KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

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DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

ENZYMATIC HYDROLYSIS OF STARCH IN TIGERNUT (*CYPERUS ESCULENTUS L.*) MILK USING TWO ENZYMES AND ITS SENSORIAL EFFECTS

BY

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A thesis submitted to the Department of Food Science and Technology, Kwame Nkrumah University of Science and Technology in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

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CERTIFICATION

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

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DEDICATION

To my siblings Elsa, Aubert, Auguste and Karol thanks for bringing laughter and joy to my life. Thank you all for your emotional, physical, spiritual support and companionship. The skyline we ought to target is far away higher. I therefore pray that we shall stretch out our hands for God to hold and guide us through His chosen paths for us, and may His light forever shine upon us, and may He be gracious to us always, Amen. To my loving parents Marguerite-Marie and Georges, without whom I would not have made it this far; thank you both for everything in my life.

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ABSTRACT

The aqueous beverage of *Cyperus esculentus* L. also known as tigernut milk has been considered as substitute to cow milk. But its starch content imparts an undesirable organoleptic attribute: chalkiness. In order to improve tigernut milk sensorial quality, the enzymes α-amylase and glucoamylase was used at 5 different combinations (1% α-amylase, 0.8% α-amylase+0.2% glucoamylase, 0.5% α-amylase+0.5% glucoamylase, 0.2% α-amylase+0.8% glucoamylase, 1% glucoamylase) for starch hydrolysis at 50°C for 4 hours. Physico-chemical properties (starch and glucose contents, pH and °Brix) were analyzed hourly. Two hydrolyzed samples with the most effective enzyme combinations were subjected to sensory analyses with the raw sample as control. The initial starch content was 33.08 ± 3.82% and decreased hourly to 19.36 ± 0.39% after four hours. However, glucose content increased from 3.66 ± 0.16% to 6.00 ± 0.5%. The pH of the fresh sample was 5.48 ± 0.02. It increased throughout hydrolysis up to 6.13 ± 0.01. The °Brix was initially between 3.17 and 4.27 and this increased to 6.13 ± 0.06. It was shown that 1% α-amylase and 0.8% α-amylase+0.2% glucoamylase have the closest and highest physicochemical results but reduction in starch content was higher in 1% α-amylase than 0.8% αamylase+0.2% glucoamylase. The optimum time for hydrolysis was found to be 3 hours. Sensory analyses showed that hydrolysis resulted in the browning of the samples, but did not affect the chalky mouthfeel to the extent that it could not be effectively noticed by respondents. Raw tigernut milk sample had the highest consumer preference, followed by 0.8% αamylase+0.2% glucoamylase hydrolyzed tigernut milk. More work should be done to meet the need of the industry in terms of chalkiness reduction by targeting enzymatic hydrolysis of cellulose and pectinase.

Key words: tigernut milk, starch, enzymatic hydrolysis, sensory attributes

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

LIST OF ABBREVIATIONS

- ANOVA----------Analysis of Variances
- B.C. ----------Before Christ
- cm----------centimetre
- conc. ----------concentrated
- FAO----------Food and Agriculture Organization
- g----------gramme
- h----------hour
- H_2SO_4----------sulphuric acid
- I_2----------iodine
- kg----------killogramme
- KI----------potassium iodide
- kJ----------kilojoule
- KNUST----------Kwane Nkrumah University of Science and Technology
- l----------litre
- m----------metre
- mg----------milligramme
- ml----------millilitre
- min----------minute(s)
- mm----------millimetre
- n.d. ----------non dated
- nm----------nanometre(s)
- NPK----------Nitro-Phospho-Potassium
- pH----------potential of hydrogen
- t/ha----------tonnes per hectare
- tr/s----------tours per second

- UHT----------Ultra High Temperature
- USD----------United States of America Dollars
- w/w----------weight per weight
- °Brix----------degree Brix - °C----------
  ----------degree Celsius
- %----------percentage
- / ----------per
- ± ----------more or less
- ~ ----------approximately
- + ----------plus
- α----------alpha
- β----------beta
- µm----------micrometre
- × ----------multiply by

CONTRIBUTION TO SCIENCE

This study:

- Provides first-hand information on the starch content of some tigernuts (*Cyperus esculentus* L) produced in Ghana.
- Provides baseline data on enzymatic hydrolysis of tigernut milk starch.

