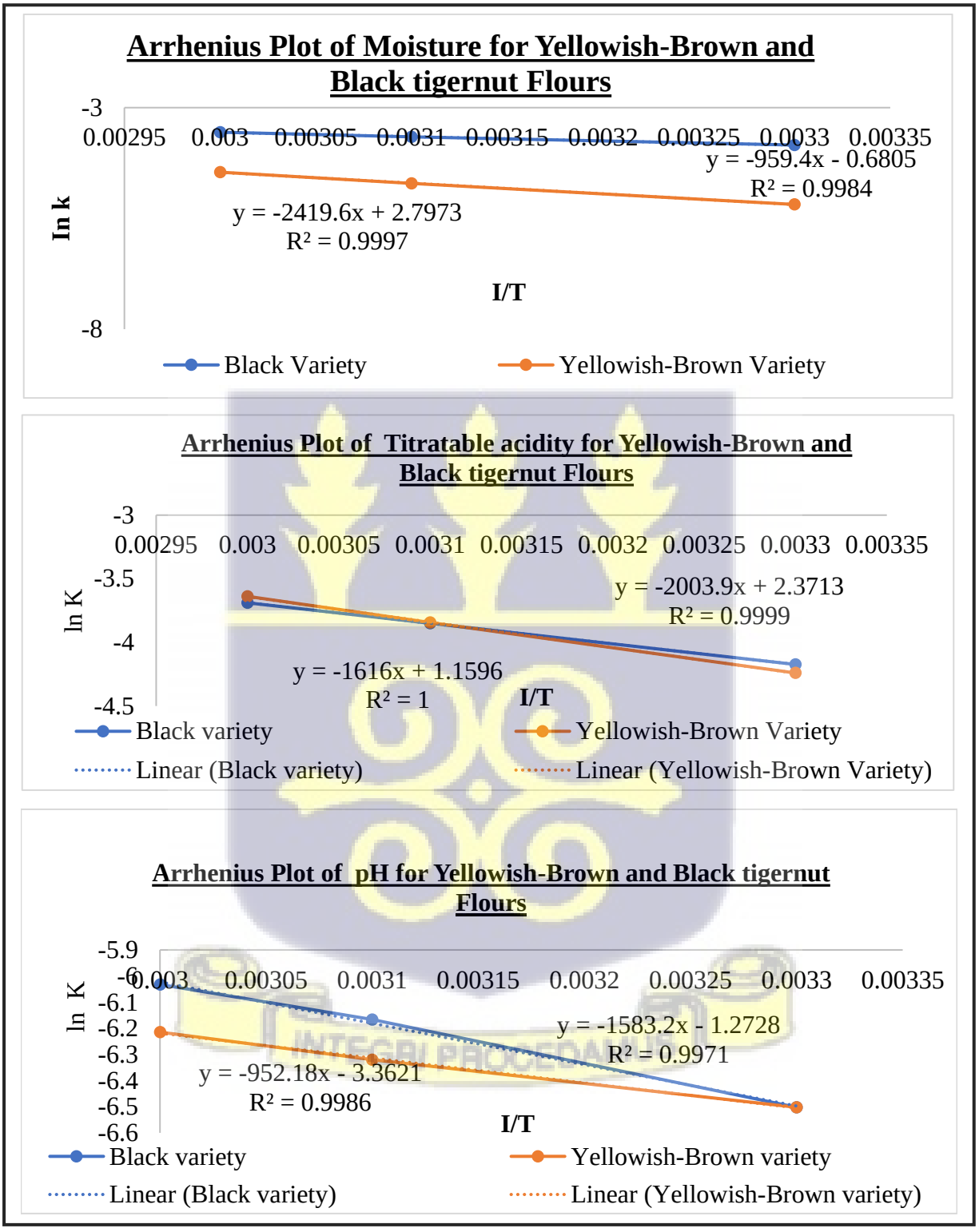


Figure 5.6: Arrhenius plots of the tigernut flours

The Arrhenius equation, corresponding R^2 values and Residual mean square errors of each parameter and variety of tigernut were compared and represented in Table 5-8.

Table 5-8: R^2 value and Residual Mean Square Errors (RMSE) for each parameter for the Tigernut Flour

Variety	Parameter	Arrhenius equation	R^2	RMSE
Yellowish-brown	Moisture	$\ln k = -2419.6x + 2.7973$	0.9997	0.7940
	pH	$\ln k = -952.18x - 3.3621$	0.9986	0.9245
	Titratable acidity	$\ln k = -2003.9\,x + 2.3713$	0.9999	0.8330
Black	Moisture	$\ln k = -959.4x - 0.6805$	0.9984	0.9239
	pH	$\ln k = -1583.2x - 1.2728$	0.9971	0.8709
	Titratable acidity	$\ln k = -1616\,x + 1.1596$	1	0.8678

The shelf-life determination of the tigernut flour was estimated based on the parameter of quality which experienced the fastest decline, that is, the parameter with the highest correlation coefficient value (R^2) (Pulungan et al., 2018) as well as the parameter with a relatively lower Residual Mean Square Error (RMSE) (Sumit & Kumar, 2012). In this study, the parameter with the largest R^2 value for the black and yellowish-brown tigernut flours was titratable acidity (0.9998 for yellowish-brown and 0.9999 for black variety) (Table 5-8).

For the black tigernut flour, the titratable acidity had the lowest RMSE (0.8678) whilst it was the second lowest value for the yellowish-brown tigernut flour (0.8330) indicating further that the TTA is an appropriate indicator for the determination of shelf life of the tigernut flour.

Therefore, tigernut flour spoilage (the decrease in the appearance quality, texture and aroma) during storage under elevated temperatures was found be directly related with increasing titratable acidity. This is consistent with the fact that, higher temperatures lead to higher degree

of lipid oxidation (Liu et al., 2019). Increased lipid oxidation results in the increase in the production of hydroperoxide, which subsequently forms into secondary products of oxidation such as aldehydes and ketones. This results in the development of unpleasant smell and the loss in colour quality. Also, higher storage temperatures result in the incomplete hydrolysis of triacylglycerols (TAG) which leads to the production of free fatty acids (Navneet, 2018). The tigernut flours are prone to lipid autoxidation due to their high fat content and unsaturated fatty acids constituents.

The activation energy of the yellowish-brown and black varieties was calculated as 3209.38 cal/mol and 3979.75 cal/mol respectively, which were used to estimate the shelf life of the tigernut flours at lower temperatures. The lower temperatures were picked to represent ideal refrigeration temperatures (4°C) and ideal dry and cool room temperature for flour (10°C) (Ovca, 2021). Table 5-9 contains the reported shelf life of the flour of the yellowish-brown and black varieties.

Table 5-9: Estimating the shelf life of the tigernut flour using the titratable acidity

Variety	Storage (°C)	K value	Shelf life	
			Days	Months
Yellowish-brown	30	0.0144	29	0.97
	10	0.0106	47	1.6
	4	0.0093	55	1.8
Black	30	0.01540	29	0.97
	10	0.01060	42	1.2
	4	0.00933	47	1.6

From Table 5-9, it was observed that reduction in storage temperature increased the shelf life of the tigernut flours of both cultivars. It is therefore advisable to store the flour at relatively

low temperatures as this will decrease lipid oxidation and the subsequent reduction in the amount of titratable acidity.

5.7 Conclusion

The sugar composition profile of the two varieties of tigernut was studied and it was evident that both varieties had notable quantity of natural sugars, and this quality may make them suitable for inclusion into diets requiring a natural sweet taste and food applications such as flavouring and sweetening agents etc. On the other hand, in terms of the total starch profile, the yellowish-brown variety had a higher total starch content as against the black variety. The specific components within this total starch that gives tigernut its functional properties are the amylose and amylopectin components, with both varieties having significantly more amylose components than amylopectin. This quality may suggest that tigernut starch may be used as a source of flour in baking as it properties may enhance the formation of films and results in less open breadcrumbs. Dietary fibre of tigernuts, in the form of resistant starches were found to be in amounts that were noteworthy. Tigernuts can therefore be incorporated into the diet of diabetic patients since these resistant starches will not be broken down into sugars in the body but will rather reduce the glycaemic index of their foods.

The pasting properties of tigernut showed that the yellowish-brown variety had a higher water-retaining ability and viscosity at heating and holding cycles as compared to the black variety, making flours obtained from the tubers to be resistant to swelling. The high amylose content contributes to the trait of resistance to swelling in tigernut starches and indicates that the starches within the tuber have the capacity to resist decomposition during heating and contribute to retrogradation. The emulsion capacity of tigernut flour were remarkably high. This makes it stand a chance in food applications such as the manufacture of coffee whiteners, cakes and sweets. In addition, the foam capacity and stability were also notably high. Feasible food applications such as baking of pastries such as cakes and bread require this quality as well

as in ice cream making. The bulk density of both tigernut flour was comparatively low. These can therefore be used in weaning food products for children with underdeveloped digestive system as the low bulk density aids with digestion, while providing massive energy and dietary benefits. The water absorption and oil absorption properties of tigernut flours were high. Although the high-water absorption observed in the tigernut flours reduces the preservation of nutrients, the high oil absorption of the flours may enable them to retain flavours and enhance food mouthfeels.

In determining the shelf life of tigernut flour, rancidity indicators (titratable acidity, pH and moisture) were the spoilage parameters considered. For all storage conditions (30^{o}C, 50^{o}C, 60^{o}C), pH decreased while titratable acidity increased. The presence of oxygen triggered autoxidation and the onset of rancidity, hence the increase in acidity. Titratable acidity was identified as the shelf-life indicator of tigernut flour as it had the highest R^2 value with a corresponding low RSME value. Between the two varieties however, the yellowish-brown stayed longer on the shelf as compared to the black variety, mainly due to the higher fat content of the black variety, hence, rancidity was triggered faster. Using the Arrhenius equation, it was predicted that the shelf life of the tigernut flour would increase with decreasing temperature (29 days shelf life for both varieties when stored at 30^{o}C compared to 55 days and 47 days for yellowish-brown and black varieties respectively when stored at 4 ^{o}C).

CHAPTER SIX

6. OBJECTIVE 4: Effects of roasting and addition of alpha amylase on the functional properties of the tigernut milk

6.1 Introduction

Application of heat treatments to tigernuts are noted to have effects on the tuber. Heat treatment does not only affect the quality taste and flavour of the milk but also the composition of the milk and its shelf-life.

In a study conducted by Asante et al. (2014) various cooking methods or application of heat treatments (boiling and roasting) along with soaking and its effects on the tigernuts and its milk yield were analysed. The various cooking or heat treatment methods used included soaking of the tubers only before extraction of milk, cooking before soaking and soaking before cooking or applying heat treatments, where cooking stands for either boiling or roasting.

Results from the study showed that tubers that were boiled before soaking gave the highest milk yield (Asante et al., 2014). This is because boiling facilitates "better opening of cellular pores and weakening of cell walls after milling of tubers". On the other hand, tubers that were soaked before roasting yielded the lowest amount of milk (Asante et al., 2014). The milk from this source however had the most acceptable flavour because roasting improves food flavour (Asante et al., 2014). Taste and flavour of milk from tigernut tubers that were boiled did not have high acceptability as compared to milk from tubers that were roasted before soaking. Aside affecting yields of tigernut milk, heat treatments also affect the composition as well. Sanchez-Zapata et al. (2012) states that the phytochemical composition of raw and roasted tigernuts differed from each other. While raw tigernuts contained higher levels of alkaloids,

sterols and resins and lower levels of cyanogenic glycosides, roasted tigernuts contained only alkaloids and cyanogenic glycosides (Sanchez-Zapata et al., 2012).

According to Djomdi et al. (2020), starch gelatinization due to boiling leads to sedimentation of tigernut milk and may affect the shelf-life and stability of tigernut milk. The use of enzymes in the food industry has been widely exploited to increase the food applications of foods containing large amounts of starches (Fernandes, 2010). The choice of enzyme used in food processing is widely dependent on the food property that is desired (Park et al., 2018). Djomdi et al., (2020) conducted a study in which starch in tigernut milk samples obtained from sprouted tigernuts, native tigernuts and tigernuts that had been soaked in Vitamin C for some time were hydrolysed. Two amylase sources were used which included Termamyl, (an alpha amylase, which is also a commercial enzyme) and amylolytic extracts from the sprouted tigernut tubers. Results from this study revealed a considerable reduction in the starch content of the tigernut milk obtained from the sprouted tigernuts. About 66.66% of the starch content had been converted to reducing sugars (which were not specified). There was, however, an increase in the amount of protein and ascorbic acid present in the tigernut milk. Enzymes are proteins; thus, this change could be attributed to the synthesis of enzyme (Termamyl) (Djomdi et al., 2020).

Kazeem et al. (2013) noted that "alpha-amylase is a noteworthy enzyme in the pancreatic juice and saliva which breaks down large insoluble starch molecules into much smaller and absorbable molecules". Tapsoba (2016) stated that alpha amylase is usually obtained from *Aspergillus orizae*, *Bacillus amyloliquefaciens* and *Bacillus licheniformis*. According to Butterworth et al. (2011) alpha-amylase catalyses the initial step in the digestion of starch. In this case, α-amylase progressively hydrolyses the polysaccharide which leads to the production of maltotriose, limit dextrin and maltose as the main products. The inhibitors of α-amylase are primary proteins and hydrolysis products and the main food applications of α-amylase include

liquefaction of starch, maltose manufacturing, manufacturing of high fructose syrups, manufacturing of oligosaccharides mixture, maltotetraose or G4 syrup manufacture, among others (Tapsoba, 2016). There is limited literature on the addition of alpha amylase to tigernut milk extract. There is also limited literature on the effect of roasting of dried tigernut prior to milk extraction as previous studies have been limited to roasting prior to sprouting which was followed by milk extraction. The purpose of this study was to evaluate the effect of roasting and addition of alpha amylase on the functional properties of the tigernut milk.

6.2 Materials and Methods

6.2.1 Sources of materials

Pre-sorted and cleaned tigernut purchased from markets and streets of the Greater Accra region (Table 3-1- and Table 3-2) were used for the preparation of the tigernut milk extracts.

6.2.2 Sample Preparation of Variants of Tigernut Milk

For each variety of tigernut, the following variants of milk were prepared:

- Fresh tigernut milk;
- Fresh tigernut milk + 0.2% alpha amylase;
- Fresh Tigernut Milk + 0.5% alpha amylase;
- Roasted Tigernut milk;
- Roasted tigernut milk + 0.2% alpha amylase and
- Roasted Tigernut Milk + 0.5% alpha amylase.

Figure 6.1 highlights the process flow for the preparation of the variants of tigernut milk.

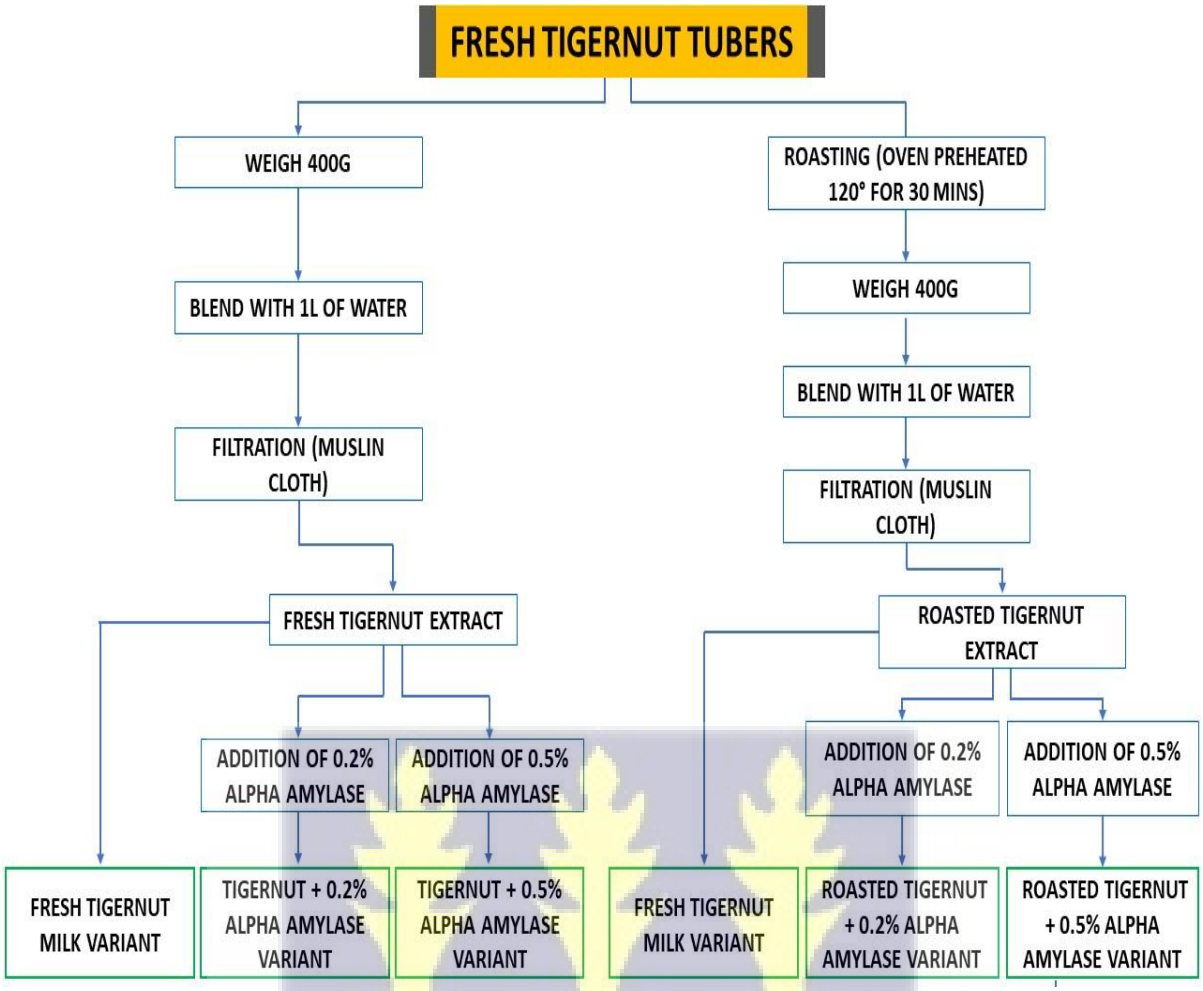


Figure 6.1: Process flow for the preparation of tigernut milk variants used in this study

a) Fresh Tigernut Milk

400g of previously soaked tigernuts (black and yellowish-brown varieties) was blended in an electric blender (Toni 767 Heavy Duty Commercial Grinder Blender) with 1000ml of clean water for 60 seconds. The slurry obtained was paced in a muslin cloth and the milk was squeezed out.

b) Roasted Tigernut Milk

Each variant of clean tigernut was placed in an oven to roast (180°C for 30 minutes). 400g of each variety of roasted and cooled tigernut was blended in an electric blender (Toni 767 Heavy

Duty Commercial Grinder Blender - Germany) together with 1000ml of water for 60 seconds. The slurry obtained was placed in a muslin cloth and the milk was squeezed out.

c) Alpha-amylase hydrolysed variants of tigernut milk

To prepare the tigernut milk + 0.2% alpha amylase variant, 250g of the fresh tigernut milk was homogenised in a blender (Toni 767 Heavy Duty Commercial Grinder Blender - Germany) with 0.5g (0.2%) of alpha amylase. The tigernut milk + 0.5% alpha amylase tigernut milk variant was prepared by blending 1.25g (0.5%) of alpha amylase with 250g of the fresh tigernut milk variants in a blender (Toni 767 Heavy Duty Commercial Grinder Blender - Germany). These same procedures were repeated with the roasted tigernut milk variants to make the roasted tigernut milk + 0.2% alpha amylase and roasted tigernut milk + 0.5% alpha amylase variants.

6.3 Physico-functional properties

All six variants of tigernut milk of both the yellowish-brown and black varieties were subjected to the following analysis to determine the functional properties of each variant: total solids, pH, titratable acidity, emulsion activity, emulsion stability, foaming capability, foaming stability, % brix, colour and flow behaviour.

6.3.1 Determination of total solids in tigernut milk

The Association of Official Analytical Chemists (A.O.A.C) (1990) for total solids determination was used for this analysis with slight modifications. The total solids were calculated after the moisture determination in section 8.5.2.1 using Equation 6.1

$$\%\ \text{of moisture} = \frac{\text{Wet weight (g)} - \text{Dried weight}}{\text{Sample weight (g)}} \qquad \textbf{Equation 6-1}$$

6.3.2 Determination of pH of tigernut milk

The pH of the tigernut milk samples were determined using the A.O.A.C (1990) method. The standard Metrohm 876 Dosimat plus pH meter was well calibrated using a buffer solution of pH of 4 and 7.

6.3.3 Determination of titratable acidity (TA) of tigernut milk

The percentage titratable acidity of each tigernut milk was determined using procedures outlined by Association of Official Analytical Chemist (A.O.A.C) (2012) for total titratable acidity determination with slight alterations.

The sample was mixed thoroughly by pouring it from one container to another. The sample was warmed in a water bath to a temperature of 20 ℃. 20 ml of the milk sample was pipetted into conical flasks after which six drops of phenolphthalein indicator solution was added. The sample was titrated with 0.1 M solution of a standard solution of sodium hydroxide with continuous stirring of the sample with a glass rod until the appearance of a faint pink colour that remained for 30 seconds was observed. The volume of the sodium hydroxide used to reach the end point was recorded. The average titratable acidity was obtained from triplicate determination and calculated utilising the Equation 6-2:

$$\text{TA (\% Oleic Acid)} = \frac{\text{Average titre value x 0.1 M x 90 x 100}}{\text{Volume of milk used x 1000}} \qquad \textbf{Equation 6-2}$$

6.3.4 Determination of %brix of tigernut milk

The % brix of each tigernut milk sample was determined using the refractometric method. The display screen was properly cleaned and standardized using an amount of distilled water at 20 ℃ to 1.333 Refractive index. The double prism of the refractometer was opened with the aid of a screw head and a drop of the tigernut milk was placed on the prism. The % brix readings were taken after the instrument had been left to stand for 1 minute to allow the temperature of

the test sample to be the same as the instrument. The prism was cleaned between readings by wiping off milk with a cotton pad moistened with distilled water and allowing it to dry.

6.3.5 Determination of emulsion capacity of tigernut milk

Pearce and Kinsella's (1978) technique was used for emulsion capacity and stability determination. 50 ml of the tigernut milk sample was homogenised in a blender for 60 seconds with the addition of 30 ml of olive oil. This was transferred into two 50 ml centrifuge tubes, kept in a temperature-controlled water bath at a temperature of 80 °C for 20 minutes and then centrifuged for 30 minutes. The volume of oil separated from the emulsified layer and liquid layer was measured and emulsion capacity calculated using Equation 6-3:

$$\text{Emulsion capacity} = \frac{\text{Volume of oil emulsified}}{\text{Volume of sample}} \text{ x } 100 \qquad \textbf{Equation 6-3}$$

6.3.6 Determination of emulsion stability of tigernut milk

Emulsion stability of tigernut milk was determined by consistently blending 50 ml of sample with 30 ml of olive oil. The emulsion was carefully poured into two 50 ml centrifuge tubes. The total volume, emulsified layer, total oil, and liquid separated during the standing period at room temperature was measured at 60 minutes. Emulsion stability was calculated as measured and emulsion capacity calculated using Equation 6-4:

$$\text{Emulsion stabilty} = \frac{\text{Height of oil emulsified layer}}{\text{Height of whole solution}} \text{ x } 100 \qquad \textbf{Equation 6-4}$$

6.3.7 Determination of foam capability and stability of tigernut milk

Coffman and Garcia's (1977) method was used for the determination of the foam capacity and stability, with minor amendments. Tigernut milk (100 ml) was homogenised with the aid of a

blender and immediately transferred into a 250 ml glass measuring cylinder. The volume increase was recorded and measured as the initial foam volume.

$$\text{Foaming capacity} = \frac{\text{Va} - \text{Vb}}{\text{Vb}} \text{ x } 100 \qquad \textbf{Equation 6-5}$$

Where: Va = volume after homogenization

Vb = volume before homogenization or initial foam volume

To determine the foam stability, 100 ml of the blended homogenized samples was allowed to stand for 60 minutes. The total volume was read after 15 minutes interval. The percentage decrease in foam volume was calculated as foam stability (Equation 6-6).

$$\text{Foam stability} = \frac{\text{Vt x } 100}{\text{Vo}} \qquad \textbf{Equation 6-6}$$

Where Vt = foam volume at 60min

V_o = initial foam volume

6.3.8 Determination colour of tigernut milk

The Chroma meter cr-400 (Konica Minolta Inc, Osaka Japan) was used to evaluate the colour of all 12 variants of tigernut milk. Each milk sample (150ml) was placed in a beaker and the measuring head of the Chroma meter was placed vertically above the sample whiles in display screen. The measure button was pressed after the ready light was switched one. The L*, a*, and b* measurement was recorded, and values compared.

6.3.9 Determination of flow behaviour of tigernut milk

A modified form of the method for the determination of flow behaviour used by Singh and Kaur (2004) (rotary viscometer method) was used for the flow behaviour studies of the tigernut milk. The Thermo Scientific Haake Viscotester D (Thermo Fisher Scientific, Massachusetts, United States) was used in this study. The L2 spindle size was preselected from a few trials.

The spindle was placed in each of the sample and the viscosity was measured at 4 different speeds: 10 rpm, 30 rpm, 50 rpm and 100 rpm to determine the flow behaviour of the tigernut milk.