PAPER PRESENTATIONS FROM STUDY

- Tapsoba A. R. B., Oduro I. and Wireko-Manu F. D. B. (2014). Effect of two different enzymes on the starch and glucose content of tigernut milk during hydrolysis. A poster presented at a one-day Research Conference of the Graduate Students Association of Ghana (GRASAG).
- Tapsoba A. R. B., Oduro I. and Wireko-Manu F. D. B. (2014). Enzymatic hydrolysis of starch in tigernut milk using α-amylase and amyloglucosidase to increase upon sweetness. A poster presented at the Annual Ghana Biomedical Convention (GBC).

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Dairy milk is considered to be very nutritious, containing most essential macro and micro nutrients essential in human nutrition. The Food and Agriculture Organization has stated that milk plays an important role in human health, welfare and development (FAO, 2013). Milk is one of the major protein sources, among others such as meat, eggs and fish, plus milk products (Steinke *et al.*, 1991). These protein sources are not affordable for most households in the developing world. Furthermore, due to some physiological, health and religious reasons some people do not consume cow‘s milk. Consequently, it is indispensable to bring in cheaper plantbased protein alternatives.

A number of plant-based milk products, including soy milk, almond milk and coconut milk are being consumed and recommended by vegetarian organizations (Hackett, n.d.; Kay, 1987). Tigernut milk has been of research interest in recent studies in Ghana. The milk is derived from the tubers of *Cyperus esculentus* L. which is believed to possess some health benefits including the prevention of colon cancer, coronary heart diseases, obesity, diabetes and gastro-intestinal diseases (Anderson *et al*., 2009). These health beneficial properties of tigernut are attributed to the high dietary fibre content of about 9.8 % (w/w) (Pascual *et al.*, 2000) . The nuts are also known to have high protein (3.78 – 9.70%), lipid (22.0 – 35.43%) and carbohydrate (47.9 –

75.88%). Starch accounts for 17.2-39.2% of the total carbohydrate content of the nuts (Umerie *et al.*, 1997; Karababa *et al.*, 2001; Turesson *et al*., 2010).

Tigernut milk, obtained by aqueous extraction has a milky-like appearance and has a very short shelf-life when produced traditionally (Selma *et al.*, 2003). Tigernut milk is very nutritious. Indeed it has been shown to contain 0.1% dietary fibre, 2.5-2.6% fat, 48% carbohydrates with starch minimum content of 2.2-2.4%, 4.5 - 12.0% total soluble solids (°Brix) and total ash content of about 0.24% (Pascual *et al*. 2000; Cortés *et al*. 2005; Corrales *et al*. 2012). Some studies have been done on the use of tigernut milk in the chocolate industry in Ghana as an alternative to dairy milk in some cocoa-based products. However, the starch imparts some undesirable sensory attributes to the products. Hydrolysis of the starch in tigernut milk is necessary to overcome the sensory challenges related to starch content in tigernut milk. There are different methods of starch hydrolysis but enzymatic hydrolysis has been reported to be safest and best for the consumer and the product (Taherzadeh and Karimi, 2007).

1.2 Problem statement

A major setback to the use of tigernut milk in the local beverage industry is its starch content which is believed to impart undesirable sensory attributes to the final product and also limit heat treatment of the product. There is dearth of information on the starch content of the tigernut varieties produced in Ghana and their effect on the sensory attributes of the milk such as jellification upon heating above 70 °C (Corrales *et al*., 2012) and chalky mouthfeel in their use in the beverage industry. Unlike other plant-based milk sources such as coconut milk and soymilk with relatively lower starch content (Anonymous, 2004), some varieties of tigernut have starch content ranging between 2.2 – 2.4% in their milk necessitating the need for the hydrolysis of the starch.

1.3 Justification

Investigating the possible ways of improving tigernut milk would greatly enhance its potential use in the beverage industry and other food applications that require heating. Chalkiness and starch jellification could be reduced with reduction in the starch content of tigernut milk and could improve sensory quality.

Enzymatic hydrolysis is a safe method of starch hydrolysis which is yet to be fully exploited in tigernut milk. Exploration of enzymatic hydrolysis of starch in tigernut milk could open doors for more work to be done in the field for the improvement of the tigernut milk industry. Other benefits of starch hydrolysis such as increased glucose content may provide sweeter tigernut milk varieties for the local market. These naturally sweeter tigernut milks could be marketed to health conscious consumers who prefer little to no added sugars or sweeteners in their beverages. This could also positively impart its use in foods that require some level of sweetness.

Improvement in the tigernut milk quality and diverse tigernut milk products which this study is expected to drive in the long term, would increase demand for tigernuts in the food industry and help to improve livelihood of rural and small scale farmers in tigernut production through increased production and income.

1.4 Objectives

The objective of this research is to investigate enzymatic starch hydrolysis of tigernut milk using two different enzymes.

Specifically the study seeks:

1. To evaluate starch and glucose contents of tigernut milk at different enzyme concentration, combination and hydrolysis time
2. To assess the effect of enzymatic starch hydrolysis on the chalkiness, sweetness, flavour and colour of the tigernut milk and consumer acceptability of the hydrolyzed products

CHAPTER TWO

2.0 LITTERATURE REVIEW

2.1 History of tigernut

Tigernut, *Cyperus esculentus* L. is also known as chufa, yellow nutsedge, earth almond or tiger nut. Sometimes it is also called rush-nut. In French, it is called ―pois sucré‖ or ―souchet comestible‖. In West Africa, it is known as ―atadwe‖ in Twi (Ghana) and also as ―tchogɔn‖ in Diula/Bambara (Mali, Côte d'Ivoire and Burkina Faso). It grows wild as a weed, but is also grown as a crop (Pascual *et al*., 2000). Since ancient times tigernut tuber has been regarded a foodstuff; it was cultivated along the Nile in ancient Egypt, millennia BC. Tigernut is considered to have its origins in the Mediterranean area and western Asia but has spread (mainly as a weed) to many parts of the world during the Arabian expansion in the Middle Ages (Pascual *et al*., 2000).

Presently, tigernut is abundantly cultivated in Spain, especially in Valencia. It is also grown in other countries throughout the world in Asia, Europe, and America. In Africa, countries that are into tigernut breeding include Burkina Faso, Cameroon, Côte d'Ivoire, Mali, Niger, Nigeria, Togo and Ghana among others. In many parts of Ghana, the crop is widely cultivated. It is nationally well known to be grown in Kwahu, Techiman, Bodweasae (Yeboah, 2014) as well as in Twifo Praso, in the Central Region of Ghana.

2.2 Agro-economic facts of tigernut

A grass-like perennial sedge, with 10 to 90 cm long narrow dark-green leaves (Defelice, 2002), tigernut like any other plant requires some specifications of cultivation. It also needs some good

harvesting method and a proper postharvest handling to minimize loses and to keep a certain level of quality.

2.2.1 Cultivation of tigernut

Tigernut grows very well in moderately high temperatures with a well-distributed and moderate rainfall. It gives a maximum yield in light sandy loams of pH range between 5.5 and 6.5 although it has been recorded to grow well in a wide range of soil types particularly sand, sandy loam, sandy gravel loam, muck, clay-loam, clay and compost (Dakogre, 2008).

The nuts are soaked in water for 1 to 4 days to facilitate germination before planting. It can be planted after soaking or kept (for 7-12 days) until sprouting starts before planting. Pre-sprouting before planting ensures uniform emergence and a perfect stand. Tigernut is planted in the major season between March and April, and in the minor season from August to September (Tetteh and Ofori, 1998).

There is little information on fertilizer requirements for tigernut production but it is known that nitrogen in natural conditions is often limiting. In Ghana, NPK 15:15 is mostly used by breeders for tigernut cultivation though some farmers desist from application due to taste issues (Tetteh and Ofori, 1998). Weedicide and insecticide commonly used for tigernut cultivation are Gamazone and lamda-super respectively. According to some breeders in the Central Region of Ghana, the use of 2 litres of lamda-super/ acre for the breeding time is sufficient.

2.2.2 Types and yield of tigernut

There are different types of tigernut depending on the shape and size, and also on the colour. Oladele and Aina (2007) mentioned three different types of tigernut based on the colour of the rhizomes: yellow, brown and black. When it comes to the shape and size (mainly the size), there

are also three types of tigernut categorized: the mini (6-8 mm), the standard (8-12 mm) and the large (>12 mm) (Dakogre, 2008). In the category of large tigernut, Pascual *et al*. (2000) mentioned three sub-categories (Figure 2.1) which are:

- 'Gegant Africana' (GA) which is characterized by its large size (length 17.3 mm). The unit weight of it tubers is 1.09 g.
- 'Llargueta Alboraia' (LA) produces oval tubers (length 15.2 mm). It yields more than the two other cultivars 18.525 t/ha.
- 'Ametlla Bonrepos' (AB) produces spherical tubers (length 12.4 mm). It has a good tuber yield of 15.977 t/ha.