6.3.9.1 Calculation of Power -law model's coefficients and thixotropic index of tigernut milk

The flow behaviour of the samples was investigated. For a non-Newtonian fluid, the arrangement of the polymer chain is the predominant factor that influences the fluid behaviour (Grabowski and Schmidt, 1994). The flow behaviour and the consistency indices were calculated from the power law equation in Equation 6-7 (Grabowski and Schmidt, 1994) to determine the extent to which the samples exhibited non-Newtonian behaviour.

$$\tau = k\,\gamma^{n} \qquad \textbf{Equation 6-7}$$

Where;

T is the shear stress; K is the flow behaviour index; γ is the shear rate and n is the flow behaviour index

- The thixotropic index was calculated using Equation 6-8

$$\frac{\text{Viscocity at low speed}}{\text{Viscocity at high speed}} \qquad \textbf{Equation 6-8}$$

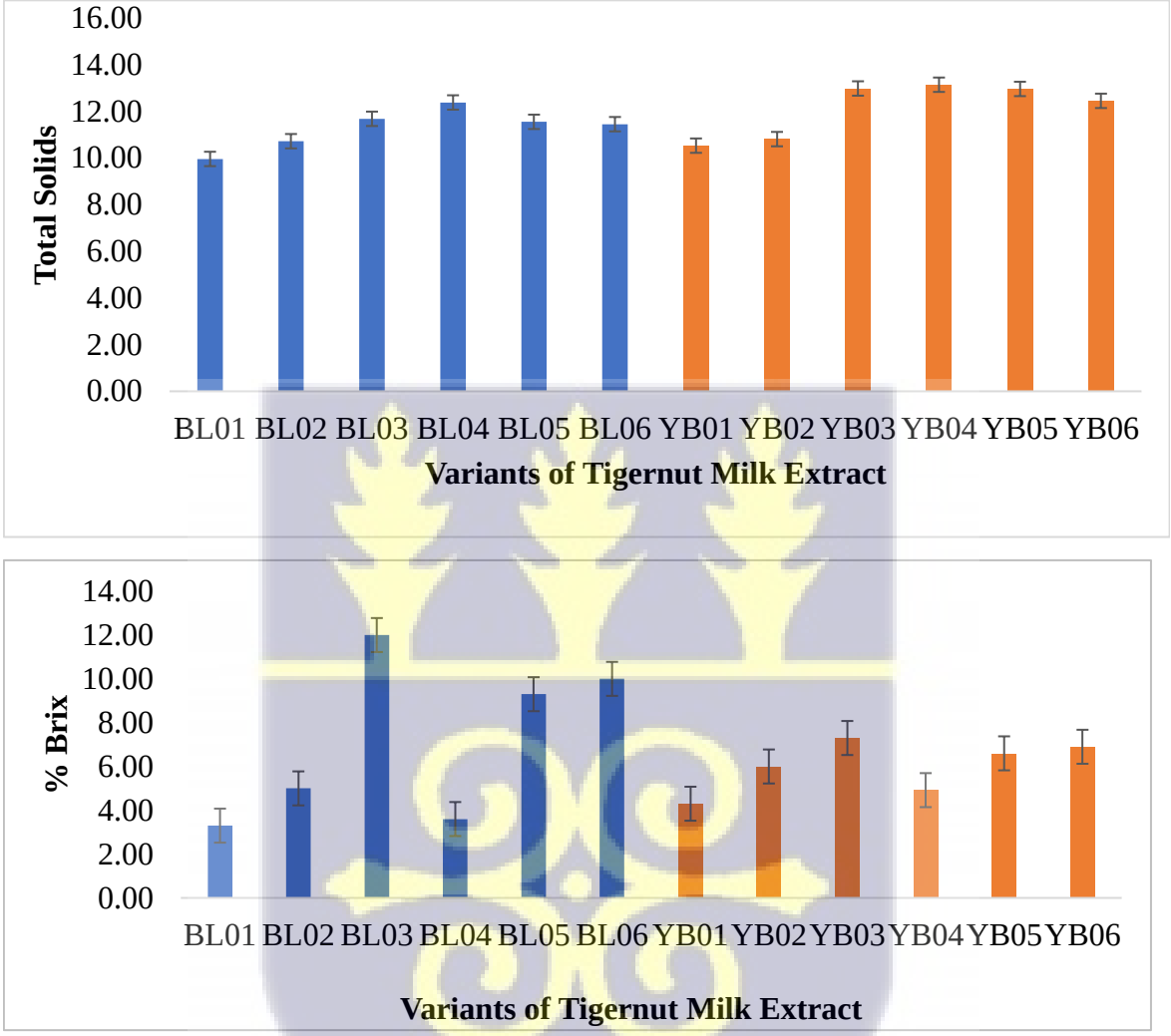
6.4 Statistical Analyses

The Analysis of Variance test was used to establish the significant difference in the functional properties of tigernut milk with respect tigernut variety, heat treatment method (roasting) and enzyme concentration.

6.5 Results and Discussion

6.1.1. Total Solids and % Brix of tigernut milk variants

Figure 6.2 shows the total solids and % brix of the variants of tigernut milk



BL01= Black Fresh;	YB01= Yellowish Brown Fresh
BL02= Black Fresh + 0.2% Alpha Amylase	YB02= Yellowish Brown Fresh + 0.2% Alpha Amylase
BL03= Black Fresh + 0.5% Alpha Amylase;	YB03= Yellowish Brown Fresh + 0.5% Alpha Amylase
BL04= Black Roasted	YB04= Yellowish Brown Roasted
BL05= Black roasted+ 0.2% Alpha Amylase	YB05= Yellowish Brown Fresh + 0.2% Alpha Amylase
BL06= Black roasted+ 0.5% Alpha Amylase	YB06= Yellowish Roasted + 0.5% Alpha Amylase

Figure 6.2: Refractive Index and % brix of Variants of Tigernut Milk

The total solids of milk extracted from the fresh yellowish-brown variety (10.53%) was higher than that obtained from the black tigernut variety (9.96%). This may be because the moisture content of the yellowish-brown variety is less than in the black variety (Suleiman et al., 2018). The total solids of the fresh tigernut milk extract obtained from both varieties (9.96% for black and 10.53% for the yellowish-brown tigernut milk) was significantly ($p<0/05$) lower than those obtained from the roasted tigernuts for both varieties (12.38% for black and 13.14% for the yellowish-brown tigernut milk). The increase in total solids of the roasted tigernut milk samples may have arisen as a result of the formation of new compounds during the heating process. This process is known as dextrinization, where heat breaks down the structure of starch into dextrins (Guha et al., 1997). This increases the soluble materials in the milk, thus increasing the total solids. According to da Rosa Zavareze and Dias, (2011), during heating, glycosidic linkages are also broken, contributing to the increase in total solids. Furthermore, the heating process makes compounds more readily extractable due to the destruction of the cellular matrix (Vignoli et al., 2014).

Addition of 0.2% α-amylase to the fresh tigernut milk did not significantly increase the total solids of fresh tigernut milk of both varieties. However, a significant increase in total solids was only observed in samples that were pre-treated by roasting. A significant increase was observed in the total solids of the roasted variants of yellowish-brown variety. Addition of 0.5% α-amylase significantly increased the total solids of both fresh and roasted variants of the tigernut milk extracts. The general increase in total solids with increasing level of α-amylase is attributed to the increased rate of hydrolysis due to increased enzyme concentration. The increased quantity of α-amylase could also be a reason for the increased total solids in the milk extract, as it is insoluble in the extract. Singh and Kayastha (2014) explain their mechanism as cleaving to the α-1,4 bonds of glucose units in starch and other polysaccharides to produce

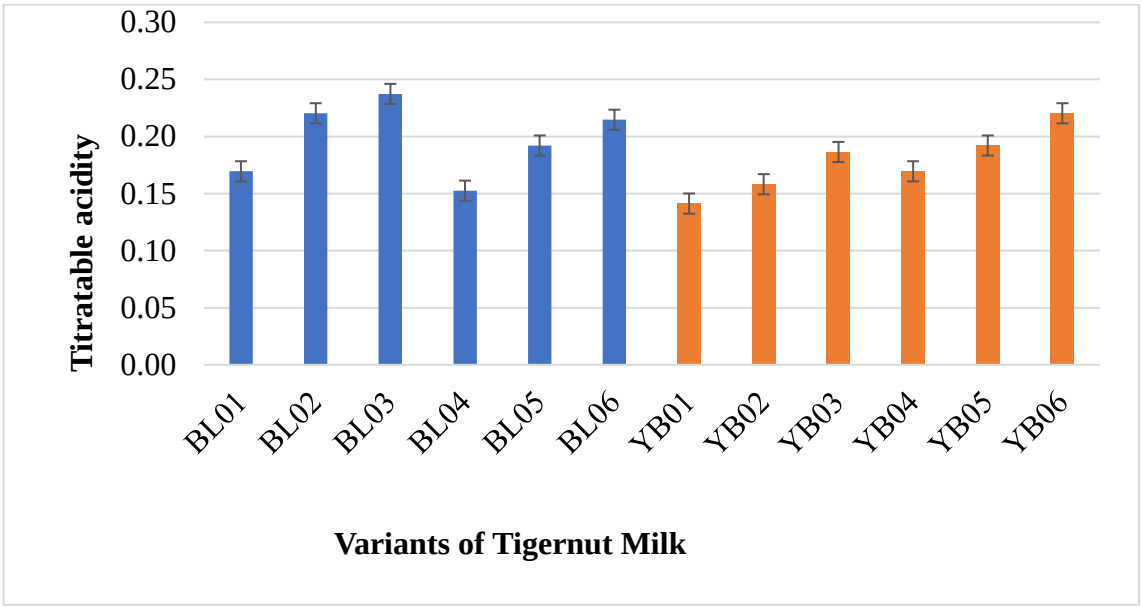
shorter chains of maltose and maltodextrins, thereby increasing the total solids in the milk extracts.

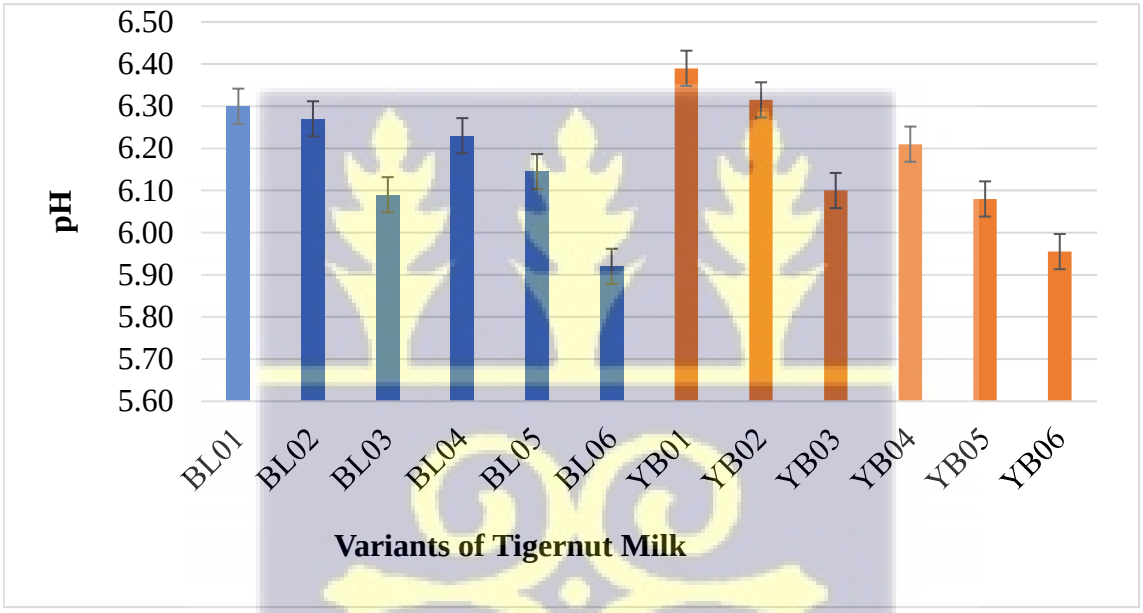
It was observed that the brix of milk obtained from the yellowish-brown variety (4.3%) was significantly ($p<0.05$) higher than that obtained from the black variety (3.3%). The trend is as a result of the higher carbohydrate content of the yellowish-brown variety as compared to that of the black variety (Suleiman et al., 2018). Addition of 0.5% α amylase significantly increased the sweetness of both the fresh and roasted tigernut milk of the black variety. However, there was no significant increase in brix of either the fresh or the roasted of the yellowish-brown variety. The increase in brix caused by the addition of 0.2% of α amylase to the fresh milk of both varieties was insignificant. The brix of the roasted variants of both tigernut varieties were also higher (3.6% for black and 4.92% for yellowish-brown) than the non-roasted (fresh) tigernut milk variants (3.3% for black and 4.3% for yellowish-brown). Owusu-Mensah et al., (2016) attributed this to the increase in maltose content by the oven roasting process and the start of the natural hydrolysis process of the maltose to glucose.

It was also observed that the change in brix of the black variety with the addition of α-amylase was higher than that which was observed with the yellowish–brown variety. This is because the black tigernut tuber contains significantly more amylose than the yellowish-brown variety (Suleiman et al., 2018). According to Tomasik and Horton (2012), the breakdown of amylose occurs more readily than that of amylopectin. This makes tigernut milk suitable as a natural drink with no added sugar as well as a potential flavouring ingredient to produce yoghurt and ice cream due to its natural sweet taste (El-Shenawy et al., 2016).

6.1.2. pH and titratable acidity of tigernut milk

Figure 6.3 shows the pH and titratable acidity of the tigernut milk variants in this study.





BL01= Black Fresh;	YB01= Yellowish Brown Fresh
BL02= Black Fresh + 0.2% Alpha Amylase	YB02= Yellowish Brown Fresh + 0.2% Alpha Amylase
BL03= Black Fresh + 0.5% Alpha Amylase;	YB03= Yellowish Brown Fresh + 0.5% Alpha Amylase
BL04= Black Roasted	YB04= Yellowish Brown Roasted
BL05= Black roasted+ 0.2% Alpha Amylase	YB05= Yellowish Brown Fresh + 0.2% Alpha Amylase
BL06= Black roasted+ 0.5% Alpha Amylase	YB06= Yellowish Roasted + 0.5% Alpha Amylase

Figure 6.3: pH and Titratable acidity of Variants of Tigernut Milk extracts

There was an observed decrease in pH in the roasted yellowish-brown sample analysed. The pH of fresh tigernut milk of the black variety (6.3) was lower than that of the fresh yellowish-brown variety (6.39). However, the titratable acidity of the fresh milk of the black variety (0.169) was higher than that of the yellowish-brown variety (0.158). This trend can be ascribed to the higher fat (fatty acids) content of the black variety (Emurotu, 2017), which makes it more acidic (lower pH with higher titratable acidity).

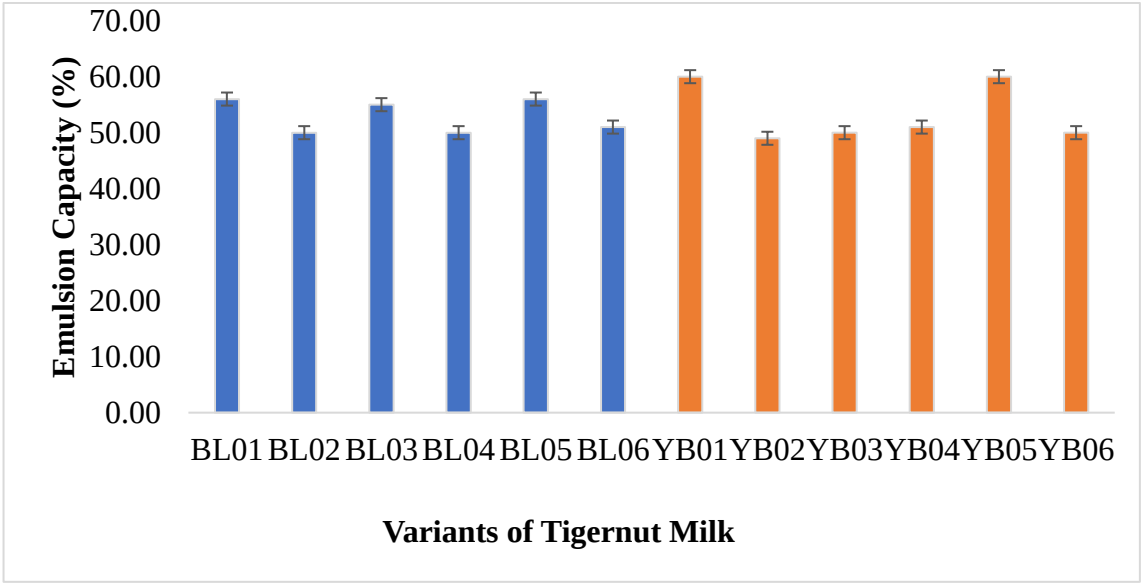
The pH of fresh tigernut milk variants of both yellowish-brown and black varieties were significantly higher with a corresponding lower titratable acidity than their roasted variants. However, the difference in titratable acidity of both varieties of fresh and roasted variants was not significant ($p=0.833$) (Appendix 4). Milk is an oil in water emulsion and therefore, has a large proportion of oils. Roasting, which is a thermal processing and hence involves the addition of heat, facilitates the breakdown and subsequent release of free fatty acids. The decrease in pH and corresponding higher titratable acidity in the roasted variants is accounted for by the formation of aliphatic acids such as glycolic, formic acid during roasting (Ginz et al., 2000).

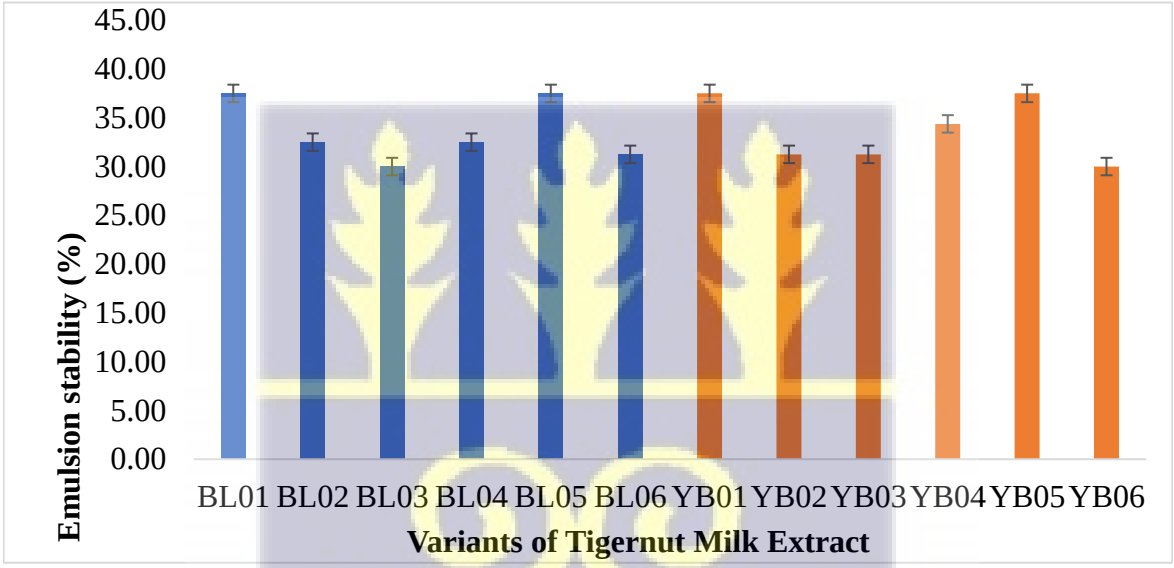
Addition of 0.2% α-amylase significantly changed the pH of the fresh tigernut milk of both variants (Appendix 4Table). However, the change observed in the titratable acidity of the fresh tigernut milk variants of both varieties with the addition of 0.2% α-amylase was not significant. Addition of 0.5% α-amylase to fresh tigernut milk of both variants caused a significant decrease in pH and corresponding significant increase in titratable acidity. This notwithstanding, both the pH and titratable acidity of the roasted variants changed significantly with the addition of 0.2% and 0.5% α-amylase (Appendix 4). This may serve as an advantage against the growth of microbes, as the decrease in pH and increase in titratable acidity with the addition of α-amylase and roasting process serves as a conducive environment to prevent the increase of microorganisms, thereby increasing shelf life.

6.1.3. Emulsion capacity and Emulsion stability of tigernut milk

The emulsion capacity and emulsion stability of the various tigernut milks are shown in Figure 6.4. The emulsion capacity of the fresh yellowish-brown tigernut milk (60) was higher than that of the fresh black tigernut milk (56). This can be attributed to the higher protein content to emulsify the relatively lower fat content of the fresh yellowish-brown tigernut (21.9% fat and 7.70% protein) as compared to that of the fresh black tigernut (23.1% fat and 7.50% protein) (Emurotu, 2017).

It was also observed that the addition of increasing amounts of alpha amylase to the fresh tigernut milk of both varieties directly increased the emulsion capacity of the resulting tigernut milk. However, for the roasted tigernut milk, the emulsion capacity increased with 0.2% addition of alpha amylase but reduced when 0.5% alpha amylase was added. Since the tigernut milk is an oil in water emulsion, there is the need for emulsification of the oil and water phases. Roasting has been reported to increase emulsifying capacity of proteins through Glycosylation reaction (Hernández-Gracia et al., 2016). This occurs when protein side chains undergo alterations in the presence of reducing sugars during a heating process, resulting in the formation of N-glycosides. The resulting protein-sugar conjugates combine the solvation properties of sugars with the emulsifying capacity of proteins increasing the emulsifying properties of the proteins (Du et al., 2013). Furthermore, the increase in sugar concentration as a result of the hydrolysis action of alpha amylase further increases the emulsion properties of the milk solution as increased carbohydrate concentration has been found to increase emulsion properties (Herceg et al., 2007). Therefore, it can be deduced that the addition of 0.5% α-amylase to roasted tigernut milk would result in higher hydrolysis than the same concentration in fresh tigernut variants.





BL01= Black Fresh;	YB01= Yellowish Brown Fresh
BL02= Black Fresh + 0.2% Alpha Amylase	YB02= Yellowish Brown Fresh + 0.2% Alpha Amylase
BL03= Black Fresh + 0.5% Alpha Amylase;	YB03= Yellowish Brown Fresh + 0.5% Alpha Amylase
BL04= Black Roasted	YB04= Yellowish Brown Roasted
BL05= Black roasted+ 0.2% Alpha Amylase	YB05= Yellowish Brown Fresh + 0.2% Alpha Amylase
BL06= Black roasted+ 0.5% Alpha Amylase	YB06= Yellowish Roasted + 0.5% Alpha Amylase

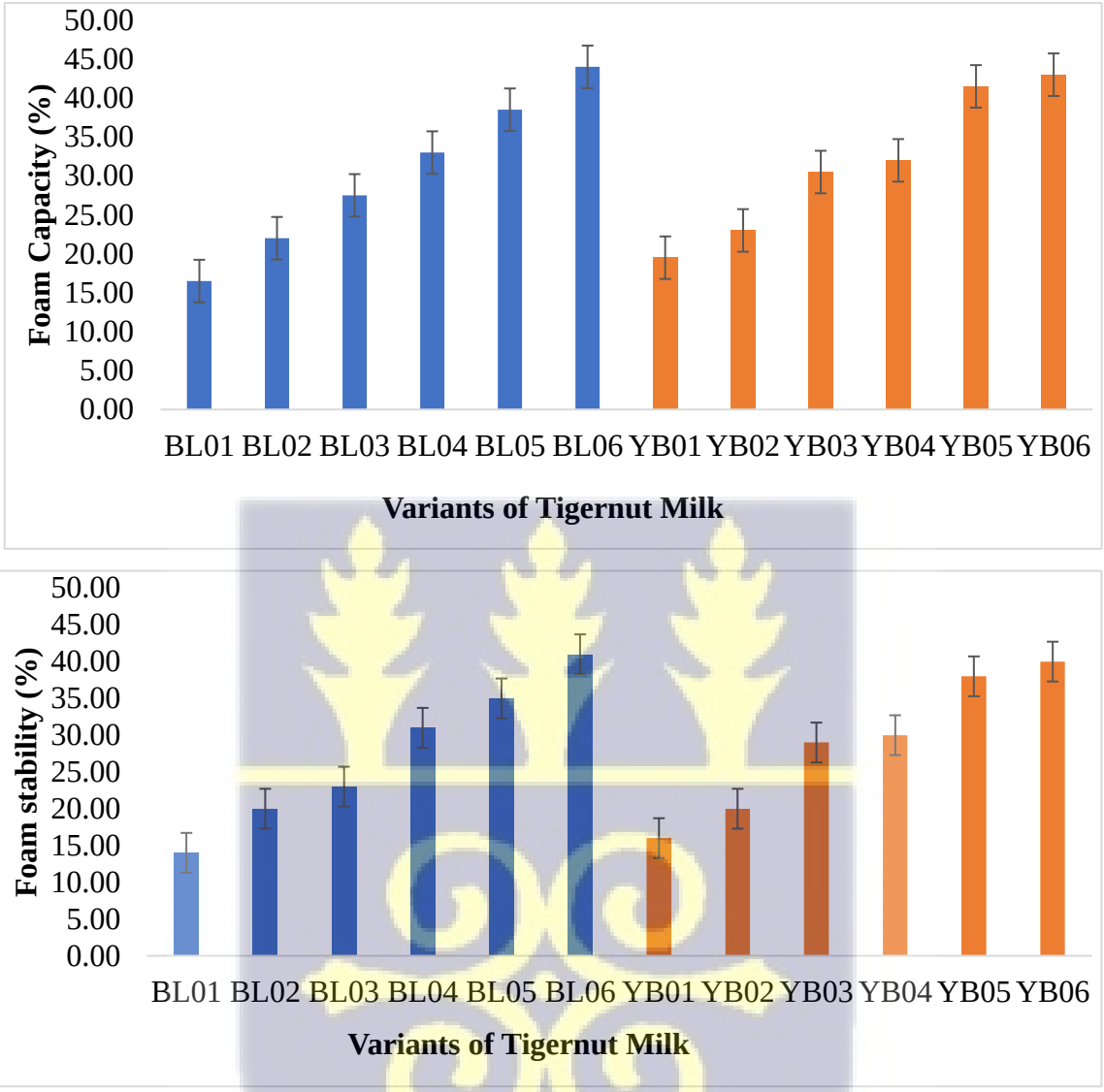
Figure 6.4: Emulsion Capacity and Emulsion Stability of Variants of Tigernut Milk

Higher hydrolysis induces unduly decreased surface hydrophobicity and increase surface load, which might negatively affect the emulsifying activity as suggested by Liu et al. (2019). This reason can also be attributed to the decreased emulsion stability of all variants in comparism to the fresh tigernut milk variant.

Whereas most of the variants were significantly different ($p<0/05$) in their emulsifying stability as compared to the fresh tigernut milk variants, the emulsion stability was not significantly different between the fresh samples and the roasted samples with addition of 0.2% α-amylase (Appendix 4). For this reason, the roasted variant with 0.2% α-amylase is recommended as it is closest to the emulsion stability of the fresh without the unwanted effect of starch sediments. This could serve as an advantage for processing tigernut milk since a lower concentration of enzyme, which is an added production/conversion cost, is needed to achieve the desired emulsion capacity and stability.

6.1.4. Foam capacity and foam stability of tigernut milk

Figure 6.5 shows the variation in foam capacity and foam stability of tigernut milk.