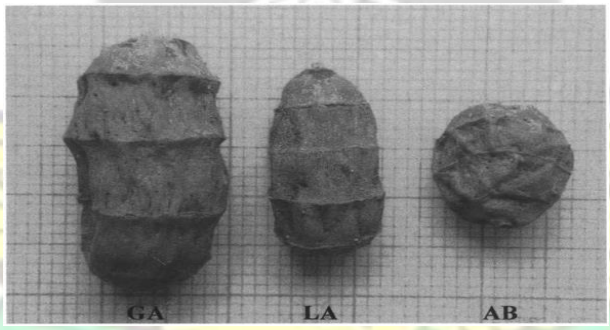


Plate 2.1: Tubers of 'Gegant Africana' (GA), 'Llargueta Alboraia' (LA) and 'Ametlla Bonrepos' (AB) cultivars (Pascual et al., 2000).

In terms of general yield, irrespective of the cultivar variety, tigernut has a yield ranging between 0.8 to 14 t/ha. This wide variation of yield depends on the types of soil, on the scale of cultivation (whether small or large-industrial) (Kay, 1987). In Ghana, a survey conducted by Tetteh and Ofori (1998) in the Kwahu South District, revealed a yield range of 2.3-11.3 t/ha.

2.2.3 Harvesting, handling and drying of tigernut

Tigernut usually requires 3 to 4 months to mature. Maturity signs of the crop show on the leaves. Basically the leaves stop growing, start yellowing and dry up. Harvesting is the most difficult aspect of tigernut production according to Dakogre (2008). Harvesting of tigernut is usually done manually by pulling from the ground; and this causes physical damage to the tubers, thus reducing their quality. However, mechanical harvesting is done in developed countries such as Canada and Florida, USA (Reid *et al*. 1972). In Florida, groundnut harvesters are sometimes used (Kay, 1987).

When it comes to handling, tubers are separated from the dried plant and spread thinly on the floor of sheds. Tubers for human consumption are washed under running water and then dried either in the sun or artificially, after which they are graded and stored (Kay, 1987). Tetteh and Ofori (1998) reported from their survey that some farmers claimed that the washed tubers rot easily, even though there was no evidence to prove it according to the authors.

Drying of tigernut is done to extend its shelf-life by preventing spoilage due to bacterial contamination. Sun drying is hard to achieve during the rainy season, hence the need for an artificial one. Moreover, sun drying method has been reported to be slow and also exposes the product to higher risk of microbiological contamination (Musa *et al.,* 2005). Oven dryers are recommended for faster and more hygienic results (Karanthos and Belessiotis, 1995). Drying affects the products sensory attributes such as colour, texture and flavour most of the time, due to the high temperatures applied (Musa *et al*., 2005). The use of mild drying temperatures can help minimize these changes by reducing the extent of Maillard browning. Tunde-Akintunde and Oke (2012) used temperature set at 55°C when using ovens for tigernut drying. According to Practical Action (2014) the acceptable moisture content for tigernuts is 18%.

2.2.4 Economical value of tigernut: Marketing

Marketing of tigernut is generally poor in Africa. Usually, farmers sell their nuts to middlemen who in turn sell to retailers in the urban centres. Contrary to the general perception that tigernut production and industry at large is not a lucrative business, studies indicate tigernut is two times more expensive than rice in some parts of Ghana (Opare, 2005), making it a crop of economic importance especially in regions where it is abundant. The country earned about twenty-five million US dollars from tigernut exportation in 2003 (Table 2.1), signifying the potential of the crop on the international market as well (Dakogre, 2008).

In Burkina Faso, a company named HOCHATA EXPORT® exports average of 12 tonnes of tigernut to Spain annually and also commercializes locally the non-alcoholic beverage (Personal communication, 2014).

Plate 2.2: Grading machine operating from HOCHATA EXPORT®

Table 2.1: Tigernut exportation from Ghana (1988 to 2003)

Year	Quantity (tonnes)	Value (USD)
1988	0.432	100.030
1989	6.640	1,593.600
1990	-	-
1991	15.872	3,749.080

1992	1.195	309.780
1993	0.200	44.340
1994	0.930	266.980
1995	1.250	385.570
1996	2.550	562.600
1997	8.616	6,118.770
1998	6.292	8,687.780
1999	121.923	149,636.760
2000	66.627	52,763.900
2001	103.421	18,008.000
2002	35.802	12,326.780
2003	63.462	25,130.820

Source: Ghana Export Promotion Council (Dakogre, 2008)

2.3 Nutritional composition, health benefits and food technology applications of tigernut

2.3.1 Proximate composition

Tigernut is characterized by a good nutritional profile. It has been quoted by several authors (Table 2.2) to protein content ranging from 3.98-9.70%, fat content of 24.00-35.43%; while carbohydrate content ranges between 22.08 and 75.88%. Oladele and Aina (2007) and Salau *et al*. (2012) evaluated the energy value of tigernuts to be 1511 kJ/100g and 1754.98 kJ/100g respectively. Tigernut tubers are also rich in fiber, ranging from 6.26 to 13.00 % (Oladele and Aina, 2007). Asante *et al.* (2014) reported that tigernuts from the Twifo-Praso in the Central region of Ghana contained 17.66% fat, 5.66% protein, 1.23% ash, 64.16% carbohydrate, 11.62% fiber and an energy value of 1867.07 kJ/100g.

Table 2.2: Proximate composition and energy value of tigernut

Parameters	Umerie, *et al.* (1997)	Oladele & Aina (2007)	Turesson *et al.* (2010)	Salau *et al.* (2012)	Asante *et al.* (2014a)
Crude Protein (%)	3,98	9,7	-	9,15	5,66
Fat (%)	-	35,43	24	33,33	17,66
Carbohydrate (%)	75,88	46,99	-	22,08	64,16
Starch (%)	-	-	32	-	-
Crude fiber (%)	12,88	6,26	-	11,11	11,62
Moisture (%)	-	3,78	-	8,66	-
Ash (%)	-	-	-	22,33	1,23
Energy value (kJ/100g)	-	1511	-	1754,98	1867,07

2.3.2 Mineral composition

Tigernut contains some good proportions of essential minerals like zinc, magnesium, potassium (Glew *et al*., 2006; Oladele and Aina, 2007; Asante *et al*., 2014) among others as shown in Table 2.3 below.

Table 2.3: Mineral composition of tigernut

Minerals (mg/100g)	Glew *et al*. (2006)	Oladele & Aina (2007)	Asante *et al*. (2014)
Calcium	18.80	140.0	1.68
Sodium	8.21	235.00	48.45
Potassium	557.30	255.00	805.20
Magnesium	76.30	56.30	64.00
Manganese	1.19	38.41	0.10
Phosphorus	193.70	121.00	39.83
Iron	5.29	0.80	0.39
Zinc	1.12	0.01	3.84
Copper	0.24	0.01	0.35

2.3.3 Amino-acid profile of tigernut roots

Tigernut has been demonstrated to contain high amounts of essential amino acids. The essential amino acids in the tuber meets the recommended levels as set by WHO/FAO standard. Prominent among these amino acids are leucine, phenylalanine+tyrosine and lysine (Glew et al., 2006).

2.3.4 Health benefits

Tigernut has been reported to be high in dietary fiber content (Toràn and Rovira, 2003), which could be effective in the treatment and prevention of many diseases including colon cancer (Adejuyitan *et al.*, 2009), coronary heart disease (Chukwuma *et al.*, 2010), obesity, diabetes, gastrointestinal disorders (Anderson *et al*., 2009). Tigernut ―milk‖ has been reported to be used in the treatment of flatulence, indigestion, diarrhea, and dysentery, and its starch content presumably provides prebiotic properties for colon bacteria (Toràn and Rovira, 2003). Tigernut milk has been found to be good for preventing arteriosclerosis, since its consumption can help prevent heart problems and thrombosis and activate blood circulation (Chukwuma *et al*., 2010). Tigernut milk can be drunk by diabetics for its content in low-glycemic carbohydrates (mainly starch) and due to its arginine which liberates hormones that produce insulin (Toràn and Rovira, 2003).