BL01= Black Fresh;	YB01= Yellowish Brown Fresh
BL02= Black Fresh + 0.2% Alpha Amylase	YB02= Yellowish Brown Fresh + 0.2% Alpha Amylase
BL03= Black Fresh + 0.5% Alpha Amylase;	YB03= Yellowish Brown Fresh + 0.5% Alpha Amylase
BL04= Black Roasted	YB04= Yellowish Brown Roasted
BL05= Black roasted+ 0.2% Alpha Amylase	YB05= Yellowish Brown Fresh + 0.2% Alpha Amylase
BL06= Black roasted+ 0.5% Alpha Amylase	YB06= Yellowish Roasted + 0.5% Alpha Amylase

Figure 6.5: Foam capacity and Foam stability of Variants of Tigernut Milk

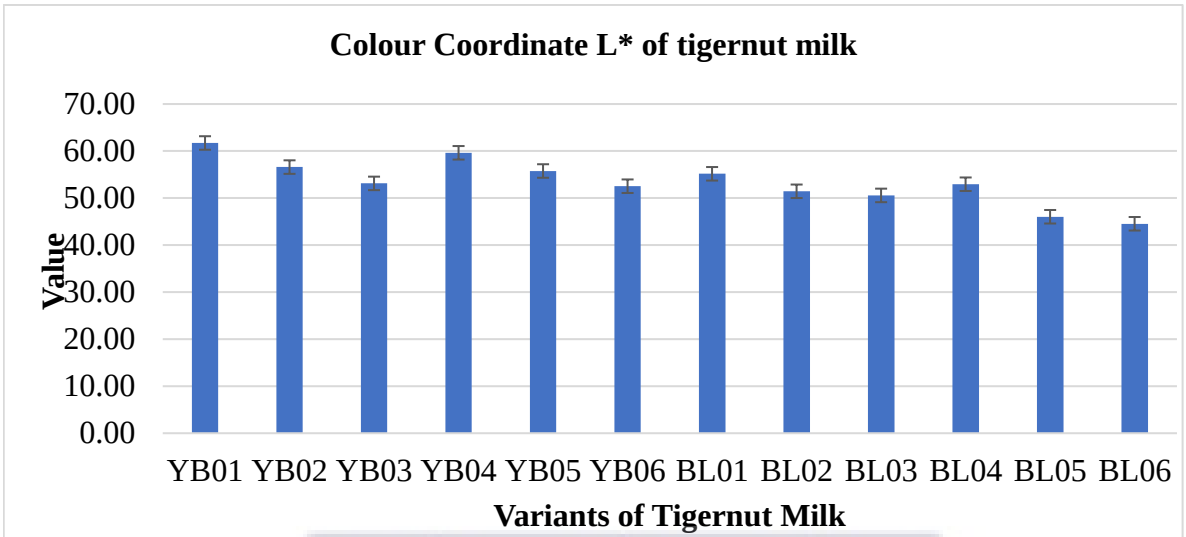
Fresh yellowish-brown tigernut milk had foam capacity (19.5%) and stability (16%) higher than that of the fresh black tigernut milk (16.5% and 14% respectively). This may be as a result of the higher protein levels of the yellowish-brown tigernut milk as compared to that of the black (7.70 % for yellowish-brown and 7.50% for black tigernut (Emurotu, 2017)). Proteins reduce the surface tension at the liquid-air interface, increasing the possibility of solution foaming, and under certain conditions (Asghari et al., 2016).

There was observed significant ($p<0.05$) increase of the foaming capacity and stability of roasted variants of both varieties (Appendix 4). Research has shown that, heat treatment denatures the proteins, thereby converting it into their soluble forms (Chukwuma et al., 2016). This may account for the increase in the foaming properties. It was further observed that, the foam capacity and stability of all the variants of tigernut milk decreased with increasing α-amylase. The presence of carbohydrates has been reported to enhance the foaming protein suspensions (Herceg et al., 2007). The α-amylase is responsible for hydrolysing the starch molecules into smaller chains, thereby increasing the amount of starchy (maltose) molecules in the milk samples. This accounts for why addition of the enzymes further enhanced the foaming properties of the milk.

All the changes that occurred with the addition of the α-amylase and the roasting were significant except the foam stability of the yellowish-brown tigernut milk with the addition of the 0.2% α-amylase to the fresh samples (Appendix 4).

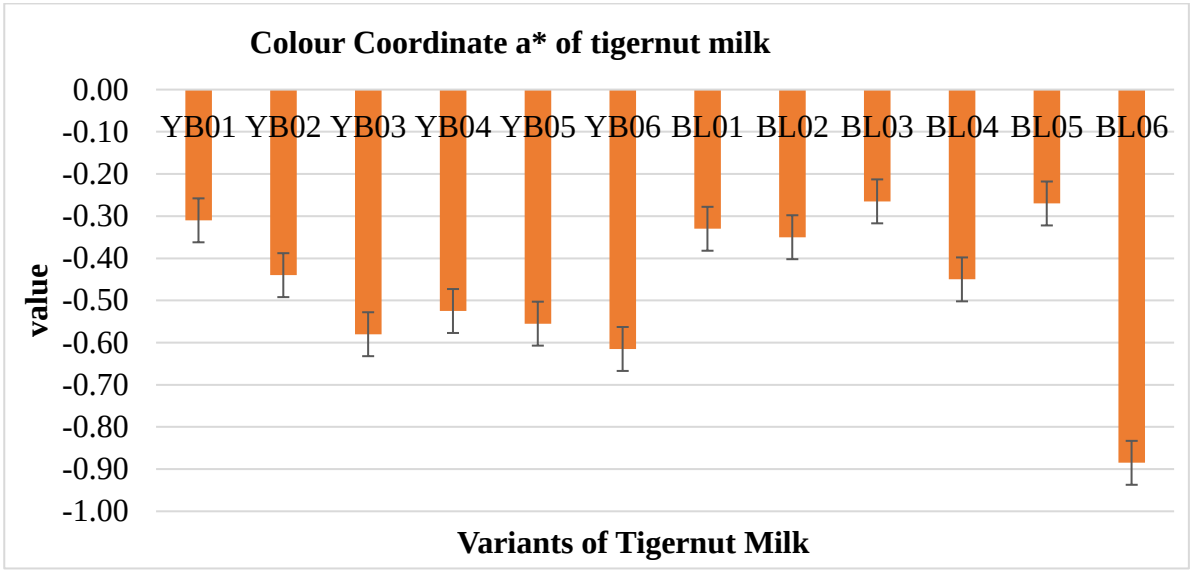
6.1.5. Colour of tigernut milk variants

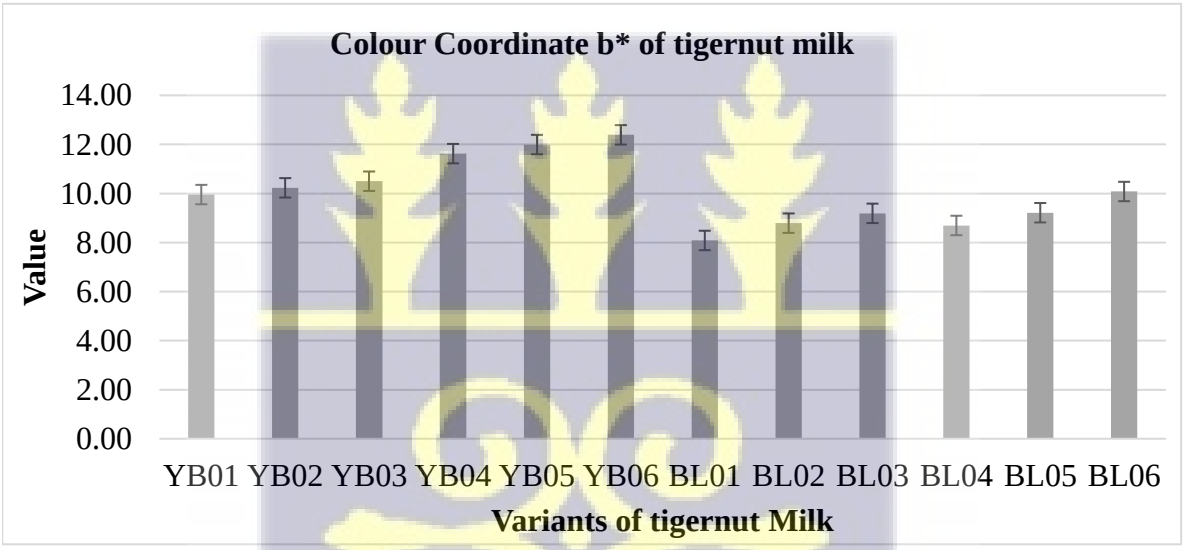
The colour of the various tigernut milk are shown in Figure 6.6 and Figure 6.7



BL01= Black Fresh;	YB01= Yellowish Brown Fresh
BL02= Black Fresh + 0.2% Alpha Amylase	YB02= Yellowish Brown Fresh + 0.2% Alpha Amylase
BL03= Black Fresh + 0.5% Alpha Amylase;	YB03= Yellowish Brown Fresh + 0.5% Alpha Amylase
BL04= Black Roasted	YB04= Yellowish Brown Roasted
BL05= Black roasted+ 0.2% Alpha Amylase	YB05= Yellowish Brown Fresh + 0.2% Alpha Amylase
BL06= Black roasted+ 0.5% Alpha Amylase	YB06= Yellowish Roasted + 0.5% Alpha Amylase

Figure 6.6: Colour Coordinate L* of tigernut milk





BL01= Black Fresh;	YB01= Yellowish Brown Fresh
BL02= Black Fresh + 0.2% Alpha Amylase	YB02= Yellowish Brown Fresh + 0.2% Alpha Amylase
BL03= Black Fresh + 0.5% Alpha Amylase;	YB03= Yellowish Brown Fresh + 0.5% Alpha Amylase
BL04= Black Roasted	YB04= Yellowish Brown Roasted
BL05= Black roasted+ 0.2% Alpha Amylase	YB05= Yellowish Brown Fresh + 0.2% Alpha Amylase
BL06= Black roasted+ 0.5% Alpha Amylase	YB06= Yellowish Roasted + 0.5% Alpha Amylase

Figure 6.7: Colour Coordinate a* and b* of tigernut milk

The whiteness colour, L*, of the milk obtained from the fresh yellowish-brown tigernut was lower than that obtained from the fresh black tigernut, whilst it had lower a* and b* values. The influence of roasting and α-amylase addition on the colour property of the tigernut milk were examined. From the measurements obtained, it was observed that, the roasted samples had lower L* (Lightness) inferring that they were darker samples. In contrast, the values of a* (redness) and b* (yellowness) of the roasted samples were higher than the fresh samples for both varieties. Grigioni et al. (2009) associated the decrease in the L* values to Millard reaction which leads to the formation of brown colouring. The brown colouring occurs as a result of the formation melanoidins (browning compounds) (Shimamura & Hiroyuki, 2012) produced because of the chemical interaction between milk proteins and sugars (glucose, fructose and sucrose) (Hernández-Gracia et al., 2016). The subsequent effect of this reaction is evident in the a* and b* values which was observed in this study.

Also, the fresh and roasted samples for both the black and yellowish-brown cultivars containing the α-amylase showed lower values for L* and higher values for a* and b* as compared with samples without the enzyme. This phenomenon is because the action of the α-amylase on the starch molecules leads to the production of more glucose molecules. This increased amount of glucose molecules enhances the formation of the Millard reaction as glucose is considered a precursor for the reaction (Ganesan & Benjakul, 2014).

The values for the roasted samples containing the enzymes for both varieties recorded the lowest and highest values for L* a* and b* respectively. This is because of the increased Millard reaction in these samples as a result of the combined effect of the roasting and the α-amylase. The colour of the α-amylase (brown in colour) could be a contributing factor for the deeper cream colour of the samples containing it.

From the Appendix 4, there was significant difference between all colour categories. The lightness (L*) of the fresh samples compared to all other variants of tigernut milk were significantly higher except those of the roasted samples. Addition of 0.2% alpha amylase did not significantly change the a* and b* colour constituents of fresh tigernut milk. This shows that it is difficult to maintain the colour of the fresh tigernut milk variant once heat and other ingredients are included in the manufacturing process. Comparing the L*, a* and b* colour constituents of tigernut milk to other milks, the L* colour constituent of dairy milks ranged from 66.5 to 95.9, showing that the tigernut milk was darker in colour. The a* and b* values for dairy milks range from -0.2 to 20.6 and -7.3 to 28.8 respectively. The a* and b* values are more closely related to those of tigernut milk than the L* colour constituent.

6.1.6. Flow behaviour of tigernut milk variants

The flow behaviour of all variants of the black and yellowish-brown tigernut milk were analysed at 10, 30, 50 and 100 rpm and results are presented in Figure 6.8 and Figure 6.9 respectively.

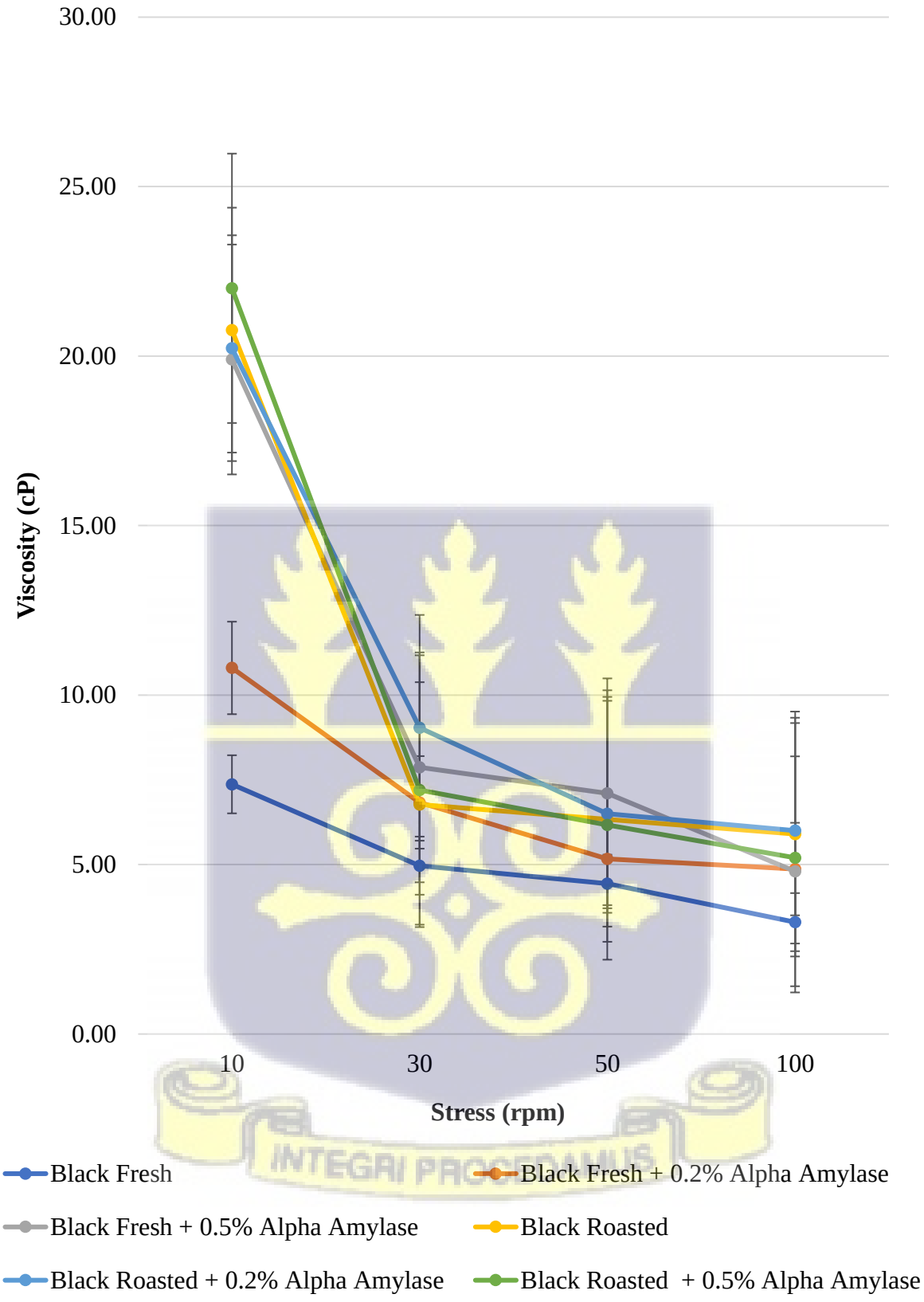


Figure 6.8: Flow Behavior of Black Tigernut Milk Variants

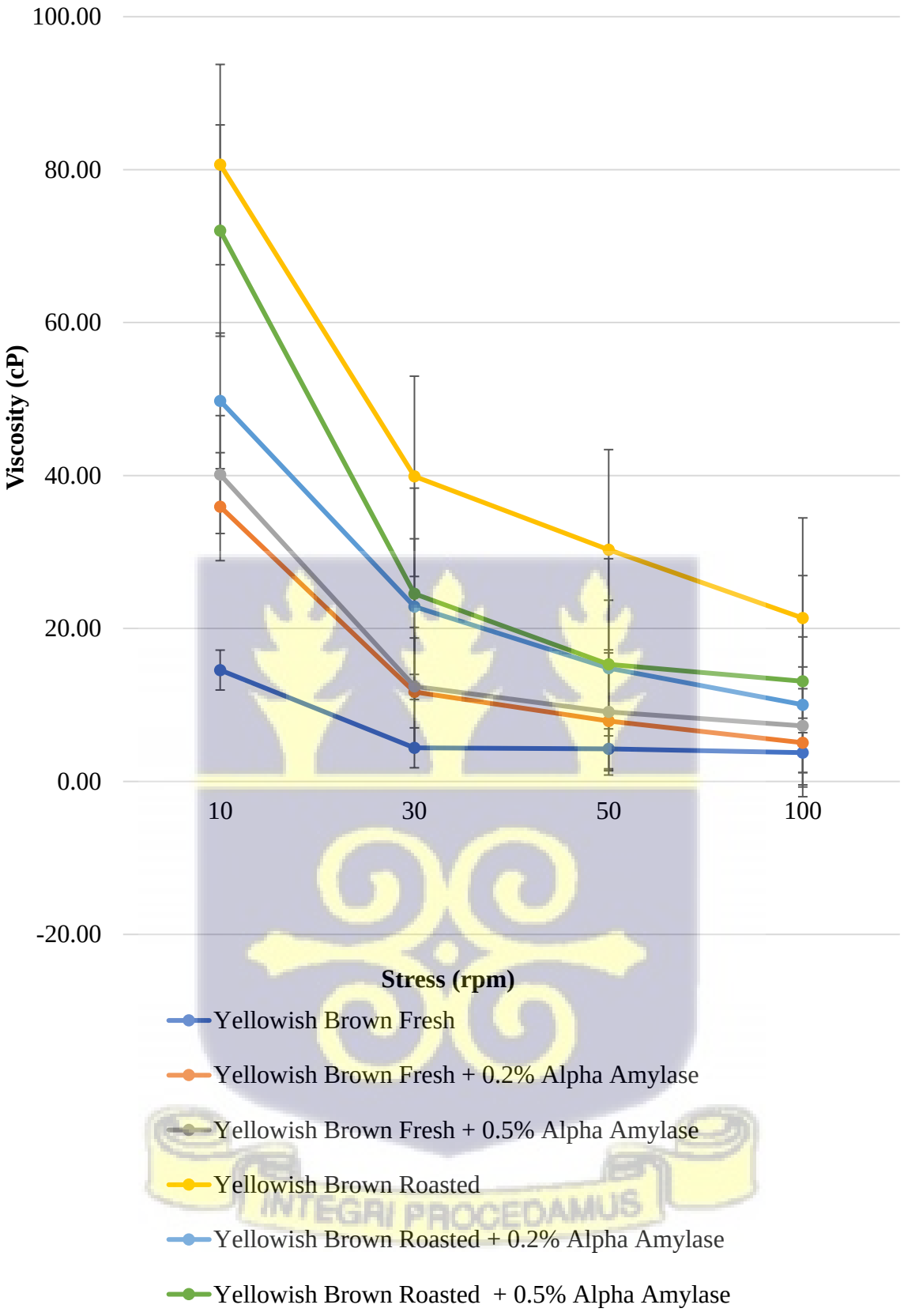


Figure 6.9: Flow Behavior of Yellowish-Brown Tigernut Milk Variants

It was observed that, the viscosity of the fresh yellowish-brown tigernut milk (14.57cP) was significantly higher than that of the fresh black tigernut milk variant (7.37cP) at 10rpm. This could be because the yellowish-brown tigernut tuber contained more starch than the black tigernut tuber (Emurotu, 2017).

The influence of roasting and effect of the addition of α-amylase on the flow properties of the milk variants were investigated. Viscosity was found higher in the roasted samples than the fresh samples for both varieties. This is because heating is known to break down starch molecules and this greatly affects how well they can flow. Samples with α-amylase showed a further increase in viscosity and restricting flow. This can be accounted for by the fact that, the addition of the amylase further hydrolyses the starch molecules thus allowing the broken-down starch molecules to flow in the fluid and causing restrictions in the flow.

It was observed that, the viscosities of all the samples reduced with increasing stress (speed), which classifies them as non-Newtonian fluids according to Eberhard et al., (2019). In non-Newtonian fluids, there is no linear correlation between shear stress and shear rate due to deformation during processing and the complicated structure of the components in the materials. To determine the extent to which the tigernut milk exhibited non-Newtonian behaviour (change with increasing stress), the flow behaviour index (k) and the consistency index (n) were determined. A graph of log τ(shear stress) was plotted against log γ (shear rate) from power law model (Equation 6-9) to graphically determine n and k from the slope and intercept of the graph respectively for all the samples analysed. Table 6-1 summarizes the values of n, k and R^2 (Statistical correlation coefficient).

Table 6-1: Flow behaviour index, consistency index and R^2 of tigernut milk

Samples	Flow behaviour index (k)	Consistency index (n) (Pa-s)	R^2
Black Fresh	0.34	0.0162	0.9931
Black Fresh + 0.2% Alpha Amylase	0.36	0.0240	0.9472
Black Fresh + 0.5% Alpha Amylase	0.61	0.0744	0.9607
Black Roasted	0.61	0.0617	0.8187
Black roasted+ 0.2% Alpha Amylase	0.55	0.0653	0.9288
Black roasted+ 0.5% Alpha Amylase	0.64	0.0811	0.8957
Yellowish Brown Fresh	0.59	0.0466	0.8248
Yellowish Brown Fresh + 0.2% Alpha Amylase	0.86	0.2412	0.9865
Yellowish Brown Fresh + 0.5% Alpha Amylase	0.76	0.2003	0.9388
Yellowish Brown Roasted	0.58	0.2982	0.9960
Yellowish Brown Roasted + 0.2% Alpha Amylase	0.71	0.2500	0.9962
Yellowish Roasted + 0.5% Alpha Amylase	0.77	0.3783	0.9446

From Table 6-1, shear-thinning was observed in all the samples as the flow behaviour index (n) was less than 1 in all sample variants, reinforcing the non-Newtonian behaviour of the milk. Chamberlain et al., (1999) established that the more Newtonian a fluid is, the larger the deviation of the flow behaviour index from 1. It was also observed that, flow behaviour index increased with the addition of alpha amylase and roasting (application of heat). This suggests that heat treated tigernut milk (roasting) caused the milk to be much closer to a Newtonian fluid compared to the fresh tigernut milk. Additionally, the breakdown of the starch into smaller chains also caused similar effects.

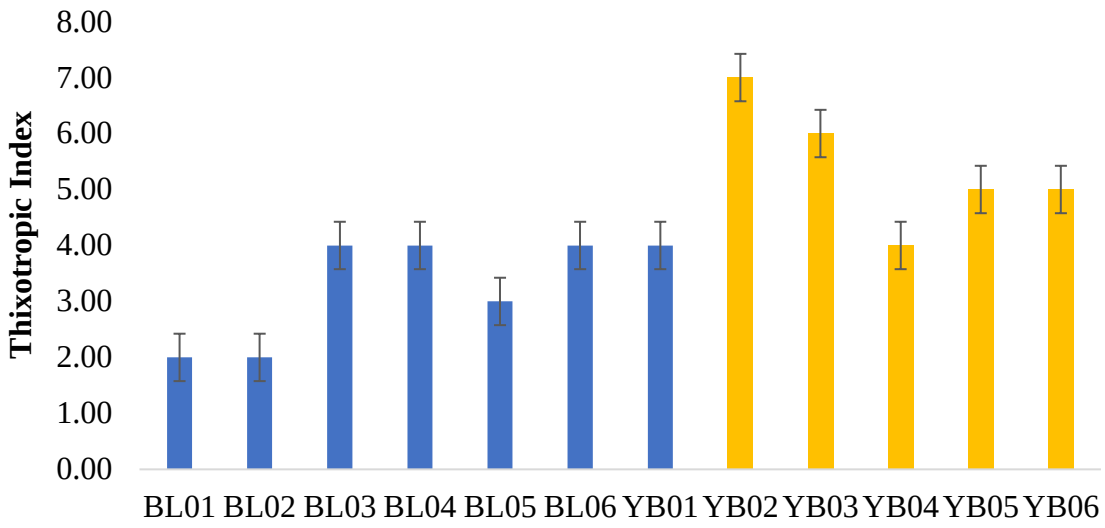
The arrangement of the polymer chains is the main parameter that affects fluid behaviour of non-Newtonian fluids. Roasting and the addition of alpha amylase increased the shear rate of the milk, which in turn increased the number of polymer segments, and consequently decreased viscosity, reiterating the non-Newtonian behaviour of the milk.

From the results obtained, it was observed that, in both tigernut varieties, the consistency index increased when the heat was applied. Also, a difference was observed in the consistency index of the two varieties with the yellowish-brown variants being higher than the black variants. According to Kermani et al., (2016), a high consistency index indicates that samples have a low resistance to flow. This is because the increased temperature leads to increased movement of molecules, which in turn increases the spacing between molecules and as such decreases flow resistance. The consistency index also increased with the addition of alpha amylase and heat (roasting). According to Müller et al., (2020), samples with enzyme application had a significantly lower apparent viscosity. Granule size also influences consistency index. Chamberlain et al., (1999) states that consistency index is exponentially correlated to granule size. When granules are far apart and properly distributed, there is no significant interaction and as such consistency is unaffected. However, once they are closely packed together, there is an increase in molecular interaction and subsequent effect on the consistency (Okechukwu and Rao, 1995). Micrographs of starch granules of yellowish-brown varieties, according to Akonor et al., (2019), showed a uniform amount of loosely packed granules, both small and large while the black variety was dominated by small granules that were crowded and closely packed. According to their study, both varieties had starch granules which were round and smooth even though the black variety had more smaller granules, which affected the consistency index.

Pearson's R^2 value usually ranges from -1 to 1. According to Elblbesy and Hereba, (2016), a R^2 value of -1 shows a negative linear correlation, a R^2 value of 0 indicates no correlation and

a R^2 value of 1 indicates a positive linear correlation. From the results obtained in this study, the fresh black variety had a R^2 value close to 1, showing a strong correlation between its variables. This however decreased with the addition of alpha amylase and further decreased when roasted, showing that, pre-treatment decreased the correlation between the rheology variables. The opposite was observed for the yellowish-brown variety, where the fresh sample had a lower R^2 value which further increased with the addition of alpha amylase and with heat in the form of roasting.