2.3.5 Utilization of tigernut and its by-products

Tigernut is a crop full of potential. It could be useful as a good source of dietary fiber in the food industry because of its large amount of dietary fiber and the pleasant, nutty flavor (SánchezZapata *et al*., 2009).

At the department of Food Science and technology in Kwame Nkrumah University of Science and Technology, it has been used for the production of digestive biscuits and also incorporated into yam flour to make porridge.

Oil extracted from tigernut tubers have been found to compare well with olive oil (Eteshola and Oraedu, 1996; Umerie *et al.*, 1997; Turesson *et al.*, 2010; Muhammad *et al*., 2011; Salau *et al*., 2012). Tigernut oil could be used as a potential supplement to or substitute for olive oil given its fatty acid composition and other physico-chemical properties. It could also be useful as diesel fuel due to its low viscosity value (Ofoefule *et al.*, 2013).

Abo-El-Fetoh *et al.* (2010) used tigernut starch in combination with sweet potato and corn starches to thicken pudding. Tigernut starch also found uses in the pharmaceutical industry, as an excipient (Manek *et al*., 2012; Builders *et al*, 2013; Kenneth *et al*., 2014).

Kay (1987) reported that tigernut can be used for animal feed and are also used in certain types of confectionery, often as a substitute for almonds. The ground tubers are sometimes used as a substitute or adulterant of coffee and cocoa.

By-products of tigernut processing such as the fibre and liquid are used in the food industry to serve technological and functional purposes (Salau *et al*., 2012; Sánchez-zapata *et al.*, 2013; Sánchez-Zapata *et al*., 2013b). Sanchez-Zapata *et al*., (2010) found that burgers with tigernut fibre incorporated were perceived as less greasy, less juicy and grainier; with less meaty flavor than the controls. Such burgers were also found to be more nutritious with better cooking characteristics. Tigernut liquid, collected after production of tigernut milk have shown good properties for the cultivation of probiotics (Sánchez-Zapata *et al*., 2013).

2.4 Plant-based milk

Plant-based milk is a milk-like products derived from a plant source. It represents a possible way of meeting the demands for proteins, vitamins, minerals, carbohydrates and health beneficial ingredients. The commercial label ―milk‖ is given to such beverages by manufacturers though it is not legally accepted. These beverages, obtained from oil seeds and legumes (Aidoo *et al.*, 2010) are either emulsions or suspensions.

Different production methods have been used in the extraction of plant milk. Production generally starts with sorting and removal of foreign materials that can impart negatively on the overall quality of the beverage. Soaking, cooking and addition of chemicals are some off the processes in tigernut milk production (Chan and Beuchat, 1992; Tunde-Akintunde and Souley, 2009). It has been reported that water uptake during soaking directly impacts positively on the texture and the grinding properties of the plant material as well as the milk solid (Djomdi *et al.*, 2007; Djomdi *et al*., 2013). Heating is of great impact on microbial load reducing. Heating or pasteurization in the extraction process reduces microbial load in the beverage. Heat also affects the flavour (more flavour) and colour (darker) of the final product as well as the milk solids (Asante *et al.*, 2014).

Production of plant-based milk can be done in a number of ways depending on the type of the plant material used and the test objective. It can be done by blending the wet (fresh or soaked) plant material with water; or by milling the dried plant material to get a powder which can be dissolved in water and sieved. This process was reported by Aidoo *et al*. (2010) and is being used by several women associations in Burkina Faso as a way of promoting tigernut milk production.

As compared to cow or any other animal milk, plant-based milks are cholesterol-free and lactose-free products. According to Yadav *et al.*, 2003 soymilk is one plant-based milk that has a protein content (3.12%) similar to cow milk protein content (3.18%). Cow milk is also relatively higher in

calcium, riboflavin and vitamin B12 than plant-based milk. These facts have led to the fortification of plant-based milk by manufacturers and the development of composite plant-based milk. One popular composite plant-based milk is cowpea-peanut milk (Aidoo *et al*., 2010) in which cowpea with its low energy content is added to peanut to balance the energy deficiency.

Generally, plant-based beverages have the characteristic flavour and colour of the particular plant material from which it is extracted. Plant-based milks extracted from unroasted peanuts or soybeans are characterized by their strong flavour, suspension instability and chalky mouthfeel (Kuntz *et al.*, 1978). Quasem *et al.* (2009) reported that the beany flavour of sesame seed milk can be reduced by heat treatment.