To confirm the extent to which the viscosities of the tigernut milk extract changed over time, the thixotropic index was calculated using the viscosities at 100rpm and 10 rpm and the results are represented in Figure 6.10. When thixotropic fluids are at rest, they tend to form a system within their molecular structure which increases the viscosity of liquids. Hence, in order to change this viscous liquid back to solid, an external force is needed to break the intermolecular system in order to reduce the viscosity. This is however dependent on time because once viscosity reduces the fluid at rest, the strong intermolecular structure is reformed (Schramm, 2000). The average thixotropic index of all the samples was 4 ±1.4, whereas that of the black and yellowish-brown varieties were 3 ± 0.89 and 5 ±1.23 respectively. According to Basu et al. (2007), fluids with thixotropic index greater than one are classified as thixotropic. This means that, samples exhibit a stable form whilst at rest but becomes more fluid when agitated and over time This behaviour of the liquid can inform about the type of packaging suitable for tigernut milk. Tigernut milk should be packaged in containers/bottles with wider necks to prevent spillage as agitation and capillary pressure can cause them to rise when the bottle lids are open. Although milk is a Newtonian fluid, the thixotropic behaviour of tigernut milk make them suitable for yoghurt, pudding and ice cream manufacture (Khetra et al., 2018).



BL01= Black Fresh;	YB01= Yellowish Brown Fresh
BL02= Black Fresh + 0.2% Alpha Amylase	YB02= Yellowish Brown Fresh + 0.2% Alpha Amylase
BL03= Black Fresh + 0.5% Alpha Amylase;	YB03= Yellowish Brown Fresh + 0.5% Alpha Amylase
BL04= Black Roasted	YB04= Yellowish Brown Roasted
BL05= Black roasted+ 0.2% Alpha Amylase	YB05= Yellowish Brown Fresh + 0.2% Alpha Amylase
BL06= Black roasted+ 0.5% Alpha Amylase	YB06= Yellowish Roasted + 0.5% Alpha Amylase

Figure 6.10:Thixotropic index of all Tigernut Milk Variants

6.6 Conclusion

Between the two tigernut variants, the yellowish-brown variety had a higher total solids value when compared to the black variety. The fresh milk however had lower total solids as against the roasted milk. A significant increase in total solids was only observed in samples that were pre-treated by roasting. Once heat is applied, separation is observed in the milk and an emulsifier, in this case, α-amylase was added. This also contributed to the increased total solids observed.

The percentage brix was higher in the yellowish-brown variety as opposed to the black variety. Roasting increased the milk extract of the tubers and as such, coupled with the addition of α-amylase, roasting tigernuts caused a significant increase in the brix of milk from tigernuts.

Roasting lowered the pH while increasing the titratable acidity of tigernut milk as against the fresh milk. The addition of α-amylase caused a significant decrease in pH but increase in titratable acidity, which is a good control for preventing the growth of microbes and providing a conducive environment to help minimize oxidation of lipids in the milk (metal ion chelation). The emulsion capacity and stability of the milk from fresh tigernuts increased with increasing doses of α-amylase while that of the roasted milk increased with decreasing doses of α-amylase but decreased with increasing doses of α-amylase. This is an advantage in commercializing tigernut milk where a little boost from α-amylase can provide the desired emulsion capacity and stability. Foam capacity and stability also increased significantly in roasted milk for both tigernut varieties, as the heating process causes deformation of the proteins rendering them more soluble. However, the addition of α-amylase decreased the foaming capacity and stability of all analysed samples. Roasting and the addition of α-amylase impacted the colour of tigernut milk due to chemical reactions that occur during these processing methods as well as physical impacts of the brown colour of α-amylase. Roasting and the addition of α-amylase decreased the viscosity of the milk due to the breakdown of starch molecules. Increasing speed and time further decreases viscosity and as such based on the desired viscosity, commercial production of milk of tigernut must control parameters such as speed of machines, during of processing, as well as concentrations of food additives such as α-amylase.

CHAPTER SEVEN

7. OBJECTIVE 5: To evaluate the polyphenol content and stability of tigernut oil during heating at different temperatures

7.1 Introduction

Vegetable oils are indispensable in achieving human dietary requirements and are exploited in many food applications (Idouraine et al., 1996). Unrefined vegetable oils contain rich bioactive hydrophobic components from the nut and seeds, and it is another reason they are drawing increasing attention for various health benefits. The cake obtained after extracting oil from oilseeds is employed in compounding animal feed due to their rich protein content (McKevith, 2005).

Vegetable oils can be grouped into two types; the edible oils and the industrial oils. The edible oils are usually food grade oils, and are typically odourless unsaturated fats, used in frying and as an ingredient in numerous food products, particularly where a healthy oil is needed. Industrial oils are usually inedible and comprise of high concentrations of compounds crucial for certain industrial procedures. Fats and oils make up about 40% of the calories in the diet of a regular person and as such, they are known to be essential nutrients. They also serve as a carrier of fat-soluble vitamins. Edible vegetable oils are used in food applications as salad dressings or cooking oils or may be hardened, through hydrogenation, to produce margarine and shortening.

Several physicochemical properties of edible oil are used to assess the compositional quality of oils (Mousavi et al., 2012; Ceriani et al., 2008). These include peroxide value, flash point, acid value, saponification value, free fatty acid, smoke point, iodine value, ester value among others. Various studies have evaluated the effect of temperature on the iodine and peroxide

value of oil as well as the stability and viscosity to evaluate the quality and functionality of the oil (Li et al., 2010; Farhoosh et al., 2008; Jinfeng et al., 2011). One of the most common techniques used in food preparation is deep frying. When oil is used in frying repeatedly, several oxidative and thermal responses are triggered which causes a transformation in the sensory, nutritional and physicochemical properties of the oil (Che Man & Jasvir, 2000). During frying, the structure of oil transforms leading to changes in the flavour and stability of its compounds caused by hydrolytic, oxidative and polymerization procedures (Gloria and Aguilera, 1998). During deep frying, factors like frying condition, reduction in the oxidative stability, original quality of frying oil determine the diverse responses of the oil (Choe and Min, 2007).

Available literature has attributed the goodness of tigernut to its phenol content. However, there is limited information on the phenol content of Ghanaian tigernut. Polyphenols have also been noted to be affected by processing. Edible oils possess functional and phenolic properties, which may be a basis for which they are patronized. Most Ghanaian diets require the use of edible oils in the preparation of daily household meals, which include the application of heat. Structural breakdown of the oil easily occurs since there is a rapid reaction between lipids and other organic compounds and atmospheric oxygen. Consequently, it is imperative to monitor the quality of oil used in cooking in order to prevent the use of deteriorated oil which will have negative health effects to the consumer (Vijayan et al., 1996). The objective of this study was to evaluate the polyphenol content and stability of tigernut oil during heating at different temperatures.

7.2 Materials and Methods

7.2.1 Sample preparation: Soxhlet extraction and fat content determination of tigernut tubers

Tigernut samples purchased from traders (wholesalers/retailers) in the Greater Accra Region (Table 3-1 & Table 3-2) were used for the preparation and analysis of the oil.

AOAC Method 920.39 (Soxhlet extraction method with diethyl ether) was used for the oil extraction from tigernut tubers. This was done in the Botany Laboratory of the University of Ghana. A pre-heated 2000ml volumetric flask was accurately weighed and the mass recorded. 4kg of each variety of pre-sorted and cleaned, crushed and dried. The dried tigernut was measured and placed into separate 25× 10 cm paper thimbles. A ball of small glass wool was used to cover the samples in the thimbles. This was done to prevent sample loss. Petroleum ether (100ml) with boiling point 40 - 60°C was added carefully to a pre-weighed extraction flask. A Soxhlet extraction set-up was mounted and the samples were refluxed for 5 hours at a 50°C on the heating mantle. The extraction process was performed on each tigernut sample.

The solvent for each sample was recovered by distillation after the extraction process. The oil produced by the samples in the flasks was heated in a temperature-controlled oven at 60°C to evaporate the solvent. The flasks were positioned in a desiccator and cooled to room temperature. The flasks were weighed to determine the weight of the oil collected for each sample. For each sample, the fat content was determined according to the Equation 7-1.

$$\%\text{Fat (dry basis)} = \frac{\text{Oil collected (g)x 10}}{\text{Weight of sample (g)}}$$

$$\%\text{Fat (dry basis)} = \frac{(\text{Weight of flask} + \text{oil}) - \text{wt. of flask (g)x100}}{\text{Weight of sample (g)}}$$

Equation 7-1

7.3 Methods

7.3.1 Determination of the compositional properties of tigernut oil

7.3.1.1 Fatty Acid Profile

Association of Official Analytical Chemist (A.O.A.C) method 2012.13 for fatty acid determination of fats and oils was the reference method used for the analysis of Fatty Acids in the tigernut samples. 200mg of powdered tigernut samples of each variety was weighed into a dried 25ml centrifuge tube. 2ml of water was added and mixed to ensure that the samples were uniformly dissolved. 5ml of an internal standard (C11:0 FAME + C13:0 TAG, each at 2 mg/mL in methyl tert butyl ether) was added.

To each tube, 10 ml of 5% (w/v) methanolic sodium methoxide solution was added after which the tubes were capped tightly and vortexed for 15 seconds. 2ml of hexane and 10ml of a neutralization solution (10% disodium a hydrogen citrate/15% sodium chloride in water) was added after 180 and 210 seconds respectively. The tubes were shaken carefully with a vortex mixer and at 1750rpm, centrifuged for 10 minutes. Using a pipette, 200 µl of the clear supernatant of each solution was transferred into 10ml flasks and diluted with hexane to the mark. The samples were analysed using Clarus SQ 8 GC/MS Gas chromatography (Perkin Elmer, London U.K). To quantify the peaks, the region under each fatty acid peak was compared to the total area of all fatty acid peaks (Bado et al., 2015). The average value for each constituent fatty acid was recorded.

7.3.1.2 Determination of the smoke point of tigernut oil

The smoke point of the oil was determined using the AOCS Cc 9a-48 method, Cleveland Open Cup. The oil was heated and when a blue smoke was noticed, the temperature was recorded as the smoke point of the oil.

7.3.1.3 Determination of Flash point of tigernut oil

The procedure used for the determination of the flash point was adopted from the Kanna et al. (2017) with slight alteration using the Pensky-Martin apparatus. Samples were dehydrated with calcium chloride to get rid of any free or dissolved water. The apparatus was thoroughly cleaned and dried after which, the cup was filled with oil to be tested up to the level shown by the filling mark. A thermometer was inserted, and the test flame turned on, which was adjusted to a diameter of 1.0 mm. The sample was heated so that the temperature increased to about 5 to 6℃ per minute. The turning device was turned from one to two revolutions per second as the heating continued. The test flame was applied when the temperature of the sample was a value below 17 ℃ less than the flash point. At every 10 ℃ increase in temperature, the agitation was discontinued, and the test flame was applied by opening the device, which regulates the shutter and lowering of the test flame into the shuttle opening. The flash point was determined as the temperature shown by the thermometer at the time the flame application caused an evident flash in the interior of the cup.

7.3.2 Determination of the changes in the polyphenol content and compositional properties of tigernut oil at different temperatures

Functional properties and phenolic content of tigernut oil of both cultivars were determined at room temperature. The oils were further subjected to the following temperatures (50^{o}C, 110^{o}C, 300^{o}C) for 30 minutes and the same analyses were conducted to determine if there would be any alteration on the parameters due to the increasing heat.

7.3.2.1 Determination of Polyphenol content of tigernut tubers and tigernut oil (fresh and after heat applications)

The total phenolic content, catechin, gallic acid and quercetin of both tigernut tubers and fresh tigernut oil were determined. The process of analysis included sample extraction, processing and High-Performance Liquid Chromatography (HPLC).

- **Extraction**

The extraction procedure was based on QUECHERS with slight modifications. 2g of previously dried and milled Tigernut samples were placed into 50ml centrifuge tubes. 5ml of distilled water was measured was added to each centrifuge tube and allowed to wet and soak for 10mins. Using a dispenser, 5ml of 1% acetic acid in acetonitrile was added and agitated for 15mins. Sodium Chloride (0.5g) and Magnesium Sulphate (2g) were added to the tubes, which were agitated for 2mins and centrifuged at 1968g force for 10mins. The supernant was further cleaned up using dispersive-SPE (C18, PSA, $MgSO_4$). For HPLC analysis 20 µl of the clean-up extract was used.

- **Total phenol content determination**

The total phenol content of the tigernut extracts was determined spectrophotometrically utilizing a modified method of Folin Ciocalteau assay as described by Singleton et al. (1999) with slight modifications. The external calibration was performed using varying concentrations of gallic acid; 0.00, 0.25, 0.50, 0.75 and 1mM. 8.0 ml of distilled water was added to 100 µl of the extracts of each tigernut sample in a flask. 500 µl of the Folin Ciocalteau reagent was added after which the contents of the flask were vortexed for 10 minutes. 1.5 ml of 20% sodium bicarbonate (Na_2CO_3) was added and the contents incubated for 2 hours. The absorbance was measured at a wavelength of 765 nm using a Shimadzu spectrometer (Mettler Toledo-Columbus, USA) at room temperature. The measurements were performed in triplicates. The total phenol content was evaluated as mg of gallic acid equivalence (mM GAE) through extrapolation from the gallic acid calibration curve,

- **HPLC analysis (Catechin and Gallic acid)**

Catechin and Gallic analysis were done in the Cecil-Adept® Binary Pump HPLC system (Cecil Instruments Ltd, Cambridge, U.K.) adjusted to a rate of 1.0 ml/ min and connected to a Wave Quest® CE 4300 DAD Detector tuned to a wavelength of 280 nm. The mobile phase used for

the analysis was 1% Acetic Acid: Acetonitrile (80: 20 v/v). Waters® SunFire™ C18, 15cm x 4.6mm, 5µm column was used at a column temperature of 40 °C.

- **HPLC Analysis (Quercetin)**

Quercetin analysis was done in HPLC system (Cecil-Adept® Binary Pump HPLC, Cambridge, U.K.) adjusted to a flow rate of 1.0 ml/ min and connected to a Wave Quest® CE 4300 DAD Detector tuned to a wavelength of 370nm αα. The mobile phase used for the analysis was 0.4% Phosphoric Acid: Methanol (51: 49 v/v). Waters® SunFire™ C18, 15cm x 4.6mm, 5µm column was used at a column temperature of 40 °C.

7.3.3 Determination of the functional properties of tigernut oil

7.3.3.1 Determination of Peroxide Value (PV) of tigernut oil

The AOAC Official method 995.33 was used to determine the peroxide value (PV). 5.00 ±0.05 g of the test sample was weighed into a 250 ml glass stoppered Erlenmeyer flask. 30 ml of CH_3COOH-$CHCl_3$ was added and the mixture was swirled to dissolve. After the swirling, 0.5 ml saturated KI solution was added using a Mohr pipet. The solution was allowed to stand with occasional swirling for 1 minute after which 30 ml of water was added. The solution was slowly titrated with 0.1 M $Na_2S_2O_3$ whiles shaking vigorously until the yellow colour almost faded. 1% starch solution was added after which the titration was resumed with vigorous shaking until all the I_2 was liberated from the chloroform ($CHCl_3$) layer with the disappearance of the blue colour. The procedure was carried out in duplicate portions. The peroxide value (milli equivalence peroxide/kg oil or fat) was calculated using the Equation 7-2.

$$PV = S \times M \times 1000g \text{ sample} \qquad \textbf{Equation 7-2}$$

where S= ml $Na_2S_2O_3$ (blank corrected) and M = molarity $Na_2S_2O_3$ solution.

7.3.3.2 Determination of the saponification value of tigernut oils

The American Organization of Analytical Chemists (A.O.A.C) official method 920.160 for determination of saponification value of fats and oils was used for this analysis with slight modifications.

5 g of the oil samples was weighed accurately and filtered into a 250 ml Erlenmeyer flask to expurgate any impurities and traces of moisture. 50 ml of alcoholic potassium hydroxide (KOH) solution was pipetted into the flask. The flask was connected to an air condenser after which the mixture was boiled 30 minutes to ensure that the fat was completely saponified. The mixture was allowed to cool to ambient temperature and titrated with 0.5 M HCl, using phenolphthalein as indicator. The determination was done in triplicates. Blank determinations were also conducted to act as a check for accuracy.

The determination of the saponification value was obtained from the calculation Equation 7-3:

$$\text{Saponification Value} = 28.05\,(B - S) / g\ \text{oil} \qquad \textbf{Equation 7-3}$$

Where B = volume of 0.5 HCl required by blank, S= volume of 0.5M HCl required for the sample.

7.3.3.3 Determination of the iodine value

The iodine value of the tigernut oil samples was determined using the American Organization of Analytical Chemists (A.O.A.C) official method 993.20 for iodine value determination of fats and oils with slight alterations.

Accurate amounts of the dry oil as indicated in the Table 7-1 were each weighed into a 500 ml conical flask with a glass stopper. 20 ml of carbon tetrachloride was pipetted and added to the conical flasks and the contents mixed well. Two blank determinations were prepared to run with each sample group. 15 ml of cyclohexane-acetic solvent was added to each test sample and swirled to ensure that they dissolved completely. 20 ml of Wijs solution was dispensed

into the flasks and swirled again to mix. The timer was set for 1.0 or 2.0 hours depended on the iodine value of the sample. The flasks were stored in the dark at 25 ± 5℃ for duration of reaction.

Potassium iodide (KI) solution (20ml) followed by 150 ml of recently boiled and cooled water was added to the flasks after the storage period and mixed well. The released iodine was titrated immediately with standardized sodium, thiosulphate solution (Na_2SO_3), using starch as indicator at the end, until the blue colour formed disappeared while thoroughly shaking the flask.

Table 7-1: Required quantity of dry oil for estimation of iodine value

Expected value	Weight to be taken for estimation (g)	
	Maximum	Minimum
3	10.58	8.46
10	3.17	2.54
20	1.59	1.27
40	0.79	0.63
80	0.40	0.32
120	0.28	0.32
160	0.20	0.16
200	0.16	0.13

Calculation of iodine value is done using Equation 7-4.

$$Iodine\ Value = \frac{(B - S) \times M \times 12.69}{\text{Weight of oil}}$$

Equation 7-4

Where B = titration of blank (ml)

S = titration of test sample (ml)

M = molarity of Na_2SO_3 solution

7.3.3.4 Acid value determination.

The acid value of the samples was determine using the American Association of Analytical Chemists (A.O.A.C) 940.28 method for the determination of free fatty acids of oils. 7.05 g of well mixed oil sample was weighed into a 250 ml conical flask. 50 ml of alcohol previously neutralized by adding 2 ml of phenolphthalein solution and enough amount of 0.1M NaOH to GENERATE a faint permanent pink colour was added to the flask. The resultant mixture was titrated with 0.25M NaOH whiles vigorously shaking the flask until a faint pink permanent colour appeared. The determination was done in triplicates. The acid value is calculated using the Equation 7-5.

$$\text{Acid value} = \%\ \text{free fatty acids (as oleic)} \times 1.99 \qquad \textbf{Equation 7-5}$$

7.3.3.5 Determination of Ester value of Tigernut oil

The Ester value is calculated by subtracting the acid value of oils from the corresponding saponification value of the corresponding oils.

7.4 Statistical analysis

The Kruskal Wallis test and Mann-Whitney test were used to determine the significant difference in the functional and phenolic properties of tigernut oil during heating at different temperatures because the data were not normally distributed.

7.5 Results and Discussion

7.5.1 Oil yield from Tigernut tubers

The percentage yield of oil extracted from 1000g of the tubers for black and yellowish-brown variety was 17% and 15% respectively. Yellowish-brown tigernut yielded more oil due to its higher oil content. For a tuber, the percentage yield was considered good for both varieties. However, in comparison to other vegetable sources, the percentage yields reported in this determination were relatively lower than that of sunflower oil (41.3%) (Abitogun et al., 2008), coconut oil (42.37%) (Adeyanju et al., 2016) and ground nut oil (37.80 ± 2.21%) (Nkafamiya et al.,2010).

7.5.2 Fatty acid profile of Tigernut

The fatty acid constituents of the tigernut cultivars were analysed and reported in Table 7-2.

Table 7-2: Free Fatty Acids composition of tigernut tubers

Free Fatty Acid (%)	Black Variety	Yellowish-Brown Variety
Myristic acid	0.13 ± 0.21^{a}	0.17 ± 0.32^{b}
Palmitic acid	14.68 ± 0.07^{a}	13.74 ± 0.10^{b}
Palmitoleic acid	0.57 ± 0.30^{a}	0.49 ± 0.38^{b}
Stearic acid	5.13 ± 0.83^{a}	4.91 ± 0.62^{b}
Oleic acid	67.22 ± 0.12^{a}	69.23 ± 0.25^{b}
Linoleic acid	10.54 ± 0.10^{a}	9.63 ± 0.14^{b}
Linolenic Acid	0.35 ± 0.24^{a}	0.33 ± 0.33^{b}
Arachidic acid	0.56 ± 0.34^{a}	0.66 ± 0.40^{b}
Gadoleic acid	0.30 ± 0.20^{a}	0.32 ± 0.03^{b}
Behenic acid	0.22 ± 0.02^{a}	0.24 ± 0.03^{b}
Lignoceric acid	0.29 ± 0.02^{a}	0.29 ± 0.02^{a}

Values are means of triplicates and ± standard deviation. Averages for both varieties of tigernut had different superscripts, meaning that they were significantly different at $p \leq 0.05$

The tigernut oil analysis showed high quantities of Oleic acid (67.22% ± 012 and 69.23% ± 0.25) followed by Palmitic acid (14.58% ± 0.07 and 13.74% ± 0.10) and the least quantity was recorded for Myristic acid (0.13% ± 0.21 and 0.17% ± 0.32). The most abundant unsaturated fatty acids recorded was Oleic acid whereas the most abundant saturated acid was Palmitic acid. The amount of Oleic acid and Palmitic acid in the black tigernut variety were found lower than that in the yellowish-brown variety. However, the amount of Linoleic and steric acid was found higher for the black variety than for the yellowish-brown variety.

The amount of Lignoceric acid was virtually the same in both cultivars. Oleic, Linoleic, Arachidic and stearic oils reported for both varieties were found higher than those reported by Bado et al. (2015) (Oleic (64.25-65.76), Linoleic (0.14-0.17), Arachidic acid (0.57-0.68) and stearic acid (4.73-5.36). The fat contents of tigernuts are relatively higher than those of cereals but like that of soybeans (Suleiman al., 2018). The predominant quantity of monounsaturated fatty acids (Oleic acids and Palmitic acids) in both tigernut cultivars analysed in this study were found coherent with that reported by Roselló-Soto et al. (2018). Also, the fatty acid profile of tigernut has been reported to be like that of olive oil (Sabah et al., 2019; Linssen et al., 1988). The fatty acid profile of tigernut makes it a better substitute for olive oil (Sabah et al., 2019; Linssen et al. 1988). As a result of its lipid content (16-25%), tigernut seeds can be used as a substitute for animal fat to enhance the shelf life and nutritional quality of meat products (Aljuhaimi et al., 2018).

The independent samples t-test was used to establish if a significant difference exists in palmitoleic acid, palmitic acid, myristic acid, gadoleic acid, oleic acid, lignoceric acid, arachidic acid, stearic acid, behenic acid and linoleic acid across the two varieties of tigernuts. The corresponding *p-values* of the test showed that there was a significant difference in means across both black and yellowish-brown varieties of tigernuts for gadoleic acid, lignoceric acid,

behenic acid, arachidic acid, oleic acid, linoleic acid, stearic acid, myristic acid, palmitoleic acid and palmitic acid. This is consistent with that reported by Bado et al. (2015).

7.5.3 Smoke point of tigernut oil

The smoke point of the tigernut oils were analysed at ambient temperature in this experiment. The smoke point for the black variety was found to be 207 ^{o}C and that of the brown variety was 210 ℃. According to reports, most vegetable oils have smoke point in the range of 165 ℃ - 260℃ (Wang, 2002). The smoke points of the oils analysed were lower than those reported for ground nut oil (225 ℃), sunflower oil (232 ℃) but higher than coconut oil (205 ℃) and olive oil (190 ℃). According to Oladiji et al. (2010) smoke point of oils have been found as an important factor for selecting oils for frying. The smoke point of edible oils may vary depending on the source due to impurities present in them (Sarwar et al., 2016).

It is important to know the smoke point of oils used for food because overheating an oil with a low smoke point may be very harmful, as this leads to the production of free radicals which may cause health damages (Sarwar et al., 2016). The tigernut oils have relatively high smoke points and thus can be used for frying at high temperatures. The smoke point of oils is the temperature at which the oil gives out smoke (Quiles et al., 2002). Decreasing smoke point values affect the quality of the oils and as such may not be suitable for repeated frying.

7.5.4 Flash point of tigernut oil

The flash point of oils is very important as it shows the degree to which an oil or fat can be heated without decomposing. High flash point is characteristic of a good lubricant. Typically, vegetable oils have been found to have high flash points, making it possible to use them over a wide range of temperature without them giving off vapour and igniting (Vunguturi & Irfanuddin, 2017). The flash point values measured for the black and brown variety were 159 ℃ and 167 ℃ respectively. These values were lower than the values reported for Suhartono et

al. (2018) for coconut oils (270-300℃), palm nut oil (314 ℃) and pea nut oil (340℃). Other researchers like Vunguturi and Irfanuddin, (2017) reported higher flash point values for Sunflower oil (309 ℃) and Cotton seed oil (312℃). The relatively lower value of the flash point of the tigernut oils shows that, the oils cannot be used as lubricant oils at high temperatures.

7.5.5 Effect of processing on the phenolics of tigernut

Polyphenols have been found to be beneficial to human health. The changes that occur to the levels of polyphenols when tigernut oil is extracted from the tuber as well as the effect of heating on specific polyphenols in the tigernut oil were studied and results are discussed in the sections below.

7.5.6 Changes in the levels of the total phenolics of tigernut tuber as it is processed into tigernut oil

The total phenolic content of tigernut tuber and tigernut oil are shown in Table 7-3**Error! Reference source not found.**

Table 7-3: Total phenolic content of tigernut tubers and tigernut oil

Product	Variety	Total Phenols (mg GAE/g)
Tigernut Tubers	Black	53.02 ± 0.95^{a}
	Yellowish-Brown	147.93 ± 1.49^{b}
Tigernut Oil	Black	12.47 ± 0.46^{a}
	Yellowish-Brown	24.78 ± 2.00^{b}

Values are means of triplicates and ± standard deviation for n= 3. For each product, means for both varieties of tigernut with different superscripts were significantly different at $p \leq 0.05$

The total phenol content of the yellowish-brown variety (147.93 ± 1.49 mgGAE/g) was found significantly higher than that of the black variety (53.02 ± 0.95 mgGAE/g) measured at an ambient temperature of 25℃. Oladele et al. (2017) reported higher total phenolic content for the yellow variety (351 mgGAE/100 g) than the black variety (134 mgGAE/100 g). Other researchers reported lower values for the total phenolic content in tigernut (5.63-64.9 mgGAE/100 g) (Parker et al., 2000) than those reported in this analysis. Owon et al. (2013) reported higher value of 197.20 mgGAE/100 g for the total phenol content in the tigernut they analysed. Taha et al. (2012) reported lower values of TPC for peanut flour. The higher values of the total phenolic content of the tigernut makes them resistant to oxidation.

The total phenolic content (TPC) of the tigernut oils were also analysed at room temperature (25℃) (Table 7-3). The values reported in this analysis for the oils (24.78 ± 2.00 mgGAE/100 g for yellowish brown and for 12.47 ± 0.46 mgGAE/100 g black tigernut oils) were found to be lower than found in the tigernut tuber. This trend may suggest that some polyphenols present in the tigernut tuber are lipophobic and thereby were not extracted with the oils (Jiang et al., 2015). The total phenolic content of the tigernut oils found in this study were also lower that reported for olive oil (Owen et al., 2000). Xuan et al. (2018) reported lower value TPC for sunflower oil (4.39 ± 0.20 mgGAE/g) and soybean oil (3.23 ± 0.08 mgGAE/mg). The relatively higher phenolic content makes them more resistant to oxidation than the other edible oils.

Quercetin, catechin and gallic acid content of tigernut tuber and tigernut oil are shown in Table 7-4.

Table 7-4: Polyphenol content of tigernut tubers and tigernut oil

Product	Polyphenol	Variety	mg GAE/100g
Tigernut tuber	Quercetin	Yellowish-Brown	49.58 ± 0.54[a]
		Black	21.16 ± 0.92[b]
	Catechin	Yellowish-Brown	1.60 ± 0.36[a]
		Black	3.7 1 ± 0.18[b]
	Gallic acid	Yellowish-Brown	1.88 ± 0.10[a]
		Black	0.60 ±0.02 [b]
Tigernut oil	Quercetin	Yellowish-Brown	30.88 ± 0.74[a]
		Black	15.42 ± 0.10[b]
	Catechin	Yellowish-Brown	Not detected
		Black	Not detected
	Gallic acid	Yellowish-Brown	0.88 ± 0.01[a]
		Black	0.22 ± 0.014[b]

Values are means of triplicates and ± standard deviation. For each polyphenol and product, means for both varieties of tigernut with different superscripts were significantly different at $p \leq 0.05$

Quercetin recorded the highest mean levels of 21.16 mg GAE/100g ± 0.92 and 49.58 mg GAE/100g ± 0.54 for tubers of both black and yellowish-brown cultivars respectively. This same trend was observed in the tigernut oil which had mean levels of 15.42 mg GAE/100g ± 0.01 and 0.22 mg GAE/100g ± 0.001 for black and yellowish-brown cultivars respectively. The smallest level of polyphenol observed in this study for both varieties of tigernut tuber was Gallic acid. There exists a significant difference in means across both black and yellowish-brown varieties of tigernuts for quercetin, catechin and gallic acid for both tigernut tuber and tigernut oil. Catechin was not detected in tigernut oil. This may be because the tigernut may contain more hydrophilic catechin than lipophilic catechin as observed with some plants (Paximada et al., 2017).

Numerous authors have reported levels of different polyphenols in tigernuts tubers. Oladele et al. (2017) reported Quercetin concentration to be $3.76\times 10^{-3} - 60.63$ mg GAE/100 g. Also,

Gallic acid and Catechin concentration were reported to be $3.95 \times 10^{-3} - 1.74$ mg GAE/100 g and $8.83 \times 10^{-4} - 6.58$ mg GAE/100 g respectively (Oladele et al., 2017).

The role of polyphenol compounds in the inhibition of some human diseases have been recognized in numerous studies. Their antioxidant oxidative properties have been found to be important in the oxidative stability of foods (Rasouli et al., 2017). Polyphenols (quercetin, catechin and gallic acid) have been found to have curative properties of cancer and cardiovascular diseases (Rasouli et al., 2017). It has been reported that, a dietary supplement of tigernut has been proven to exhibit substantial renal and hepatoprotective properties in rat against acrylamide-induced toxicity (Roselló- Soto et al., 2018). The health benefits of Quercetin have been studied by numerous researchers due its relatively high bioavailability (Rasouli et al., 2017) as compared to other polyphenol compounds. The daily intake Quercetin is approximated to be around 5-40 mg/day (Bigelman et al., 2011). Others have reported the daily consumption of quercetin based on the intake of fruits and vegetable to an estimated value of 5-100 mg per day (Batiha et al., 2020). With the high consumption of fruits and vegetable, these levels can spike up to 200-500 mg/day (Russo et al., 2012). The mean values of both tigernut cultivars were found within the range of the reported for this analysis. In their study Han et al. (2020) performed a clinical trial test to establish the safety supplementation in patient with chronic obstructive diseases. Three groups of subjects were to different levels of Quercetin: 500, 1000 and 2000 mg/day respectively. After the clinical tests the subjects reported no serious adverse effects or significant change in lungs function and blood count. Lee et al. (2011) reported a reduction in blood pressure and blood glucose in male smokers subjected to a daily intake of 100 mg of quercetin obtained from the consumption of quercetin-onion peel extracts. The estimated daily of catechin has been approximated to be 18-50 mg/day (Manach et al., 2005). This is found lower than the mean values reported for both black and brown cultivars in this study. In their study to investigate the protective property of gallic acid

(GA) against high fat diet induced DNA damage, Setayesh et al. (2019) subject male and female mice with different doses of GA (0, 2.6-20 mg/ kg b.w./ day) for a week. The results showed a decrease in sugar levels indicating that eating of food rich in Gallic acid prevents adverse biological consequences in overweight or obese individuals. A possible combination of other foodstuffs such as banana grapes and strawberries, which have been reported to be a good source of gallic acid (Pandurangan et al., 2015) with tigernut can be a good diet for obese individuals. The daily intake of 23.7 g-189 g and 10.0 g-82.3 g for the black and yellowish-brown is enough to attain the daily requirement of catechin proposed by Peterson et al. (2005). For the catechin, the approximated intake of 485g -1577g and 1125g- 3125g accounts for the daily recommended intake of catechin proposed by Nirengi et al. (2016). Once tigernut production is boosted in Ghana, pharmaceuticals can formulate drugs based on tigernut and prescribe for the sick.

7.5.7 Effect of heating on the levels of specific polyphenols of tigernut oil

The changes in the polyphenols with respect to different temperatures are shown in Figure 7.1 and Figure 7.2.

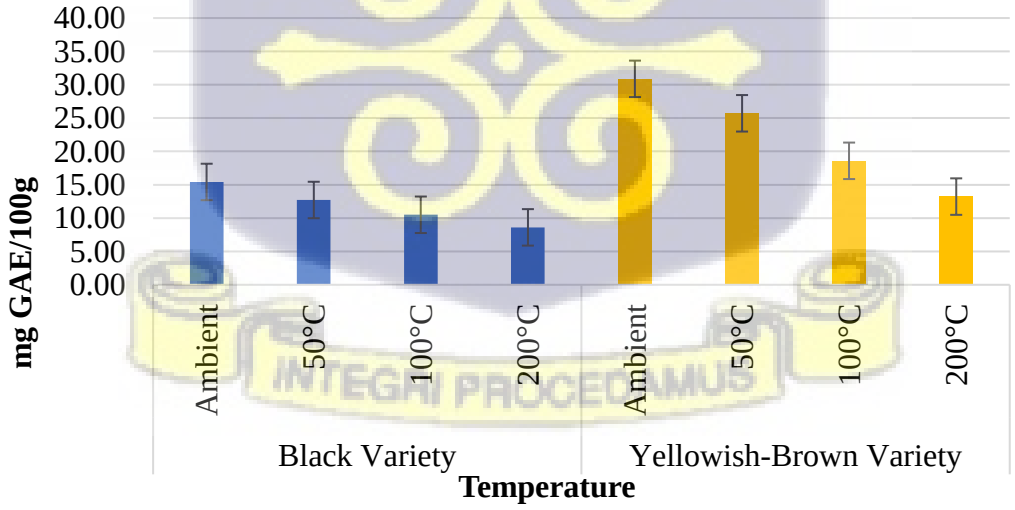


Figure 7.1: Changes in the levels of quercetin in tigernut oil at different temperatures

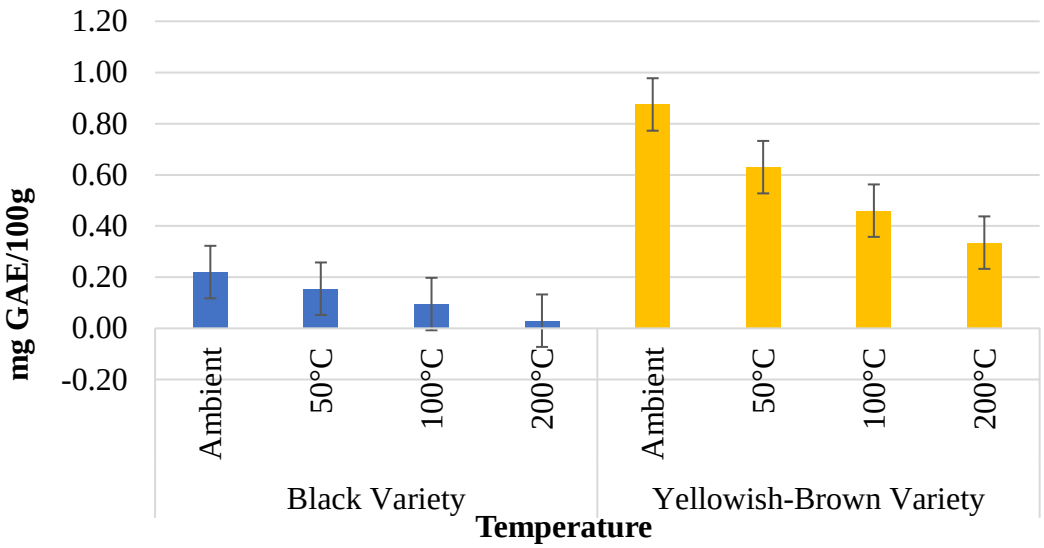


Figure 7.2: Changes in the levels of quercetin and gallic acid in tigernut oil at different temperatures

The quercetin and gallic acid content of tigernut oil of both cultivars were observed to decrease with increasing temperature from ambient temperature to 200°C for both. Other researchers reported a decrease in total phenols in heated edible oils. Prata et al. (2018) reported a decrease in total phenolic content in heated olive oil. Other researchers like Herchi et al. (2016) also reported changes (decrease) in antioxidant compounds (as gallic acid equivalence (mg/100g)) of flaxseed hull oil during heating. It can thus be concluded that, heating oil affects the levels of phenolic compounds and subsequently the antioxidant and quality of the tigernut oils. The reduction in the levels of polyphenols during heating is due to the fact that, heat treatment causes oxidation hydrolysis and polymerization of the phenolic compounds (Santos et al., 2013). Therefore, to prevent loss in the gallic and quercetin levels of the oils which contributes to their antioxidant properties, the oils should not be heated for prolonged time periods at higher temperatures.

7.1. Effect of heating on the compositional properties of tigernut oil

7.1.1. Effect of heating on the Acid Value and free fatty acids of tigernut oil

Figure 7.3 shows the acid value of the tigernut oils at different temperatures.

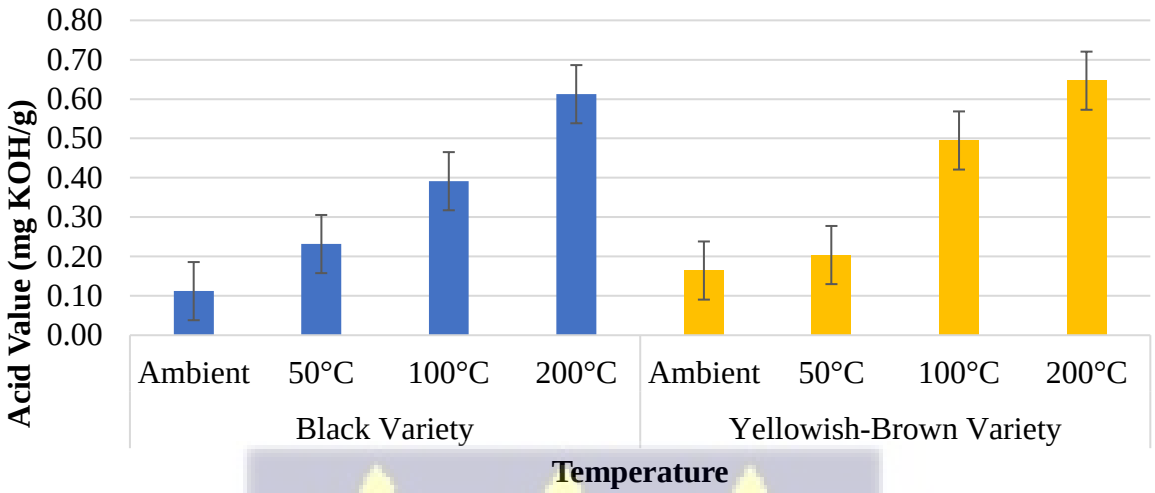


Figure 7.3: Changes in the levels of acid value in tigernut oil at different temperatures

The acid value obtained for the oils obtained in this study (0.11mg KOH/g for black variety and 0.16 mgKOH/g) shows the presence of unhydrolyzed triglycerides in the tigernut oils (El-Naggar, 2016). This means that, there is low amount of fatty acids in the oil. These values were relatively lower than that of soybean oil (1.49 ±0.32) % and sunflower oil (2.43 ± 0.32) % (Mengistie et al., 2018). It was also higher than the value reported for palm oil (1.05 mg KOH/g) and olive oil (4.2 mg KOH/g) (Hasan et al., 2016). The lower acid values of the tigernut oils make it very suitable for cooking (Sarah & Stanley, 2018) and indicates lower rate of conversion of triglycerides of oils into fatty acids and glycerol which causes rancidity (Hasan et al 2016; Zahir et al., 2014). The lower acidity value signifies the degree of the edibility of the oil (Adejuyitan, 2011). According to Al-Bachir (2015), value of the acid content in the tigernut oil indicates whether the oil can be consumed directly or suitable for industrial use. The increasing acid value with increasing temperature observed for this study is consistent with the findings that elevated temperatures increases acid values (Dawodu et al., 2015).

Figure 7.4 shows the changes in free fatty acids (FFA) with increasing temperature.

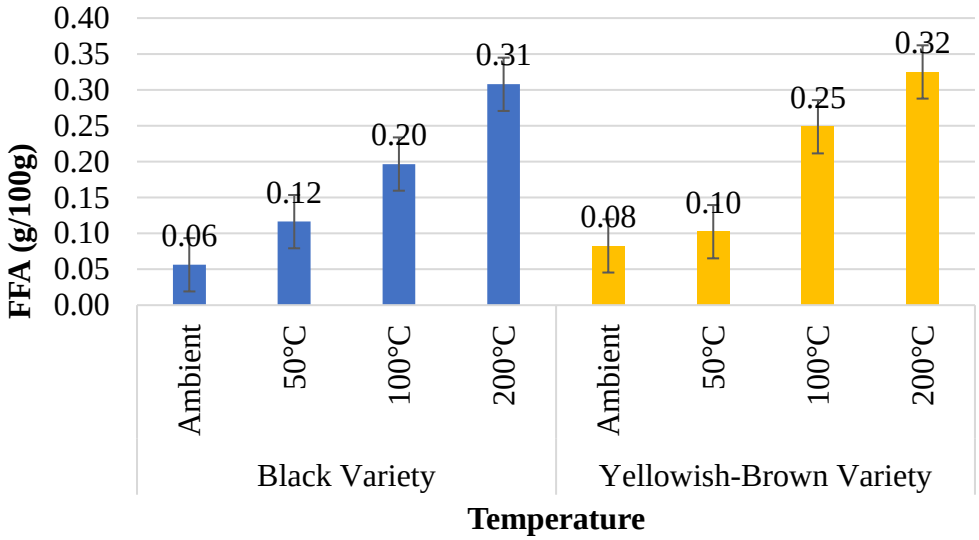


Figure 7.4: Changes in the levels of free fatty acids in tigernut oil at different temperatures

The low values reported for the free fatty acids for each variety agrees with the low values reported for their acid values. The free fatty acid determines the degree to which the glycerides in the oil has been disintegrated by lipase action, which is enhanced by light and heat (Muhammad et al., 2011; Adejuyitan et al., 2009; Adejuyitan, 2011; Eteshola and Oraedu, 1996). Therefore, as rancidity is generally accompanied by free fatty acid formation, heat treated tiger nut oil will not easily go rancid, which is a sign of longer shelf life. This conclusion was validated by Adejumo et al. (2015), whose statistical analysis also disclosed that heating temperature will considerably reduce the free fatty acid of tigernut oil. In their study, Oyedele and Ogunaike (2018), found out that free fatty acid of tigernut oil declines with a rise in heating temperature.

The FFA values in this study were however observed to increase with increasing temperature (Figure 7.4). Heating produces free fatty acids from oils (Mengistie et al.,2018). Tamzid et al. (2007) indicated that, high acid value leads to high free fatty acid and this causes rancidity of oil. According Jacome-Sosa and Parks, (2014) high levels of fatty acids in foods can cause lead

to the inability of liver to store sugar. From the analysis, heat treatment causes a significant change in both acid values and free fatty acids reducing the quality of the oils. Other oils like soybean oil and palm nut oil had free acidity values of 0.14g/100g and 0.14g/100g respectively for 220℃/30min (Guiffré et al., 2018). The values for the FFA for the tigernut oils at 200℃ was found lower than that reported by other researches like Dawodu et al. (2015) for soybean oil (3.29 ± 0.02), olive oil (1.81 ± 0.02) and 2.99 ± 0.04 for palm nut oil when heated to a temperature of 200 ℃. However, acid values reported by the same researchers for soybean oil (3.58 ± 0.02) and olive oil (7.19 ± 0.02) was found higher than those reported for the acid values in this analysis at the same temperature.

7.1.2. Effect of heating on the Peroxide value of tigernut oil

The peroxide value of oils can be used as good prediction of the quality and stability of oils (Mengistie et al., 2018; Nangbes et al., 2013). It is used to study the progression of rancidity through the assessment of the measure of peroxide produced in the product (initiation product of oxidation) (Adejumo et al., 2015).

According to Mohammed and Ali (2015), the peroxide values of oils increase with increasing temperature. This is consistent with the trend observed in this analysis (Figure 7.5).

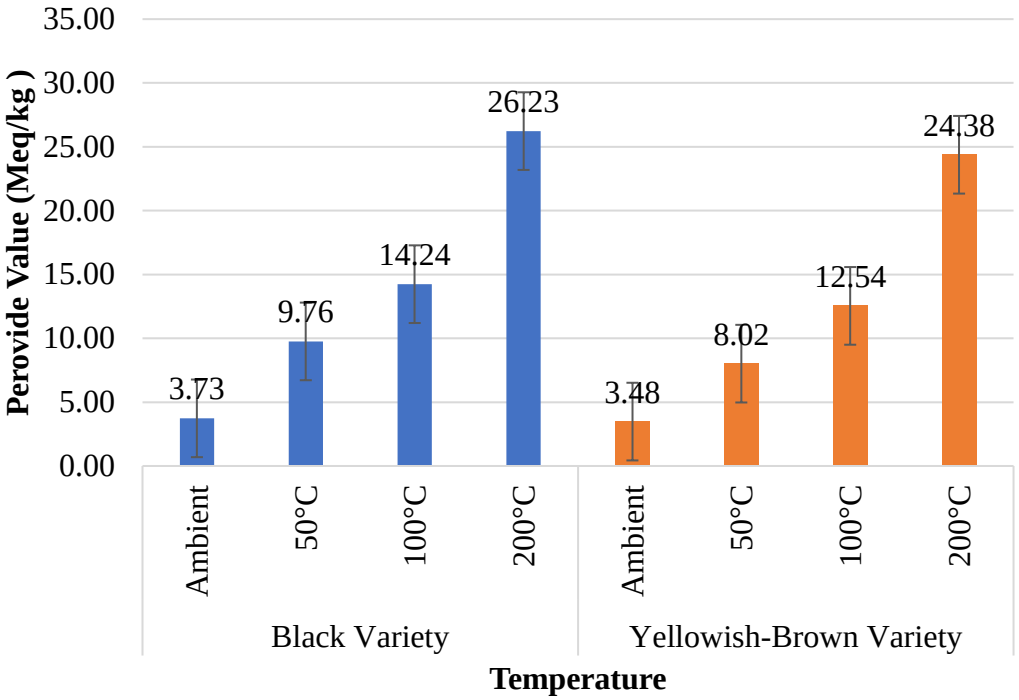


Figure 7.5: Changes in peroxide value (PV) of tigernut oil at different temperatures

The peroxide value of the tigernut oils were 3.73 meq/kg and 3.48 meq/kg respectively for the black and yellowish-brown variety. The low peroxide values reported for the oils gives an indication of their high quality and stability). These values are within the tolerable limit of 10 meq/kg as established by the Codex Alimentarius Commission (1993). High peroxide values indicate oxidation of lipids which decreases the quality of the oils (Kwon et al., 2016). Sarwar et al. (2016) reported lower values for the peroxide ground nut oil (1.6 meq/kg), sunflower oil (2.0 meq/kg) and olive oil (1.5 meq/kg). Other researchers reported higher peroxide values for soybean oil (9.26 ± 0.11) and sunflower oil (8.80 ± 0.20). In general, the results of the study as shown in Figure 7.5 highlighted the increase in peroxide values for the tigernut oils with increasing temperature. The increase in the PV value for the tigernut oils at temperatures above 50^{o}C temperatures exceeded the tolerable limit of 10 meq/kg as established by the Codex Alimentarius Commission (1993). This suggests that, using the oils for frying at higher temperatures and for long periods can be unsafe for human consumption. In similar

experiment, the peroxide value of different vegetable oils like sunflower oil and olive oil increased from 2.30±0.05 meq/kg and 5.20 ± 0.02 at 25℃ respectively to 3.49 ± 0.03 and 5.89 ± 0.03 respectively for both oils at 200 ℃ (Dawodu et al., 2015). The values are however lower than those reported in this analysis at the same temperature.

7.1.3. Effect of heating on the Saponification value and Ester value of tigernut oil

The saponification values obtained for oils of both tigernut varieties has been presented in Figure 7.6.

The saponification value provides information concerning the quality of the fatty acid available in the oil and the stability of the soap obtained from it in water. High saponification value obtained for both oils in this study suggests that, they contain relatively smaller size fatty acids while a low saponification value suggests large sized fatty acids (Muhammad et. al., 2011; Adejuyitan, 2011). The results of the analysis in Figure 7 5 showed that the saponification value of yellowish-brown tigernut oil was higher (130.93) than that of the black variety (115.36) at ambient temperature. These values were higher than that reported for sunflower oil (197.14± 0.56 mg KOH/g oil) and soybean oil (195.56 ± 1.35mg KOH/g oil) (Mengistie et al., 2018).

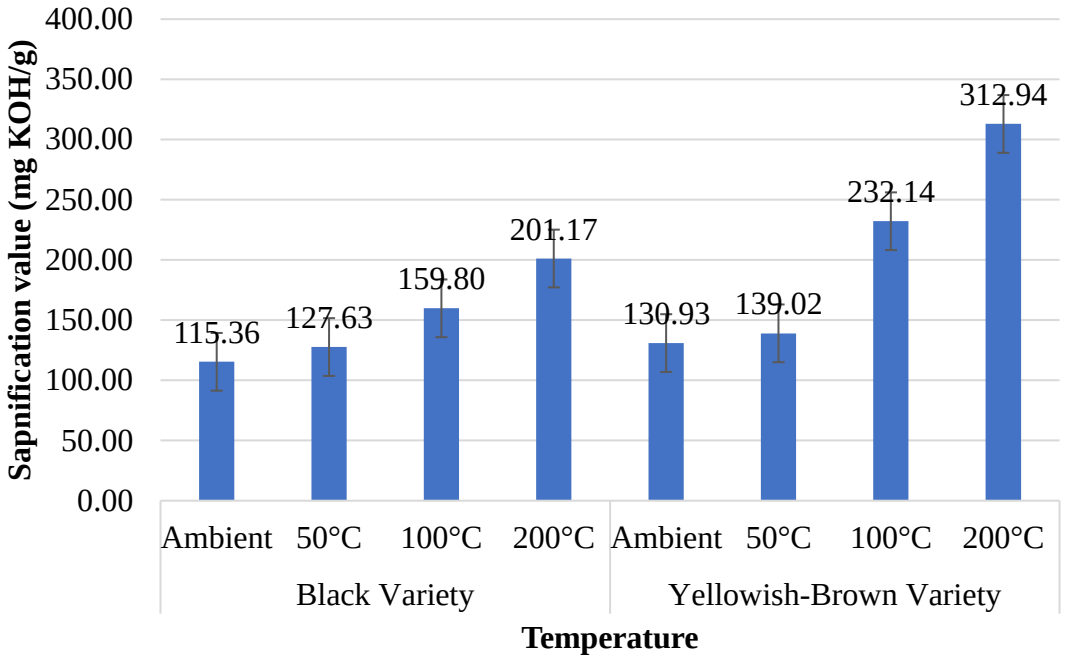


Figure 7.6: Changes in saponification value of tigernut oil at different temperatures

There was a significant increase in the saponification value for both the black and yellowish-brown tigernut variety with increasing temperature. When the oils were subjected to heat treatments, it was observed that, the saponification values increased with increasing temperature. This trend was like the observation reported by Herchi et al., (2016). In contrast, Oyedele & Ogunnaike (2018) reported a decrease in saponification with increasing drying temperatures for tigernut oil they extracted. This shows that, the quality of the oils was affected by heating. Lower saponification value implies that, the average molecular weight of fatty acids is lower, indicating that, the fat molecules did not interact with each other (Zahir et al., 2014). The fatty acid portions of oils decrease with increasing saponification values of oils (Muhammad et al., 2011). So, when tigernut oils become heated the fatty acid content diminishes as the saponification value increases reducing the quality of the oils.

The effect of temperature on the ester value of tigernut oils in this study is presented in Figure 7.7.

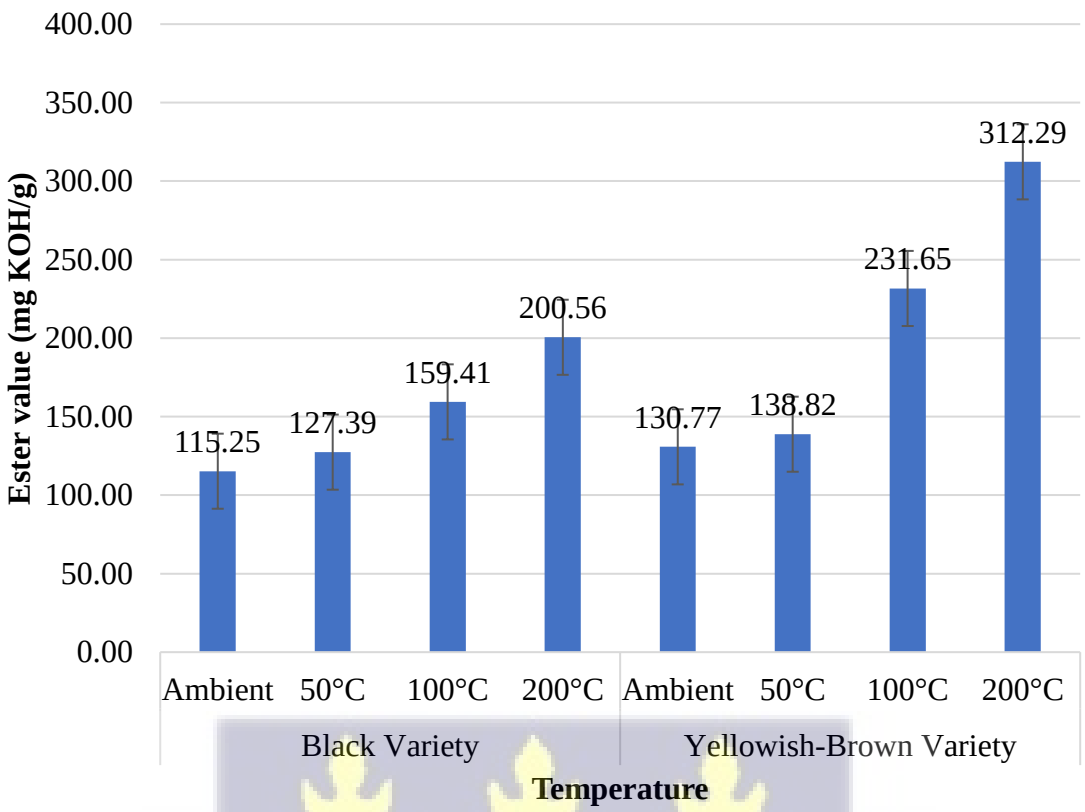


Figure 7.7: Changes in ester value of tigernut oil at different temperatures

The ester value of yellowish brown and black tigernut oils were 130.77 mg KOH/g and 115.25 mg KOH/g respectively. These values were higher that olive oil (14.92 mg KOH/g), sunflower oil (20.81 mg KOH/g) and corn oil (20.89 mg KOH/g) (Alajtal, et al., 2018). According to Alajtal, et al. (2018), oils with high ester values are not suitable for deep frying. The ester value was observed to increase with increasing temperature for both tigernut oils that were analysed. This shows that, heat treatments affect the ester values when tigernut oils are heated.

7.1.4. Effect of heating on the Iodine value of tigernut oil

The iodine value of oils determines their stability to oxidation and can be used to determine quantitatively, the overall unsaturation of the fat (Zahir et al., 2014). Figure 7.8 shows the iodine value of tigernut oil at different temperatures.

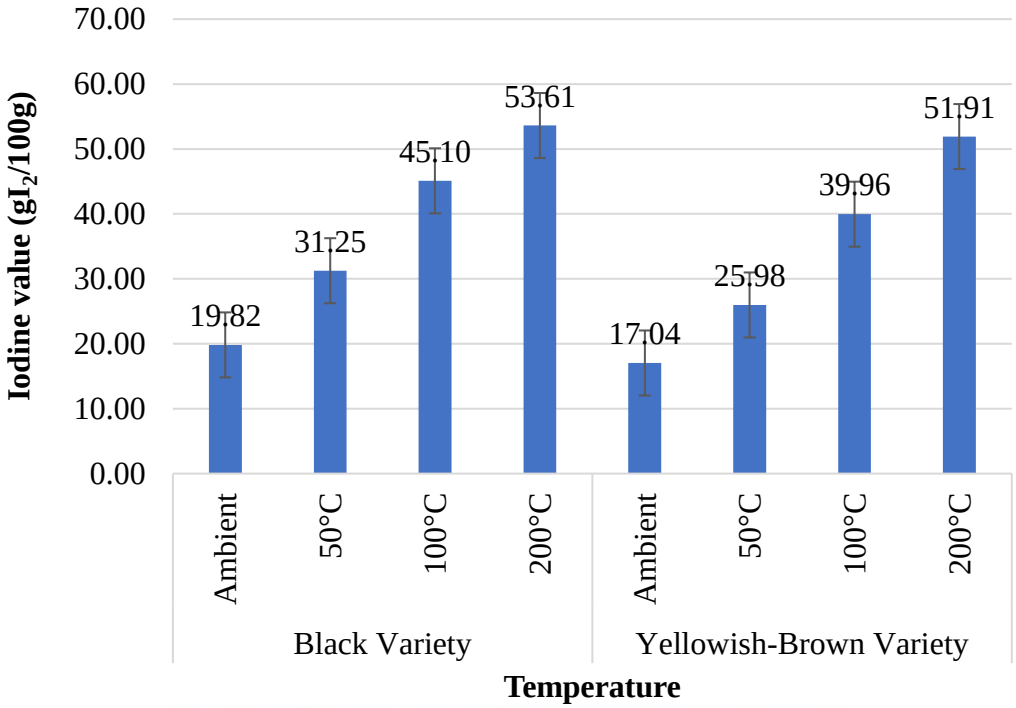


Figure 7.8: Changes in iodine value of tigernut oil at different temperatures

The iodine value of black tigernut oil extract was found to be 19.82g/100g whilst that of the yellowish-brown was found to be 17.04g/100g. This relatively low iodine values may be ascribed to the low amount of linoleic acid in both the black tigernut oil (10.54 ± 0.01) and yellowish-brown tigernut oil (9.63 ± 0.14). These values were also lower than that of olive oil (107-128) and palm nut oil (44.82 g I_2/100 g oil) (Hansen et al., 2015) but higher than that of coconut oil (6-11) (Gunstone & Harwood, 2007). According to oils that have iodine values less than 125 are non-drying oils and suitable for soap making.

The iodine value for the tigernut oils analysed in this study was found to increase with increasing temperature as depicted in the graphs below. The increase in iodine value for the tigernut oils analysed was contrast to the trend reported by Dawodu et al. (2015). They observed a decrease in iodine values for olive oil, soybean oil, sunflower oil and palm kernel oil with increasing temperature. Increasing iodine value makes the oils susceptible to oxidation since lower iodine values of the tigernut oils may contribute to its greater oxidative storage

stability (Tesfaye & Abebaw, 2016). Thus, using the oils for repeated frying affects the iodine value and reduces the quality of the tigernut oils.

7.2. Conclusion

The findings of this study established that heat causes significant alterations in the physicochemical, functional and phenolic properties of the tigernut oil. The tigernut oils analysed, had relatively high smoke points and were closely related to that of olive oil. As such, it can serve as an ideal oil for frying at low or medium-high temperatures, sautéing, preparation of sauces, and salad dressings, as overheating oils with low smoke point may cause health damages (Sarwar et al., 2016). The flash point of the tigernut oils analysed were relatively low and as such, may not be ideal to function as a lubricant, as high flash point is characteristic of a good lubricant. However, it can be combined with other high flash point oils to render it more tolerable to heat. The given oil properties suggested that tigernut oil is comparable to other vegetable oils. Subsequently, tigernut tuber oil can substitute imported olive, maize, sunflower and/or soybean oils in foods due to the high consumption of edible oils. The results of this study confirmed that acid value, free fatty acids, peroxide value, saponification value, ester value and iodine value showed significant increases to levels with increasing temperature, indicating that beyond a certain threshold, despite the ideal oil properties, matrix degradation can happen due to thermal lability.

Besides, the increasing temperatures had a destructive effect on the phenolic properties of oil, which was found as a significant reduction in the phenolic properties. In the heating process, the most significant alterations occurred in the content of gallic acid and quercetin levels in both tigernut variety. It can thus be concluded that, heating oil affects the levels of phenolic compounds and consequently, the antioxidant and quality of the tigernut oils. The reduction in the antioxidant levels due to the reduction in the levels of phenolic compounds causes

susceptibility to oxidation resulting in the rancidity of the oils. The above study shows the importance of knowing the effects of heat on the physical, chemical and phenolic composition of tigernut oils as oil is one of the main constituents in cooking. This knowledge provides the appropriate food applications and implications of tigernut oil to enhance the utilization and absorption of its food and health benefits.

CHAPTER EIGHT

8. OBJECTIVE 6: Physical and Nutritional parameters of Tigernut tuber and its products

8.1 Introduction

Tigernuts are rich in essential dietary constituents which include energy contents (sugars, fat, proteins, starch), minerals (potassium and phosphorus being the dominant ones), dietary fibre, vitamins and phytochemicals (Vitamin C, E, α-tocopherol and phytosterols). Borges et al. (2008) have stated that these nutritional benefits make them suitable for people on weight loss programs as well as people with diabetes. The rich calcium and iron content of the tubers also make them excellent for growth and development. Umerie and Enebeli, (1997) postulated that tigernuts are excellent sources of linoleic, myristic and oleic acids. Sanful, (2009) established that, fats and oils contribute 26%, glucose contributes 21%, proteins contribute 7% and starch contributes 31% to the total energy of 1635kJ. The author also added that the tubers comprise substantial fibre quantities, 14% of which are insoluble while 12% are soluble. The starch content of the tubers is mainly made of the amylose and amylopectin components which are structurally dissimilar. While amylopectin is heavily branched, amylose is linear, however, both have vital roles in the overall functional properties (gelatinization, swelling ability, viscosity, retro degradation etc.) of starch and their derivatives (Satin, 2006).

Several researchers have concluded that, tigernut oil has a comparable fatty acid composition to olive oil (Zhang et al., 1996; Arafat et al., 2009; Muhammad et al., 2011; Yeboah et al., 2011; Lopéz-Cortés et al., 2013; Adel et al., 2015). This conclusion was derived from the fact that, the unsaturated fatty acid composition of tigernut oil comprised 80% of the total oil, with 64.2 to 68.8% being primarily oleic acid. Yeboah et al. (2011) and Lopéz-Cortés et al. (2013)

found linoleic, stearic and palmitic acid as other fatty acids present in oil extracted from tigernut tubers. Even though tigernut tubers are generally low in protein compared to other macronutrients, research has reported significant quantities of essential amino acids like arginine in tigernuts (Aremu et al., 2015; Temple et al., 1990).

Food processing is the transformation of food from their raw state to an intermediate or finished product. Different methods of processing cause modifications in the nutritive constituents of food. Other processing methods are, on the other hand, essential and responsible for the bioavailability of nutrients. In terms of the nutritional quality of food, not only is the amount of minerals, vitamins and other macronutrients important but also, their bioavailability to the body. Research has postulated that the presence of food components such as tannins, fiber and phytic acid inhibit the bioavailability of certain essential minerals like zinc, calcium and iron (Reddy and Love, 1999). Thermal processing, according to Aamir et al., (2013) causes alterations in the structure of the cell walls of food and thus the overall food matrix. These modifications can release phytochemicals more readily for absorption into the body. On the other hand, thermal processing also causes the degradation of heat-sensitive nutrients in food. The objective of this study was to determine the physical properties of tigernut tuber and macro-nutrients in tigernut tuber, milk and oil.

8.2 Materials and Methods

8.3 Sources of Materials

Tigernut samples purchased from traders (wholesalers/retailers) in the Greater Accra Region (Table 3-1 & Table 3-2) were used for the physical and chemical analysis of the tigernut tubers as well as the preparation and analysis of the tigernut flour, milk extract and oil.

8.4 Sample Preparation

8.4.1 Sample Preparation of tigernut tubers

Sample preparation for the analysis of the tigernut tubers as well as the preparation of the tigernut milk and oil included sorting, washing and drying of the tigernuts.

8.4.2 Sorting

Tigernuts sampled from the markets were thoroughly cleaned by brushing off soil particles and debris. Both varieties were weighed separately. The nuts were sorted out to separate the defective or damaged ones from those in good shape.

The defective ones were weighed, and the percentage loss was determined using Equation 8-1.

$$\%\text{Loss} = \frac{\text{Wd}}{\text{Wi}}$$ **Equation 8-1**

Where Wd = weight loss defective nut

Wi = initial weight loss before sorting

8.4.2.1 Washing and Drying

The sound tigernut samples from the sorting stage were divided into four portions for each variety and for each location. The weighed-out portions were thoroughly washed in three sets of clean water. The washed samples were drained out and spread on a clean mat, which had been placed on a table in a low-ventilated room and made to dry overnight. The dried tigernut samples were sealed into nylon bags and placed in the refrigerator prior to analysis.

8.4.3 Preparation of Tigernut Milk Extract

For each variety of tigernut, fresh tigernut milk extract was prepared following the process highlighted in 6.2.2 (Sample Preparation of Variants of Tigernut Milk).

8.4.4 Oil Extraction

The tigernut oil was extracted in the Botany Laboratory of the University of Ghana using the AOAC Method 920.39 (Soxhlet extraction method with diethyl ether) as outlined in section 7.2.1 (Sample preparation).

8.5 Methods of analyses

Physical analyses were conducted on tigernut tubers. The chemical analyses were conducted on the tigernut tubers, oil, milk and flour (moisture). Accelerated keeping quality tests were also conducted on the tigernut oil and flour. All analyses were performed at the Mycotoxins and Food Analysis Laboratories in the Department of Food Science and Technology, College of Science of the Kwame Nkrumah University of Science and Technology.

8.5.1 Physical analysis of tigernut tubers

The bulk density, size and shape of tigernut tubers were determined as follows.

8.5.1.1 Bulk Density of tigernut tubers

The bulk density of the tigernut tubers was done using a well calibrated analytical mass balance and a container of predetermined volume.

The bulk density of the tigernut sample was calculated using the Equation 8-2:

$$\frac{\text{Bulk density of sample}}{\text{Volume of the container}} = \text{Weight of sample} \qquad \textbf{Equation 8-2}$$

8.5.1.2 Size and shape determination of tigernut tubers

Approximately ten Tigernut tubers were randomly picked from each sample set. The lengths of these randomly selected tubers were measured using the Vernier calliper, with the nuts held vertically between the nodes. The average measurements of each variety were compared in a Tigernut chart (Figure 8.1) and assigned as micro, standard or large.

The shape or morphology of 10 randomly selected tigernuts from each sample set (per location) were analysed and compared to a shape chart as indicated in Figure 8.1 and assigned as either Long (for cylindrically shaped tubers) or round (for spherically or circularly shaped tubers) (TTSL, 2020)

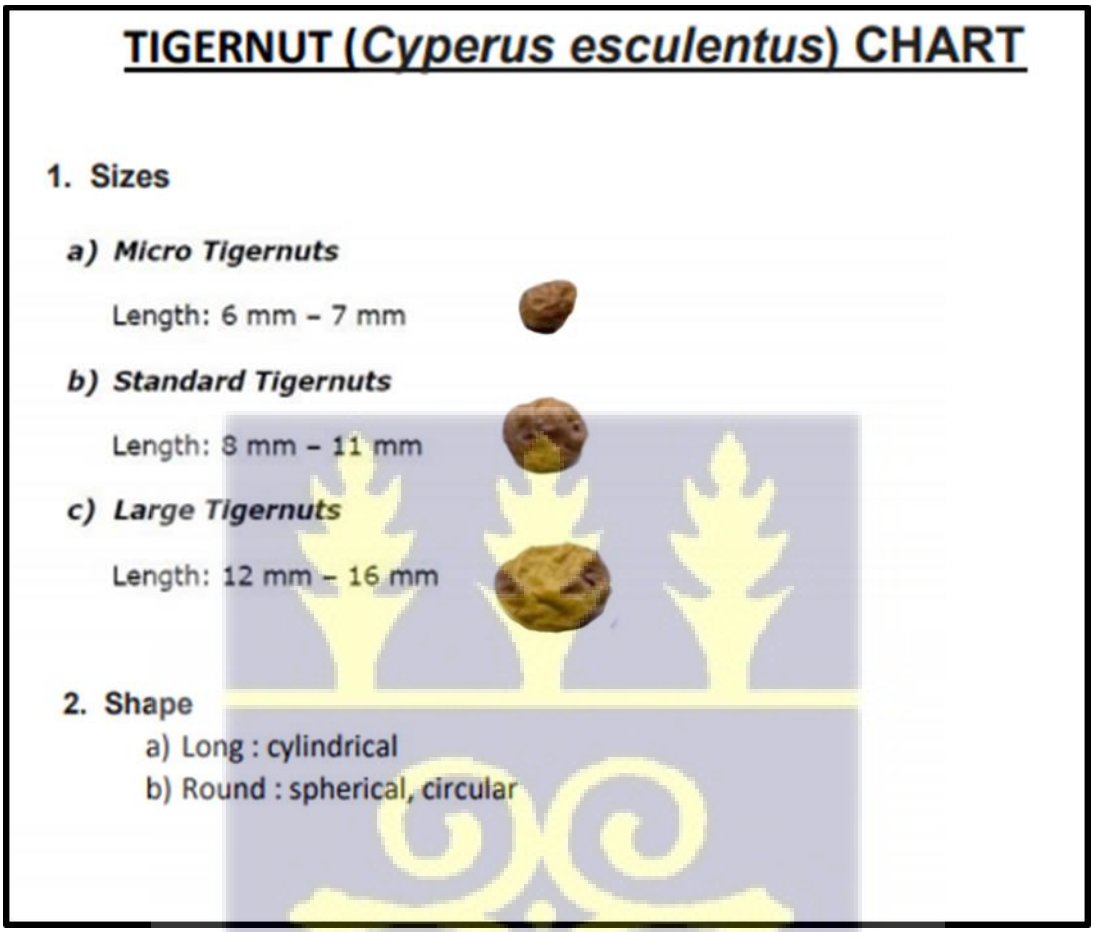


Figure 8.1: Tigernut shape and Size chart

Source: TTSL (2020)

8.5.2 Changes in the proximate composition of tigernut tuber when processed into its milk and oil

The crude fibre, moisture, carbohydrate, fat, protein and ash content of the following samples were determined using the AOAC (2012) methods and compared to ascertain their differences as tigernut tubers are transformed into products

- Tigernut tubers,
- Fresh tigernut milk extract and
- Tigernut oil

8.5.2.1 Moisture Content determination of tigernut tubers, fresh tigernut milk and tigernut oil

The moisture and total solids of the samples were obtained using the difference in weight loss after the sample had been heated in a temperature-controlled oven at 105℃. 5g of each sample was approximately and carefully transferred onto two separate dishes that had already been dried and weighed. The dishes were placed in an oven to be heated at a controlled temperature of 105℃ for 3 hours. After heating, the dishes were taken out and put in a desiccator at room temperature to allow for cooling to take place. Samples were subjected to another 30 minutes of drying and followed by cooling and reweighing. The procedures were done repeatedly until a constant weight was achieved. Equation 8-3 was used for the calculation of the moisture content in the samples.

$$\%\ \text{of moisture content} = \frac{\text{Wet weight (g)} - \text{Dried weight}}{\text{Sample weight (g)}} \qquad \textbf{Equation 8-3}$$

8.5.3 Mineral Ash content of tigernut tubers, fresh tigernut milk extract and tigernut oil

To determine the mineral ash composition of each sample, 5g of the tigernut samples was weighed and placed into an already weighed tarred crucibles. The crucibles were incinerated for 12 hours at a controlled temperature of 600°C. The crucibles were taken out and left to cool in a desiccator before weighing. The ash content of the samples was worked out according to the Equation 8-4

$$\% \text{ Ash} = \frac{\text{Weight of ash (g) x 100}}{\text{Weight of sample (g)}}$$

$$\% \text{ Ash} = \frac{(\text{Weight of crucible} + \text{ash}) - (\text{Weight of empty crucile (g) x 100}}{(\text{Weight of crucible} + \text{sample}) - (\text{Weight of empty crucibe (g)})}$$

Equation 8-4

8.5.4 Crude Fibre Determination of tigernut tubers and fresh tigernut milk

To determine the crude fibre, 2g of the sample was weighed for each sample and transferred into separate Erlenmeyer flasks (750ml). 200ml of 1.25% H_2SO_4 was carefully pipetted and added to the flasks. This was done to partition the samples. Each was immediately connected to a Liebig reflux condenser and set on a temperature-controlled hot plate. Boiling was allowed for 30 minutes for each flask, after which they were filtered separately through a clean linen cloth placed in a filter and washed with a large volume of water. 200ml of a 1.25% of NaOH was used to wash the filtrate back into the flasks. Each flask was then fastened to a condenser. The flask was boiled gently for 30 minutes. Each boiled solution was filtered through a Fischer crucible and washed carefully with water after which 15ml of 96% alcohol was added. The crucible and content of each sample were dried for 2 hours at a temperature of 105℃ and put into a desiccator for cooling to take place until room temperature was attained. They were weighed after drying to determine their masses. The samples in the crucible were intensively heated in a furnace for 30 minutes, after which they were cooled in a desiccator and reweighed.

The percentage of crude fibre was calculated according to the following formulae in Equation 8-5.

$$\% \text{ Crude fibre} = \frac{\text{Weight of crude (g) x 100}}{\text{Weight of sample (g)}}$$

Equation 8-5

$$\% \text{ Crude fibre} = \frac{\text{Weight of crucible} + \text{sample (before} - \text{after)ashing (g) x 100}}{\text{Weight of sample (g)}}$$

8.5.5 Determination of Protein Content (Macrokjeldahl method) tigernut tubers, fresh tigernut milk and tigernut oil

Approximately 2g of each of the samples was weighed into a cleaned digestion flask. 5 grams of selenium- based catalyst together with a few grams of boiling chips and 25 ml of concentrated H_2SO_4 were added to each sample in the flask. The flasks were shaken to ensure that, the samples were thoroughly wet. The flasks were heated separately on a digestion burner until a clear solution was produced and the boiling ceased. The digested sample solutions were cooled to room temperature, after which they were transferred into a dry 100 ml volumetric flask and topped up.

A 25ml portion of a 2% Boric acid (H_3BO_3) was carefully delivered by a pipette into two previously dried 250ml conical flasks. Three (3) drops of a mixed indicator (Methyl Red-Bromocresol Green) was added to each flask. The purpose of this mixture was to trap the ammonia in the sample that would be produced. With the aid of a pipette, 10ml of each digested sample solution was delivered into the separate decomposition flasks of the Kjeldahl unit and fixed. After fixing, the digested sample solutions were neutralized with 40% NaOH solution. The liberated Ammonia which was trapped into the collecting flasks containing the mixture of the acid and indicator, was distilled until a volume of 155 ml was collected. The distillate collected after the distillation process from each flask was titrated with 0.1 N standard HCl solution.

The total nitrogen was calculated with Equation 8-6

$$\% \text{ total nitrogen} = \frac{100 \text{ x } (Va - Vb) \text{ x } NA \text{ x } 0.01401 \text{x } 100}{W \text{ x } 10}$$

Equation 8-6

$$\text{NFE } (\%) = 100 - (\%EE + \%CP + \% \text{ CF} + \%\text{ash})$$

Where:

- Va = volume in ml of standard acid used in titration
- Vb = Volume in ml of standard acid used in the blank
- NA = normality of acid
- W = Weight of sample taken (g)
- NFE =Nitrogen free extract
- EE = Ether extract
- CP = Crude protein
- CF = Crude Fiver

8.5.6 Carbohydrate Content tigernut tubers, fresh tigernut milk extract and tigernut oil

The carbohydrate content was determined using Equation 8-7below:

$$\text{Carbohydrate } (\%) = 100 - (\%\text{moisture} + \%\text{fat} + \% \text{ Fibre} + \%\text{protein} + \%\text{ash})$$

Equation 8-7

8.6 Statistical Analyses

The Analysis of Variance test and independent samples t-test were used to determine the significant differences in macro nutrients across the tigernut value chain for the various tigernut products which includes oil and milk.

8.7 Results and Discussion

8.7.1 Physical Properties of Tigernut tuber

The physical properties of the tigernut tuber included the shape, weight and size. Sorting of tigernuts was done to separate the bad tubers from the good ones. The percentage unwholesome

tigernut after the sorting process (percentage loss) for the black tigernut was found to be 11.56 ± 0.41% whilst that of the yellowish-brown tigernut was 9.77 ± 0.92 %.

8.7.1.1 Classification of shape of Ghanaian tigernut tubers

The tubers were categorized into two types, based on their morphological variation using the shape classification of the tubers of *Cyperus esculentus* are shown in Figure 3 5. This was done on the basis on their colour (black or yellowish-brown) and shape (round or long) and results are shown in Table 8-1.

Table 8-1: Summary of results on the shape of samples

Variety	Shape	N	(%)
Black	Round	216	65.5[a]
	Long	144	34.5[a]
Yellowish-Brown	Round	217	70[a]
	Long	93	30[a]

Values are means of triplicates and ± standard deviation. Means for both varieties of tigernut with different superscripts were significantly different at p≤0.05

The types were identified as: black (round) and black (long) for the black variety, and yellowish-brown (round) and yellowish-brown (long) for the yellowish-brown variety. In the black samples collected, the black (round) type constituted 65.5% whereas the black (long) consisted 34.5%. For the yellowish-brown variety, the yellowish-brown (round) made up 70% of the samples whereas, the brown (long) variety constituted 30%. There was no significant association between the variety and shape of the tigernuts according to the Pearson Chi-square tests results of 1.509 which had a *p-value* of 0.219 (Table 8-1). The data indicates that for each variety the round shape is far more dominant, and this has implications for equipment design for processing.

8.7.1.2 Bulk density and size of Ghanaian Tigernuts tubers

Table 8-2 presents the summary of the physical dimensions (bulk density and size) of the tigernut tubers selected from the different sampling locations.

Table 8-2: Summary of the bulk density and size of Ghanaian Tigernut

Variety	N	Bulk Density (kg/m3)	Mean size (length) (mm)
Black	33	862.35 ± 148.98[a]	13.39± 1.87[a]
Yellowish-Brown	31	738.81 ± 141.41[b]	13.42 ± 1.57[a]

Values are means of triplicates and ± standard deviation. For each column, means for both varieties of tigernut with different superscripts were significantly different at $p \leq 0.05$

The mean bulk density of the black tigernut variety measured was 862.35 ± 148.98 kg/m3 whilst that of the yellowish-brown variety was 738.81 ± 141.41 kg/m3.

The average size of the two varieties were 13.39± 1.87 mm and 13.42 ± 1.57 mm for the black and yellowish-brown varieties respectively (Table 8-2). There was no significant difference between the sizes of the two varieties. The mean sizes of the tigernut tubers are greater than the approximate average length of 12.09 mm, which was reported by Coşkuner et al. (2002). According to Gambo and Da'u (2014), the dimension of tigernut tubers ranges from 8 mm to 16 mm. Coşkuner et al. (2002) asserted that, the size of tuber is proportional to the amount of water it can absorb at a room temperature.

Therefore, the bigger the tuber, the greater the amount of water it can absorb (Gambo & Da'u, 2014). The mean lengths recorded for both the black and yellowish-brown tigernut cultivars fall within the range of large tigernuts as shown in the tigernut chart (Figure 8.1). The sizes and

bulk density of tigernuts have not been found to affect the properties and nutritional values of the tubers.

8.7.2 Macro-nutritional composition of Tigernuts tubers, milk and oil

The macro-nutritional composition of the tigernuts tubers, milk and tigernut oil were determined and results are discussed in the sections below.

8.7.2.1 Moisture content

The moisture content of the tigernut tubers, milk and oil of the different varieties were studied, and the results shown in the Table 8-3.

Table 8-3: Moisture content of tigernut tubers, milk extract and tigernut oil

Product	Variety	Moisture (g/100g)
Tigernut Tubers	Black	24.44 ± 3.51[a]
	Yellowish-Brown	24.14 ± 3.41[a]
Tigernut Milk	Black	90.04±0.03[a]
	Yellowish-Brown	89.47±0.03[a]
Tigernut Oil	Black	0.054±0.00[a]
	Yellowish-Brown	0.0542±0.00[a]

Values are means of triplicates and ± standard deviation. For each property, means for both varieties of tigernut with different superscripts were significantly different at p≤0.05

The average moisture content of black and yellowish-brown varieties collected in both streets and markets was 24.44± 3.51g/100g and 24.14± 3.41 g/100g respectively. This difference in the moisture content between the varieties was not significant (p=0.619). It was also observed that, the moisture content of both tigernut cultivars was higher than that of grains such as maize (14-22%) (Weinberg et al., 2008) and cereals such as wheat (8.4%) (Nasir et al., 2003), probably due to the high sugars level in the tigernut tubers. However, the results acquired in

this study were lesser than those reported for other food tubers such as potato (78.75g/100 g) (Lombardo et al., 2012), yam (62.50g/100 g) (Abara, 2011) and sweet potato (70.54g/100g) (Olayiwola et al., 2013), (Venkatachalam & Sathe, 2006). Moisture has been reported to make food commodities susceptible to the growth of mycotoxigenic fungi (Kortei et al., 2019). The average dry matter content for roots and tubers vary depending on factors such as location, climate, cultivation practices and cultivars. Venkatachalam and Sathe (2006) posited that, low value for moisture contents in food is vital for the conservation of the quality and shelf life of food.

The moisture content of the tigernut milk (90.04 ± 0.02g/100g for black variety and for 89.47 ± 0.03g/100g yellowish-brown variety) were relatively lower than the 92.90 ± 0.02% that which was reported by Adebayo-Oyetoro et al. (2019). However, results of moisture reported in this study were higher than the 84.6 ± 0.41% reported by Ogbonna et al. (2013). Moisture content of tigernut milk is mainly due to the amount of water used in the extraction of the milk.

In this study, the moisture content of the black and yellowish-brown varieties of tigernut oil were found to be 0.0540 ± 0.0008g/100g and 0.542 ±0.007g/100g respectively (Table 5 3). Sarah & Stanley, (2018) reported higher moisture value (0.70% ±0.51). The moisture content of the oils analysed from the tigernut cultivars was higher as compared to other oils such as palm oil (0.20%), olive oil (0.47 %) and fresh soybean oil (0.31%) as reported by Hansen et al., (2016). According to the Asian and Pacific Coconut Community (APCC), 0.1-0.5% is the highest limit of moisture content of most edible oil. The values obtained in this study means they are bordering on the maximum moisture content an edible can have. High moisture content in oil reduces its quality (colour, peroxide value, free fatty acids, acid value, iodine and saponification value etc.) as well as the shelf life of the oil. However, according to Birnin-Yauri and Garba, (2011), the moisture content makes tigernut oil suitable for baking, frying and the manufacture of soaps.

Moisture levels of the two varieties of tigernut tubers from market and streets were compared and results presented in Table 8-4.

Table 8-4: Moisture content of Tigernuts purchased from markets and streets

Location	Variety	Moisture (g/100g)
Markets	Black	24. 26 ± 3.22[a]
	Yellowish-Brown	23.69 ± 2.26[a]
Streets	Black	24.62 ±3.81[a]
	Yellowish-Brown	24.58 ± 4.26[a]

Values are means of triplicates and ± standard deviation. For each location, means for both varieties of tigernut with different superscripts were significantly different at $p \leq 0.05$

The market and street samples of the black variety had moisture content of 24.26 ± 3.22g/100g and 24.62 ±3.81g/100g respectively (Table 8-4). The market and street samples of the yellowish-brown variety had moisture content of 23.69 ± 2.25g/100g and 24.58 ± 4.26g/100g respectively. From Table 8-4, it was observed that the moisture content of the tigernut cultivars sampled from the streets was higher than those sampled from the markets, although not significantly different. This slight difference could be attributed to the polyethene packaging of the street-selling units (Figure 8.2), which sweats due to the heat, whereas the market samples were packed in pans and exposed to the air (Figure 8.3).

Figure 8.2: Display of tigernut in the market

Figure 8.3: Packaging of tigernut in Polyethene bags by street hawkers

8.7.2.2 Mineral Ash content of Tigernut tubers, milk extract and tigernut oil

According to Ismail (2017), the ash content is referred to the inorganic residue that remains after the organic matter has been subjected to intense heat for a period. It is an implication of the mineral content in the tigernuts. Table 8-5 shows the ash content of tiger tuber, tigernut milk extract and tigernut oil.

The mean ash content obtained for both black and yellowish-brown varieties of tigernut tubers were (1.58 ± 0.80g/100g) and (1.61 ± 0.04g/100g). Pomeranz and Clinton (1981) postulated that ash content below the ranges of 1.5-2.5 % are usually not considered suitable for animal feed.

Table 8-5: Mineral Ash composition of tigernut tuber, milk and oil

Product	Variety	Mineral Ash (g/100g)
Tigernut Tubers	Black	1.58±0.08[a]
	Yellowish-Brown	1.61±0.43[a]
Tigernut Milk	Black	0.19±0.00[a]
	Yellowish-Brown	0.20±0.00[b]
Tigernut Oil	Black	0.07±0.007[a]
	Yellowish-Brown	0.13±0.003[b]

Values are means of triplicates and ± standard deviation. For each product type, means for both varieties of tigernut with different superscripts were significantly different at p≤0.05

The ash content for the tigernut of both varieties in this study fell within the range and therefore can be considered suitable for human and animal food and feed. The ash content reflects the quantity of minerals found in food commodities. This is very vital because minerals are essential in assessing the physicochemical properties of food and prevents growth of microorganism ("Moisture, Ash Testing in Food Processing", 2010). In their work on "Quantification of Ash and Moisture in Wheat Flour by Raman Spectroscopy", Czaja et al.

(2020) obtained ash content results for different flour samples in the ranges of 0.5–2.5%. The main mineral components in ash content of flours include zinc, potassium, iron, calcium, copper and phosphorus. Approximately 45% of ash content in flours is phosphorus, followed by roughly 38% potassium, 13% magnesium and 3% calcium. Other mineral compounds add up to 1% (Kulkarni et al., 2007; Piironen & Salmenkallio-Marttila, 2009).

The ash content of the black and yellowish-brown varieties of tigernut milk extract were 0.19 ± 0.00g/100g and 0.20 ± 0.00g/1respectively. These values were lower than values obtained by Adebayo-Oyetoro et al. (2019) (0.28 ± 0.02) and by Ogbonna et al. (2013) (1.80 ± 0.02). This trend may be because the ratio of water to tigernut tuber was less as compared to what was used in this study. The total ash content of the tigernut milk makes it a beneficial source of minerals essential for both growth and development (Ogbonna et al., 2013). Many studies have postulated the ash content of milk to be approximately within a range of as low as 0.1% and as high as 22% (American Dairy Products Institute Brochure, 2002; Cerbulis and Farrell, 1975). The values obtained in this study, although low, fall within this range and as such, indicate that tigernut milk provide some form of mineral content when consumed. However, recipe formulation should be revised by the addition of less water in order to obtain a higher milk ash content and more minerals when consumed.

The ash content of the black and yellowish-brown varieties of the tigernut oil were 0.07± 0.007g/100g and 0.13 ± 0.003g/100g respectively. These values were found to be higher than that of soybean oil (0.015 %) but lower than that of sunflower oil (0.925%) (Mengistie et al., 2018). Mengistie et al. (2018) concluded from their study that, the ash content of various edible oils ranges from 0.012 to 0.925. The results obtained in this study shows that tigernut oil has a moderately suitable mineral composition. Since the ash content is an indication of the mineral

content (Ismail, 2017), the moderate ash content of the oils makes them suitable for food preparation.

8.7.2.3 Protein content of tigernut

Table 8-6 shows the protein content of tigernut tuber, tigernut milk extract and tigernut oil of both varieties of tigernut.

Table 8-6: Protein composition of tigernut tubers, milk extract and oil

Product	Variety	Protein (g/100g)
Tigernut Tubers	Black	5.15± 0.41[a]
	Yellowish-Brown	6.49±0.27[b]
Tigernut Milk	Black	0.52±0.01[a]
	Yellowish-Brown	0.65±0.01[a]
Tigernut Oil	Black	0.24±0.00[a]
	Yellowish-Brown	0.30±0.1[a]

Values are means of triplicates and ± standard deviation. For each product type, means for both varieties of tigernut with different superscripts were significantly different at $p \leq 0.05$

The protein content of the tubers of yellowish-brown tigernut variety (6.49 ± 0.27g/100g) was significantly higher than that of the black tigernut variety (5.15 ± 0.41g/100g). The level of protein in the tigernut tubers is independent of colour or size (Bado et al., 2015). The value of the yellowish brown variety obtained from this study was lower (7.15g/100g) than those reported for tigernuts from Nigeria by Oladele and Aina (2007) but higher than those reported for Burkina Faso (3.47g/100g, 4.33g/100g and 3.3g/100g for morphotypes 1,2 and 3 respectively) (Bado et al., 2015).

The protein content of both tigernut cultivars of the tigernut tubers in this study was found to be higher than those in other tubers; 1.97% for potatoes (Lombardo et al., 2012), 2.55% for yam (Abara, 2011) and 1.40% for cassava (Maieves et al., 2012). Due to the high protein content, tigernut can be used as a natural source of plant protein (Suleiman et al., 2018). Research has shown that, tigernut can provide more than 17% and more than 26% of daily protein requirement for adults and children respectively (FAO/WHO/UNU, 2012). In this regard, tigernut can be used in food formulations or incorporated into our diets. In cases where the protein contribution is most desired, the yellow-brown variety should be used in the formulations.

The protein content of the tigernut milk from the black and yellowish-brown varieties were 0.52 ± 0.01g/100g and 0.65± 0.01g/100g respectively. However, the values obtained from other researchers such as Adebayo-Oyetoro et al. (2019) (2.11 ± 0.01 %) and Ogbonna et al. (2013) (8.19 ± 0.11%) were higher than those obtained in this study due to recipe differences. According to an article on the Nutritional Components of Milk, milk contains roughly 3.3% proteins. Even though the proteins in tigernut milk as compared to dairy milk is low, tigernut milk can be used as beverage for children and adults alike. It can also be used for tigernut yoghurt production since it contains relatively higher protein content as compared to other tubers and can give flavour to yoghurt. In their study, Wongnaa et al. (2019) conducted a survey to determine consumers' willingness to purchase tigernut yoghurt in Kumasi. Their findings showed high acceptability and willingness by people to purchase the products at a higher price (Gh¢ 3.50) as compared to the normal yoghurt sold at (Gh¢ 2.50). They found out that the willingness was a result of the fact that, people were cognizant with the nutritional health benefit of tigernut consumption and its aphrodisiac property which was one of the sole motivation factors for the men. This shows that if people are made aware of the nutritional

health benefits, tigernut yoghurt can have brighter market prospects in both the rural and urban setting.

The protein content of the tigernut oil from the black and yellowish-brown varieties were 0.24 ±0.00 g/100g and 0.30 ± 0.1g/100g respectively. Sarah & Stanley (2018) reported higher protein values of 0.37 ± 0.04 as compared to the results of this study.

8.7.2.4 Fat content of Tigernut

Table 8-7 presents the fat content of tigernut tubers, tigernut milk extract and tigernut oil.

Table 8-7: Fat composition of tigernut tubers, milk extract and oil

Product	Variety	Fat (g/100g)
Tigernut Tubers	Black	22.16 ± 0.50[a]
	Yellowish-Brown	19.82 ± 0.48[b]
Tigernut Milk	Black	1.21 ± 0.00[a]
	Yellowish-Brown	0.89 ± 0.01[a]
Tigernut Oil	Black	99.64 ± 0.008[a]
	Yellowish-Brown	99.03 ± 0.02[a]

Values are means of triplicates and ± standard deviation. For each product, means for both varieties of tigernut with different superscripts were significantly different at $p \leq 0.05$

The crude fat or lipid was the second highest constituent in the tigernut tuber samples. The values obtained from the analysis were 22.16 ± 0.50g/100g and 19.82 ± 0.48g/100g respectively for both the black and yellowish-brown varieties. The values from the analysis were lower than those reported in literature: Sabah et al. (2019) reported a crude oil value of 30. 01 ± 0.229g/100g for tigernut sampled from Egypt; Alegría-Toran and Farré-Rovira, (2003) reported values of 24.49g/100g for tigernut in Spain; Suleiman et al. (2018) also reported a value lower than those obtained in the analysis (17.0 %).

The crude lipid content obtained for tigernut tubers in this study (22.16 g/ 100g and 19.82 g/100 g for the black and yellowish-brown varieties respectively), were found to be lower than for other commodities such as peanuts (49.20 g/100g), pine nuts (68.40 g/ 100 g) and walnuts (65.20 g/ 100 g) (Sanchez-Zapata et al., 2012). The variation in the crude lipid has been connected to differences in genetic material and geographical location (Bado et al., 2015). The significantly higher fat content according to FAO/WHO/UNU, (2002) could contribute more than 73% and 49% of the daily fat requirement for both adults and children respectively.

The fat content of the tigernut milk extract were 1.21 ± 0.00g/100g and 0.89 ± 0.01g/100g for the black and yellowish-brown varieties respectively. The low-fat content of the tigernut milk extract makes it an ideal replacement for skimmed milk.

The fat content of the tigernut oil was 99.64 ± 0.008g/100gand 99.03± 0.02g/100g for the black and yellowish-brown varieties respectively. These values were lower than those reported by Sarah & Stanley (2018) (92.78 ± 0.01%). In their study to assess the use of tigernut oil emulsion as replacement for animal fat in Beef Burgers, Carvalho et al. (2020) reported a decrease in fat and protein contents in the samples with tigernut oil emulsion. They concluded that, the 100% substitute of animal fat with tigernut oil resulted in a healthier meat product with a significant reduction in saturated fat content and increase in unsaturated fatty acids. Due to the high oil content, studies have shown that, tigernut oils can be used as an alternative source of fuel, which is far cheaper and environmentally safe relative to other burning fuels (Wongnaa et al., 2019). It has also been recommended as a potential crop for biodiesel production (Zhang, 1996). Another study found that tigernut produced 1.5 metric tonnes of oil per hectare contingent on a tuber yield of 5.67t/ha and a 26.4% oil content (Makareviciene et al., 2013). This benefit of tigernut oil, if properly exploited by the government, can generate revenue for the country, which will help boost the economy. This, however, can be materialized through

the establishment of tigernut cultivation factories in districts where tigernuts are produced and harvested.

8.7.2.5 Carbohydrate content of tigernut tuber and tigernut milk

Carbohydrate constitutes the major components of tigernut tubers (Sanchez-Zapata et al., 2012). Table 8-8 presents the carbohydrate content in tigernut tubers and tigernut milk of the black and yellowish-brown varieties.

Table 8-8: Carbohydrate composition of tigernut tubers and tigernut milk

Product	Variety	Carbohydrates (g/100g)
Tigernut Tubers	Black	60.57±0.85[a]
	Yellowish-Brown	62.17±0.62[b]
Tigernut Milk	Black	1.04±0.03[a]
	Yellowish-Brown	1.69±0.03[a]

Values are means of triplicates and ± standard deviation. For each product, means for both varieties of tigernut with different superscripts were significantly different at $p \leq 0.05$

The carbohydrate content in the yellowish-brown variety of the tigernut tuber (62.17 ± 0.6g/100g) was higher than that of the black variety of the tigernut tuber (60.57 ±0.85g/100g). The values obtained for tubers of both varieties in this study were higher than those reported by Bado et al. (2015) for tigernut tubers grown in Burkina Faso (68.24±1.28 g/100g and 69.21 ±1.30g/100g respectively for black and yellowish-brown morphotypes).

Also, the reported values by Oladele and Aina (2007) for the carbohydrate content of tigernut tubers sampled from a local market in Nigeria were lower than those obtained from this study (46.99% and 41.22% respectively for the yellowish-brown and black variety respectively. This same trend was observed when compared to the carbohydrate content (45.73 ± 0.035g/100g)

of tigernut tubers from Egypt (Sabah et al., 2019). The values were however higher than those from Spain with a reported value of 43.30% (Alegría-Toran & Farré-Rovira, 2003). Generally, tigernut tubers are rich in carbohydrates. However, the disparities in profile and content has been reported to be as a result of difference in varieties and ripening stage (Sanchez-Zapata et al., 2012). The main composition of carbohydrates of tigernut tubers are starch and dietary fibre (Sanchez-Zapata et al., 2012). Tigernuts have been found to contain higher amounts of carbohydrates than other nuts. Freitas and Naves (2010) reported a value of 7.48g/100g for the carbohydrate content for peanuts. A value of 3.90g/100g by Venkatachalam and Sathe (2006) for walnuts and 6.10g/100g carbohydrate value for almonds was reported by Ros (2010). Also, the carbohydrate contents for tigernuts have been reported to be higher than those of root tubers:18.17g/100g for potato (Lombardo et al., 2012), 34.60g/100g for yam (Abara, 2011), 13.80g/100g for sweet potato (Scher et al., 2009) and 38g/100g for cassava (Maieves et al., 2012). Consumption of food with high carbohydrate content can be a very good source of energy for our daily activities. Due to its high carbohydrate content, the tigernut, if consumed, can contribute significantly more than 40% of the daily carbohydrate needs of a child (4-9 yrs.) and higher than 32% of that of an adult (FAO/WHO/UNU, 2002). Research has shown that tigernut flours contribute to the lowering of glucose levels when used in combination with other meals and as such, can be used in diets suitable for diabetic patients (Oluwajuyitan et al., 2019). The carbohydrate content of the black and yellowish-brown tigernut milk extract were 1.04 ± 0.03g/100g and 1.69± 0.03g/100g respectively. Oyetoro et al. (2019) reported higher values of carbohydrates (2.07 ± 0.01%) in tigernut milk extracts. Ogbonna et al. (2013) also reported higher values (58.01 ± 1.24%) in tigernut milk. Furthermore, since it is plant milk, tigernut milk is ideal for lactose intolerant milk consumers.

Tigernut oil in this study did not contain carbohydrates, which is consistent with results from researchers such as Sarah & Stanley, (2018).

8.7.2.6 Dietary fibre content of tigernut tuber and tigernut milk

Table 8-9 shows the results of the fibre content of tigernut tubers and tigernut milk.

Table 8-9: Dietary fibre composition of tigernut tubers and milk

Product	Variety	Dietary Fibre (g/100g)
Tigernut Tubers	Black	10.54±0.22[a]
	Yellowish-Brown	9.90±0.24[b]
Tigernut Milk	Black	7.00 ±0.01[a]
	Yellowish-Brown	7.13±0.00[a]

Values are means of triplicates and ± standard deviation for n= 3. For each product, means for both varieties of tigernut with different superscripts were significantly different at p≤0.05

The fibre content obtained for both varieties of the tigernut tubers was 10.54 ± 0.22g/100g and 9.90 ± 0.24g/100g for the black and yellowish-brown respectively The fibre content obtained from the analysis were found to be higher than those of potatoes (0.68) (Lombardo et al., 2012), yam (1.68) (Abara, 2011) and cassava (1.18) (Maieves et al., 2012). This makes tigernut ideal for weight loss. Due to the high fibre content, tigernut can be used to formulate diets for the treatment of colon cancer, heart disease, constipation and obesity (Bender, 1973; Ball, 1994). Generally, tigernuts have been reported to be high in fibre content. Tigernut as a bakery product ingredient will also present the added advantage of enriching the baked product with dietary fibre. Also, the high dietary fibre makes tigernut a good meal for diabetics, as dietary fibre is known to lower the absorption of sugars through the gut.

Milk extracts from black and yellowish-brown tigernut varieties contained 7.00 ± 0.01g/100g and 7.13 ± 0.00 g/100g respectively. The high fibre content of the tigernut milk helps in the prevention and curing of obesity, diabetes and colon cancer (Anderson et al., 1994). In comparison to other milk extracts, the tigernut milk extracts was found to have higher crude

fibre than that of recombined milk (0.50 ± 0.19%) (Ogbonna et al., 2013). Also, the crude fibre content for the tigernut milk extracts analysed in this study was found to be higher than those reported for soymilk (0.70 ± 0.04%) and almond milk (1.70 ± 0.20%) (Alozie-Yetunde & Udofia, 2015).

Tigernut oil from both varieties in this study did not contain any fibre, which is consistent to works of Sarah & Stanley, (2018).

8.8 Conclusion

The bulk density (weight) of the yellowish-brown tigernut variety was significantly lower than that of the black variety. Physically however, the black variety looks smaller in size compared to the yellowish-brown variety, showing that physical properties are dependent on external and innate conditions which may not result in a significant difference in dietary benefits.

The average moisture content of the fresh tubers of both varieties was approximately 24%. Among all analysed food derivatives of tigernut, moisture content was highest in milk extracts. Moisture of the oil was the lowest among all the food products obtained from the tubers with a moisture content of roughly 0.05%. This establishes the fact that tigernut oil is ideal for food applications such as frying, baking and soap manufacturing. The mineral content, as quantified by ash content, was approximately 1.6% for both tigernut tubers which is fitting for both human and animal consumption as minerals play crucial functions in the body. The ash content of the milk extract was low. The ash content of the oil was however relatively high, irrespective of this observation, oils do not require high ash contents to make them suitable for food application but usually, just a reasonable quantity is suitable. The protein content reported for the tigernuts was higher as against other tubers and as such can serve as a natural protein source in diet. The yellowish-brown tigernut tuber had a significantly higher protein content as compared to the black variety and can therefore be applied in the production of weaning mixes

for growing children. The average protein content of tigernut milk extract was however high and can be used in food applications such as beverage and yoghurt production. This is a viable product that will survive on the market due to the health benefits and especially aphrodisiac property of the tubers.

Crude fat was the second most abundant macronutrient in the tigernut tubers. The milk extract had a low-fat content, and this is a suitable alternative for lactose intolerant persons. The oil constituted the highest percentage of the total fat content of tigernut tuber with about 99% fat. Aside food applications, these can also be used as cheaper and environmentally friendly fuel sources. The carbohydrate component of tigernuts is mainly starch and dietary fibre. The carbohydrate component of tigernut was quite high for a tuber. The high carbohydrate content is an indication of high energy content when tigernut tubers are consumed. Due to the high dietary fibre content also, tigernut tuber is suitable for diabetics as they reduce glucose levels when incorporated with other foods. The milk extract had an adequate carbohydrate content, while no carbohydrate was found in tigernut oil. The tigernut tubers had high fibre content and as such can be used in recipe formulation for people with health conditions such as obesity, colon cancer, heart disease and constipation.

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CHAPTER NINE

9. Summary and Conclusion

Tigernut is grown on a very low scale in Ghana although the soil profile and climatic condition of the country are ideal for its cultivation. While there are occasional media awareness created on some of the nutritional and health benefits of the tuber, tigernut still remains one of the most underutilized crops in Ghana due to its limited use in food applications. Increase in the consumption would require knowledge in the safety (mycotoxin levels) of the tuber as well as the influence of stakeholders of the value chain on the mycotoxin levels. Additionally, inclusion of the tubers in the Ghanaian diet would require knowledge on the functional properties of the tuber and its products. The study aimed to characterise Ghanaian tigernut as an ingredient for possible food application.

Majority of the farmers and consumers, unlike traders (wholesalers and retailers) who participated in this study, had appreciable knowledge on mycotoxins and displayed notable attitude towards its prevention. Even though majority of the traders knew exactly how to identify and segregate bad tubers, some looked at superficial parameters such as colour, texture, general appearance etc. Some of the stakeholders confirmed that they feed their farm animals with mycotoxin contaminated tigernut tubers.

The results of this work showed that mycotoxins (Aflatoxins and ochratoxin A) progressed as tigernut tubers travelled further along the value chain. Irrespective of precautionary measures (sorting, washing with lime/water/salt) applied in the value chain, these mycotoxins still survived throughout all stages and were found in higher concentrations at the ready-to-eat stage (trade) due to the poor handling practices and packaging techniques. This postulates that once contamination occurs at the initial planting stages, progression continues throughout the lifecycle of the tuber.

The physicochemical properties of the tigernut flour such as its high amylose content contribute to the flour's resistance to swelling and may indicate that the starches within the tuber have the capacity to resist decomposition during heating and contribute to retrogradation. The high dietary fibre and resistant starch content of the flour may present an option for inclusion into diets of diabetics and weightwatchers. The tigernut flour was characterised by pasting properties that may promote its application in the baking industry. Titratable acidity was identified as the shelf-life indicator of tigernut flour and using the Arrhenius equation, it was predicted that storage at lower temperatures increase the shelf stability of the flour.

The physicochemical and functional properties of milk extracted from tigernut tubers are influenced by heat (roasting) and alpha amylase thereby, offering various food applications in the beverage industry. Percentage brix, total solids, emulsion stability and foaming stability increased whereas the pH and colour (lightness) decreased with increasing addition of alpha amylase and application of heat. Tigernut milk was observed to exhibit pseudoplastic fluid characteristics.

In this study, the tigernut tuber contained notable amounts of oil with a yield of 15% to 17%. The findings of this study confirmed that the tigernut oil could be a good substitute for olive oil. Application of heat appeared to have influenced the levels of phenolic compounds and the quality of the tigernut, as indicated by the flash and smoke points, acid value, free fatty acids, peroxide value, saponification value, ester value and iodine value.

The nutritional composition of tigernut tuber differs from its flour, milk and oil. Tigernut tubers had high fibre content whilst the tigernut flour was characterised by high starch content. The fat content of tigernut milk is comparable to that of skim milk whilst the tigernut oil had high phenolic composition (Quercetin and gallic acid).

9.1. Recommendations

- This study established the presence of aflatoxins and ochratoxin A in some tigernut tubers grown and sold in Ghana. However, countries such as Spain have identified fusarium in tigernut tubers (Sebastia et al., 2012) and other mycotoxins (aflatoxins and Ochratoxin A) in tigernut beverages (Sebastià et al., 2010). In order to understand the scale of mycotoxin occurrence and concentration in Ghanaian tigernut value chain, a full-scale mycotoxin analysis is recommended on tigernut tubers and its products.
- The results of the surveys from this work have provided baseline information on the knowledge of mycotoxin amongst stakeholders of the tigernut value chain (*Aduamoah* and *Adwoa* (farmers) and Greater Accra Region (traders and consumers)) and determine if measures were in place to mitigate the risk of fungal colonisation of tigernut along the supply chain However, tigernut is grown on a small scale in many other areas in Ghana and is sold and consumed throughout the country (Asare, et al., 2020). Surveys involving more farmers, traders and consumers from other parts of Ghana is recommended in order to give a complete and general outlook of mycotoxin knowledge and practices of stakeholders of the tigernut value chain in the country.
- Results from this study provided initial information on the shelf life and optimal storage temperatures for Ghanaian tigernut flour using the Arrhenius equation. It is recommended that further real time studies are conducted to confirm these storage conditions as well as determine the influence of packaging material on the shelf stability of tigernut flour. Furthermore, tigernut flour is proposed as a substitute for flour in the baking industry based on the properties of the flour assessed in this work. It is recommended that studies on the right recipe for composite flour for various food options are conducted in order to give ready information to consumers and the food industry.

- Results from this study provided information on the possibility of using heat (roasting) and alpha amylase to improve the fluidity of tigernut milk. However, it is recommended that further study is done to establish the ideal hydrocolloid which would guarantee the homogeneity of the milk. This is because the integrity of the interface created by the appreciable amount of oil in tigernut and the water added to make the milk (oil in water emulsion) may weaken overtime and lead to separation.
- This study used the solvent extraction method (Soxhlet method) to extract Ghanaian tigernut oil and provided baseline information on the effect of heat on the functional properties and phenolic composition of the oil. It is recommended that other edible oil extraction methods such as cold pressing are used and compared to establish the method that produces the most yield and best quality oil.

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APPENDICES

APPENDIX 1

QUESTIONNAIRE TO DOCUMENT TIGERNUT KNOWLEDGE, ATTITUDE AND PRACTICES AMONG FARMERS

ID OF RESPONDENT……...……. TOWN…….

DISTRICT OF INTERVIEW……

DEPARTMENT OF NUTRITION AND FOOD SCIENCE

P.O. BOX LG 134, LEGON, GHANA

Hello! I am a PhD student pursuing Food Science at University of Ghana. I am currently working on a project aimed at establishing knowledge of the existence and effects of mycotoxin contamination on tigernut and safety measures taken to` minimize occurrence of mycotoxin contamination among traders in Ghana. I would be grateful if you could spare 10 minutes of your precious time to complete this questionnaire.

Please be assured that any information provided will not be linked to personal information and data that will be published will not reveal the identity of participants.

SECTION I: DEMOGRAPHIC INFORMATION AND GENERAL INFORMATION

Please indicate your responses by ticking against your preferred choice(s) and provide answers where applicable.

1. What is your age?

   21-40 years [ ] 41-60yrs [ ] More than 60years[ ]

2. What is your sex?

Male [ ] Female [ ]

3. What is your marital status?

Single [ ] Married [ ] Divorced [ ] Widowed [ ] Other please specify……..[ ]

4. What is your level of education?

Elementary / Primary School [ ] Junior High School [ ]

Secondary / Technical School [ ] Vocational School [ ] None [ ]

5. What is your family size?

Less than 3 [ ] 4 – 6 [ ] Greater than 6 [ ]

6. How many dependents do you have?

Less than 3 [ ] 4 – 6 [ ] Greater than 6 [ ]

7. What is your major occupation?

Farming [ ] Other please specify…….. [ ]

8. What other occupations do you engage in?

……

9. What is your monthly income?

Less than 500 Ghana Cedis [ ] 600 – 1000 Ghana Cedis [ ] More than 1000 Ghana Cedis [ ]

SECTION II: TIGERNUT HANDLING AND SUPPLY

10. Which variety of tigernut do you cultivate?

Black [ ] Yellow [ ] Both [ ]

11. What is the approximate amount of tigernut harvested at each season?

……

12. When is the best time to harvest?

……

13. Is this period considered early harvest/ late harvest?

Early harvest [ ] Late harvest [ ]

14. Why is this harvest time chosen to harvest tigernuts?

……..

15. Is there any form of sorting/grading done after harvesting?

Yes [ ] No [ ]

16. What criteria is used for sorting/grading (what do you look out for to certify good/bad tigernuts)?

Presence of moulds [ ] Presence of insect infestation [ ] Other please specify [ ]

17. What drying method do you use?

……

18. Why do you use this method for drying?

……

19. How long does this drying method take?

Less than 1 week [ ] 2 – 3 weeks [ ] Less than 1 month [ ] More than 1 month [ ]

20. Do you have knowledge of any other drying method?

Yes [ ] No [ ]

21. If yes, please specify.

……

22. How do you identify the end of the drying process?

Hard Texture [ ] Shriveled tubers [ ] Sweet Taste [ ] Other please specify……..[ ]

23. How do you store harvested tigernuts? (Indicate storage conditions and pre-storage treatments if any)

……

24. Is there any form of cleaning of storerooms/houses before tigernuts are stored in them?

Yes [ ] No [ ]

25. In what form do you prefer to store tigernuts?

Fresh [ ] Semi-Dried [ ] Dried [ ]

26. How long do you store them after drying?

Less than 1 week [ ] 2 – 3 weeks [ ] More than a month [ ]

27. In what way do you prefer to store tigernuts?

On bare floor [ ] In jute sacks [ ] In tray / Basins [ ] Other please specify…… [ ]

28. What method of preservation do you employ during storage?

Use of chemicals [ ] No preservations [ ] Other please specify……. [

29. Do you encounter any losses during storage?

Yes [ ] No [ ]

30. If yes, what are the causes of the losses?

Moulds / Fungi [ ] Insects infestation [ ] Rodents [ ] Other please specify

31. What is the peak period of insect infestation?

Less than 1 week [ ] 2- 3 weeks 1 – 3 months [ ] More than 4 months [ ]

32. What method is used to protect the tigernuts?

Use of fertilizers [ ] Combination of chemicals [ ] Other please specify…… [ ]

33. Do you observe the presence of mouldy tigernut in your crops?

Yes [ ] No [ ]

34. How are mouldy tigernuts handled?

Animal feed [ ] Sold at low prices [ ] Discarded [ ] Other please specify……[ ]

35. Who do you sell tigernuts to?

Traders [ ] Manufacturers [ ] Consumers [ ] Other please specify [ ]

36. How do you sell your produce?

……..

SECTION III: MYCOTOXIN AWARENESS

37. Do you have any idea of the word mycotoxin/aflatoxins?

Yes [ ] No [ ]

38. Do you have any idea on effect of mouldy tigernut on human/animal health?

Yes [ ] No [ ]

39. If Yes, list some of them.

Headache [ ] Stomach upset [ ] Nausea [ ] Other please specify…… [ ]

40. What is your opinion on what causes spoilage/moulds?

Heaping of tigernut during drying [ ]

Inadequate drying between sales and retail stage [ ]

Insect infestation [ ] Use of chemicals on crops [ ]

Other please specify…….[ ]

41. Do you have any idea on measures to take to control moulds and fungi in storage?

Yes [ ] No [ ]

42. If Yes, state them

Periodic drying [ ] Proper washing with lime and dry [ ]

Proper washing with salty water [ ] Other please specify……. [ ]

43. Why do you think sorting/removal of mouldy nuts are important?

……

THANK YOU

APPENDIX 2

QUESTIONNAIRE TO DOCUMENT TIGERNUT KNOWLEDGE, ATTITUDE AND PRACTICES AMONG TRADERS

ID OF RESPONDENT…….…… **LOCATION…….**

DISTRICT OF INTERVIEW…… **LOCATION TYPE…….**

DEPARTMENT OF NUTRITION AND FOOD SCIENCE

P.O. BOX LG 134, LEGON, GHANA

Hello! I am a PhD student pursuing Food Science at University of Ghana. I am currently working on a project aimed at establishing knowledge of the existence and effects of mycotoxin contamination on tigernut and safety measures taken to` minimize occurrence of mycotoxin contamination among traders in Ghana. I would be grateful if you could spare 10 minutes of your precious time to complete this questionnaire.

Please be assured that any information provided will not be linked to personal information and data that will be published will not reveal the identity of participants.

SECTION I: DEMOGRAPHIC INFORMATION AND GENERAL INFORMATION

Please indicate your responses by ticking against your preferred choice(s).

1. What is your age?

   21-40 years [ ] 41-60yrs [ ] More than 60years[ ]

2. What is your sex?

   Male [ ] Female [ ]

3. What is your marital status?

   Single [ ] Married [ ] Divorced [ ] Widowed [ ] Other please specify……..[ ]

4. What religion do you belong to?

Christian [ ] Islam [ ] African tradition [ ] Other Specify…… [ ]

5. What region do you come from?

   Ashanti [ ] Brong Ahafo [ ] Central [ ] Eastern [ ] Greater Accra [ ]

   Northern [ ] Oti [ ] Savannah [ ] Upper East [ ] Upper West [ ] Volta [ ]

   Western [ ]

6. What is your level of education?

   Elementary / Primary School [ ] Junior High School [ ]

   Secondary / Technical School [ ] Vocational School [ ] None [ ]

7. How long have you been selling tigernuts?

   <1yr [ ] 1-5yrs [ ] 6-10yrs [ ] other please specify ……. [ ]

8. Do you belong to any traders association or group?

   [ ] Yes [ ] No

9. If yes, which association or group do you belong to?

   Specify……

10. Is the retailing of tigernuts your only business?

    Yes [ ] No[ ]

11. Who are your major customers?

Caterers [ ] Individual Home Users [ ] Other retailers[ ] Other please specify….[ ]

SECTION II: TIGERNUT SUPPLY

12. Where do you source your tigernuts from?

    Another retailer [ ] Directly from Farmer[ ] Wholesaler [ ] Other please specify…[ ]

13. Which city/ town or village does your tigernuts come from?

Kumasi [ ] Dormaa [ ] Accra [ ] Other please specify ……..[ ]

14. How often do you purchase tigernuts?

Weekly [ ] Monthly [ ] Quarterly [ ] Other please specify ……..… [ ]

15. How do you assess raw material quality?

Colour [ ] Buying from same Supplier [ ] Absence of insects activity [ ]

Texture [ ] Other please specify…….. [ ]

16. Have you ever rejected supplied tigernuts?

Yes [ ] No [ ]

17. If yes, how often in the past two months?

Once [ ] Twice [ ] Three times [ ] Four times [ ] Others please specify…….[ ]

18. If yes, what is the main issue?

Colour change [ ] Insect activity [ ] Other please specify [ ]

19. What is the approximate quantity you buy per batch?

1-5kg [ ] 6-10kg [ ] 11-15kg [ ] 16-20kg [ ] >20kg [ ]

20. In which form do you buy your tigernuts?

Fresh (undried) [ ] Semi-dried [ ] Dried [ ] Other please specify…….[ ]

SECTION III: TIGERNUT SAFETY PRACTICES

21. How do you display your tigernut?

In an open basket [ ] In tied polythene [ ] In a big open polythene [ ]

Other please specify……[ ]

22. Do you do anything to the tigernuts before and in between sales?

Yes [ ] No [ ]

23. If yes, which of the following do you do?

Soak in water overnight [ ] Wash with water [ ] Sun drying on bare floor [ ]

Other please specify……. [ ]

24. If you dry your tigernuts, how do you determine the end of drying?

Hard texture [ ] Shriveled tubers [ ] Sweet taste [ ]

25. How long does it take to completely sell out a batch?

<2 weeks [ ] 2-3 weeks [ ] <1 month [ ] >1 month

26. Where do you store tigernuts during this period?

At home [ ] Container in the market [ ] Market Store [ ] Other please specify… [ ]

27. In what form do you prefer to store tigernuts?

Basket [ ] Jute Sack [ ] Poly Sacks [ ] Tray / Basin [ ]

28. Is there any form of cleaning of storage rooms/houses before tigernuts are stored in them?

Yes [ ] No [ ]

29. What method preservation do you employ during storage?

Use of chemicals [ ] No preservations [ ] Other please specify……. [ ]

30. Do you grade your tigernuts?

Yes [ ] No [ ]

31. If yes, which parameters do you use in grading tigernuts?

Presence of moulds [ ] Presence of insect infestation [ ] Other please specify [ ]

32. What packaging material do you use in packaging tigernuts for sale?

Polythene bag [ ] Paper bag [ ] Jute sack [ ] Other please specify [ ]

33. In what units do you sell tigernuts.

Per 100g (1/2 margarine tin) [ ] Per 200 (margarine tin) [ ] Per kg (Olonka) [ ]

Other please specify……. [ ]

34. Do you frequently experience deterioration including breakages, foul smell, insect infestation, fungal growth?

Yes [ ] No [ ]

35. If yes which deterioration observation do you encounter most?

Breakages [ ] Fungal growth [ ] Rodent / Insect infestation [ ]

Other please specify…… [ ]

36. How many of your tigernuts get deteriorated per each batch of tigernut supply?

Less than 1/4 [ ] 1/4 – 1/2 [ ] More than 1/2 [ ] Other please specify……. [ ]

37. What is the peak period of deterioration after purchase?

Within 2 weeks [ ] 2 – 4 weeks [ ] >1 month [ ] Other please specify……. [ ]

SECTION IV: MYCOTOXIN AWARENESS

38. Do you observe the presence of mouldy tigernut in your crops?

Yes [ ] No [ ]

39. How are mouldy tiger nuts handled?

Animal feed [ ] Sold at low prices [ ] Discarded [ ] Other please specify……[ ]

40. Do you have any idea on effect of mouldy tigernut on human/animal health?

Yes [ ] No [ ]

41. If Yes, list some of them.

Headache [ ] Stomach upset [ ] Nausea [ ] Other please specify…… [ ]

42. What is your opinion on what causes spoilage/moulds?

Heaping of tigernut during drying [ ]

Inadequate drying between sales and retail stage [ ]

Insect infestation [ ] Use of chemicals on crops [ ]

Other please specify…….[ ]

43. Toxins in tigernuts due to moulds growth can be completely destroyed through cooking.

Agree [ ] Disagree [ ]

44. Toxins in tigernuts due to moulds growth can be completely destroyed through washing.

Agree [ ] Disagree [ ]

45. Do you have any idea on measures to take to control moulds and fungi in storage?

Yes [ ] No [ ]

46. If Yes, state them

Periodic drying [ ] Proper washing with lime and dry [ ]

Proper washing with salty water [ ] Other please specify……. [ ]

47. Do you receive advice on how to prevent moulds from growing on tigernuts?

Yes [ ] No [ ]

48. Who has the primary responsibility to ensure that tigernuts are free from toxins/fungal growth?

Farmer [ ] Trader [ ] Both [ ]

49. Why do you think sorting/removal of mouldy nuts are important?

To prevent rejection by consumers [ ] To retain consumers [ ]

Others please specify……. [ ]

SECTION V: ATTITUDE

50. I have a responsibility to ensure that tigernuts sold are safe and of good quality.

Agree [ ] Disagree [ ] Not Sure [ ]

51. Tigernuts exposed to insects and rodents are prone to contamination by fungal.

Agree [ ] Disagree [ ] Not Sure [ ]

52. Tigernuts kept in cool, dry storage facilities keeps longer than hot humid areas.

Agree [ ] Disagree [ ] Not Sure [ ]

53. Infected tigernuts must be sorted from wholesome ones frequently.

Agree [ ] Disagree [ ] Not Sure [ ]

54. Tigernuts stored in polythene bags deteriorates faster.

Agree [ ] Disagree [ ] Not Sure [ ]

55. Basins/baskets used to display tigernuts should be washed and dried to avoid contamination.

Agree [ ] Disagree [ ] Not Sure [ ]

THANK YOU

APPENDIX 3

QUESTIONNAIRE TO DOCUMENT TIGERNUT KNOWLEDGE, ATTITUDE AND PRACTICES AMONG CONSUMERS

ID OF RESPONDENT……. **DISTRICT OF INTERVIEW…….**

LOCATION TYPE…… **PLACE OF INTERVIEW……..……**

DEPARTMENT OF NUTRITION AND FOOD SCIENCE

P.O. BOX LG 134, LEGON, GHANA

Hello! I am a PhD student pursuing Food Science at University of Ghana. I am currently working on a project aimed at establishing knowledge of the existence and effects of mycotoxin contamination on tigernut and safety measures taken to` minimize occurrence of mycotoxin contamination among traders in Ghana. I would be grateful if you could spare 10 minutes of your precious time to complete this questionnaire.

Please be assured that any information provided will not be linked to personal information and data that will be published will not reveal the identity of participants.

SECTION I: DEMOGRAPHIC INFORMATION AND GENERAL INFORMATION

Please indicate your responses by ticking against your preferred choice(s).

1. What is your age?

   21-40 years [ ] 41-60yrs [ ] More than 60years[ ]

2. What is your sex?

   Male [ ] Female [ ]

3. What is your marital status?

Single [ ] Married [ ] Divorced [ ] Widowed [ ] Other please specify……..[ ]

4. What religion do you belong to?

Christian [ ] Islam [ ] African tradition [ ] Other Specify…… [ ]

5. What region do you come from?

Ashanti [ ] Brong Ahafo [ ] Central [ ] Eastern [ ] Greater Accra [ ]

Northern [ ] Oti [ ] Savannah [ ] Upper East [ ] Upper West [ ] Volta [ ]

Western [ ]

6. What is your level of education?

Elementary / Primary School [ ] Junior High School [ ]

Secondary / Technical School [ ] Vocational School [ ] Tertiary [ ] None [ ]

7. What is your employment status?

Student [ ] Employed [ ] Self-Employed [ ] Unemployed [ ]

8. Do you eat tigernuts?

Yes [ ] No [ ]

9. How long have you been consuming tigernuts?

<1yr [ ] 1-5yrs [ ] 6-10yrs [ ] other please specify ……. [ ]

SECTION II: TIGERNUT SUPPLY

10. Where do you purchase your tigernuts from?

Open market [ ] Street hawkers [ ] Other please specify…[ ]

11. How often do you eat tigernut or tigernut products?

Once a week [ ] More than once a week [ ] Occasionally [ ]

12. How many varieties do you know?

1 [ ] 2 [ ] 3 [ ] Other please specify …… [ ]

13. Which variety do your prefer?

Black [ ] Brown [ ] Both [ ]

14. Why do you prefer this variety?

Sweeter [ ] Appealing appearance [ ] More juicy [ ] Other please specify [ ]

15. What is the approximate quantity you buy per batch?

<200g [ ] 200g-500g [ ] 500g-1000g [ ] >1000g [ ]

16. In which form do you purchase your tigernuts?

Dried [ ] Overly Dried [ ] Other please specify [ ]

17. Check from the list below all the tigernut products you know.

Raw Tigernut Tuber [ ] Tigernut Porridge [ ] Tigernut Oil [ ] Tigernut flour [ ]

Tigernut Alcoholic drink [ ]

18. Are tigernut products easily accessible?

Yes [ ] No [ ]

19. Which product would you want to see more on the market?

Raw Tigernut Tuber [ ] Tigernut Porridge [ ] Tigernut Oil [ ] Tigernut flour [ ]

Tigernut Alcoholic drink [ ] Other please specify…… [ ]

20. Which is your favourite tigernut product?

Raw Tigernut Tuber [ ] Tigernut Porridge [ ] Tigernut Oil [ ] Tigernut flour [ ]

Tigernut Alcoholic drink [ ] Other please specify…… [ ]

21. Why do you eat tigernut?

As a Snack [ ] For Nutritional benefits [ ] For Health benefits

Other please specify……[ ]

22. If you chose medical reasons in the previous question, which of the following is the medical reason?

Antioxidants [ ] Control Diabetes [ ] Eye disorder [ ] Fertility [ ]

Good Digestion [ ] Improve IQ [ ] Sexual Enhancement [ ]

Other please specify…….. [ ]

23. Are you willing to try new tigernut products?

Yes [ ] No [ ]

24. What major quality criteria/parameter do you consider when buying tigernuts?

Colour [ ] Size [ ] Buying from same supplier [ ] Absence of Insect activity [ ]

Texture [ ] Absence of moulds

25. Have you refused to eat or use purchased tigernut?

Yes [ ] No [ ]

26. If yes how often in the past 12 months?

Half the time [ ] More than half the time [ ] Once in a while [ ]

27. If yes, what is the main issue?

Presence of moulds [ ] Presence of insect activities [ ] Other please specify…….[ ]

SECTION III: TIGERNUT SAFETY PRACTICES

28. Do you do anything to the tigernuts before and in between consumption?

Yes [ ] No [ ]

29. If yes, which of the following do you do?

Wash with water [ ] Wash with salty water [ ] Other please specify [ ]

30. Where do you store tigernuts before use?

Kitchen [ ] Fridge [ ] Yard [ ] Other please specify [ ]

31. In what form do you prefer to store tigernuts?

Aluminium basins [ ] Basket [ ] Polythene bag [ ]

32. Do you grade your tigernuts?

Yes [ ] No [ ]

33. If yes, which Parameter(s) is/are used in grading tigernuts?

Presence of moulds [ ] Presence of insect infestation [ ] Other please specify……[ ]

34. Do you frequently experience deterioration including breakages, foul smell, insect infestation, fungal growth?

Yes [ ] No [ ]

35. If yes which deterioration observation do you encounter most?

Fungal growth [ ] Presence of insect infestation [ ] Other please specify…….[ ]

36. How many of your tigernuts get deteriorated per each batch of tigernut supply?

Less than 1/4 [ ] About 1/2 [ ] More than 1/2 [ ] Other please specify……..[ ]

37. What is the peak period of deterioration after purchase?

Less than 1 week [ ] Within 2 weeks [ ] After 2 weeks [ ]

SECTION IV: MYCOTOXIN AWARENESS

38. Would you buy mouldy tigernut at a reduced price?

Yes [ ] No [ ]

39. Do you observe the presence of mouldy tigernuts?

Yes [ ] No [ ]

40. What do you do with mouldy tigernuts?

Bite mouldy part off [ ] Consume them anyway [ ] Discard them [ ]

Process into other product [ ] Use as animal feed [ ] Wash with salty water [ ]

Other please specify [ ]

41. Do you have any idea of the word Mycotoxin/Aflatoxins?

    Yes [ ] No [ ]

42. Do you have any idea on the effect of mouldy tigernut on human/animal health?

    Yes [ ] No [ ]

43. If Yes, please list some of them

    ……

44. Toxins in tigernuts due to molds can be completely destroyed through cooking.

    Agree [ ] Not sure [ ] Disagree [ ]

45. Toxins in tigernuts due to molds growth can be completely destroyed through washing.

    Agree [ ] Not sure [ ] Disagree [ ]

46. What is your opinion on what causes spoilage/moulds in tigernuts?

    Poor drying [ ] Hot temperatures [ ] Use of chemicals on tigernuts[ ]

47. Do you have any idea on measures to take to control moulds and fungi in storage?

    Yes [ ] No [ ]

48. If yes, please state some of them.

    ……

    ……

SECTION V: ATTITUDE

49. Who has the primary responsibility to ensure that tigernuts are free from toxins/fungal growth?

    Farmers only [ ] Traders only [ ] Both Farmer and Traders [ ] Consumers [ ]

50. Why do you think sorting/removal of mouldy nuts are important?

    ……

51. I have a responsibility to ensure that tigernuts consumed or processed are safe and of good quality.

    Agree [ ] Disagree [ ]

52. Tigernuts exposed to insects and rodents are prone to contamination by fungal.

    Agree [ ] Disagree [ ]

53. Tigernuts kept in cool, dry storage facilities keeps longer than hot humid areas.

    Agree [ ] Disagree [ ]

54. Infected tigernuts must be sorted from wholesome ones frequently.

    Agree [ ] Disagree [ ]

55. Tigernuts stored in polythene bags deteriorates faster.

    Agree [ ] Disagree [ ]

56. Basins/baskets used to display tigernuts should be washed and dried to avoid contamination.

    Agree [ ] Disagree [ ]

THANK YOU

APPENDIX 4

LSD POST-HOC ANALYSIS OF TIGERNUT MILK

Table 10-1: Significance of the changes in Total Solids and % Brix with the addition of alpha amylase and roasting to Fresh tigernut milk

Property	Variety	Fresh + 0.2% α-amylase	Fresh + 0.5% α-amylase	Roasted	Roasted + 0.2% α-amylase	Roasted + 0.5% α-amylase
Total Solids	Black	0.149	0.175	0.154	0.488	0.593
	Yellowish-Brown	0.276	0.036	0.005	0.014	0.046
% Brix	Black	0.492	0.012	0.910	0.063	0.038
	Yellowish-Brown	0.303	0.07	0.720	0.039	0.260

Table 10.2: Significance of the changes in pH and Titratable acidity with the addition of alpha amylase and roasting to Fresh tigernut milk

Property	Variety	Fresh + 0.2% α-Amylase	Fresh + 0.5% α-Amylase	Roasted	Roasted + 0.2% α-Amylase	Roasted + 0.5% α-Amylase
pH	Black	0.251	0.000	0.001	0.000	0.000
	Yellowish-Brown	0.294	0.000	0.027	0.000	0.000
Titratable acidity	Black	0.220	0.076	0.833	0.220	0.052
	Yellowish-Brown	0.220	0.035	0.833	0.157	0.024

Table 10.3: Significance of the changes in Emulsion stability with the addition of alpha amylase and roasting to Fresh tigernut milk

Property	Variety	Fresh + 0.2% Alpha Amylase	Fresh + 0.5% Alpha Amylase	Roasted	Roasted + 0.2% Alpha Amylase	Roasted + 0.5% Alpha Amylase
Emulsion stability	Black	0.00	0.000	0.00	1.000	0.000
	Yellowish-Brown	0.00	0.00	0.001	1.00	0.000

Table 10.4: Significance of the changes in Foam capacity and Foam stability with the addition of alpha amylase and roasting to Fresh tigernut milk

Property	Variety	Fresh + 0.2% Alpha Amylase	Fresh + 0.5% Alpha Amylase	Roasted	Roasted + 0.2% Alpha Amylase	Roasted + 0.5% Alpha Amylase
Foam Capacity	Black	0.011	0.00	0.000	0.000	0.00
	Yellowish-Brown	0.034	0.000	0.000	0.000	0.000
Foam stability	Black	0.016	0.000	0.000	0.000	1.000
	Yellowish-Brown	0.085	0.001	0.000	0.000	0.000

Table 10.5: Significance of the changes in colour constituent L*, a* and b* 'a' with the addition of alpha amylase and roasting to Fresh tigernut milk

Colour constituent	Variety	Fresh + 0.2% Alpha Amylase	Fresh + 0.5% Alpha Amylase	Roasted	Roasted + 0.2% Alpha Amylase	Roasted + 0.5% Alpha Amylase
L*	Black	0.1340	0.070	0.357	0.002	0.001
	Yellowish-Brown	0.047	0.003	0.385	0.024	0.002
a*	Black	0.681	0.196	0.027	0.230	0.00
	Yellowish-Brown	0.018	0.00	0.001	0.00	0.000
b*	Black	0.068	0.008	0.108	0.007	0.00
	Yellowish-Brown	0.448	0.146	0.00	0.00	0.00