2.5 Tigernut milk

Tigernut milk is the aqueous milky-like non-alcoholic beverage extracted from tigernut. The production process involves soaking and crunching/blending of the tigernut. In Spain it is known to be a low acid beverage with pH generally in the range of 6.3 to 7 and a minimum °Brix of 4 when no sugar is added. It has been stated by Cantalejo, 1996 that 1 kg of tigernut gives approximately 5.5-6 litres of beverage. Sugar is added at a rate of 150g/litre of beverage (Kay, 1987; Cantalejo, 1996; Cortés *et al*., 2005a; Corrales *et al*., 2012).

2.5.1 Types of tigernut milk and basic characteristics

There are six types of tigernut milk in Spain (Cantalejo (1996) and Cortes *et al.* (2004). They are the untreated/fresh, pasteurized, sterilized, Ultra High Temperature (UHT), condensed pasteurized and the powdered.

The untreated/fresh tigernut milk can be refrigerated or frozen, prepared exclusively with tigernut, water and sugar, with no other additives or technological treatment. Pasteurized tigernut milk is subjected to a technological process that modifies or suppresses the starch prior to the heat treatment that destroys the pathogenic germs and common bacteria. The sterilized tigernut milk is similar to the pasteurized one, but it suppresses both vegetative and the resistant forms of the germs. The condensed-pasteurized tigernut milk is characterized by a °Brix of 60 minimum.

2.5.2 Tigernut milk composition

Tigernut milk contains about 22 g/100ml of total solids generally and about 4.6g/100ml of carbohydrates. Table 2.4 gives a summary of the proximate composition of tigernut milk.

The beverage has also been found to contain 26.4 mg/100ml sodium, 10.5 mg/100ml calcium, 13.0 mg/100ml magnesium, 32.3 mg/100ml phosphorus, 0.16 mg/100ml iron, 0.57 mg/100ml zinc, 0.13 mg/100ml niacin and 0.02 mg/100ml thiamin/vitamin B1 (Toràn and Rovira, 2003).

Table 2.4: Proximate composition of tigernut milk

Parameters (g/100ml)	**Kay (1987)**	**Toràn and Rovira (2003)**	**Cortes *et al*. (2004)**
Total Solids	22.8	-	13.61-22.06
Fat	2.6	3.09	-
Reducing Sugars	0.03	-	-
Ash	0.24	0.25	-
Protein	-	0.96	0.29
Total Dietary Fibre	-	1.2-4.8	1.03
Carbohydrate	-	4.6	-

Tigernut milk is a suitable drink for celiac patients, who are not able to tolerate gluten and also for people who are lactose-intolerant who stay away from cow milk and many dairy foods

(Sánchez-Zapata *et al.*, 2012). It could also be recommended for those who have problems with digestion, flatulence, and diarrhea because it provides some digestive enzymes like catalase, lipase, and amylase (Adejuyitan, 2011).

2.5.3 Starch content of tigernut milk

The starch content of the untreated tigernut milk is mentioned by diverse authors to range from 2.2 to 4.31 g/100 ml (Kay, 1987; Cortes *et al*., 2004). Cortes *et al*. (2004) established a wide range of starch content depending on the type of tigernut milk. In their study, the minimum and maximum starch contents are 0.15 and 7.45 g/100ml found in UHT and condensed-pasteurized (without sugar) tigernut milk respectively.

2.6 Some characteristics of tigernut starch

Tigernut tubers are potentially a rich source of starch which may be obtained after the extraction of the oil (Kay, 1987).

The proximate composition of tigernut starch is as follows; moisture 9 %, nitrogenous material 0.3 %, fat traces, starch 89.8 %, cellulose 0.3 % and ash 0.5 %. It is a white flavourless product and when heated in water forms a transparent gelatinous paste (Kay, 1987). Tigernut tubers contain almost twice the quantity of starch as potato or sweet potato tubers (Coşkuner *et al.*, 2002). Starch granule size has been shown to be similar to potato starch in appearance but smaller in size. Umerie *et al*. (1997) noticed three different types of tigernut starch granule sizes which are 3-5 µm, 6-8 µm and 9-12 µm.

2.7 Starch hydrolysis

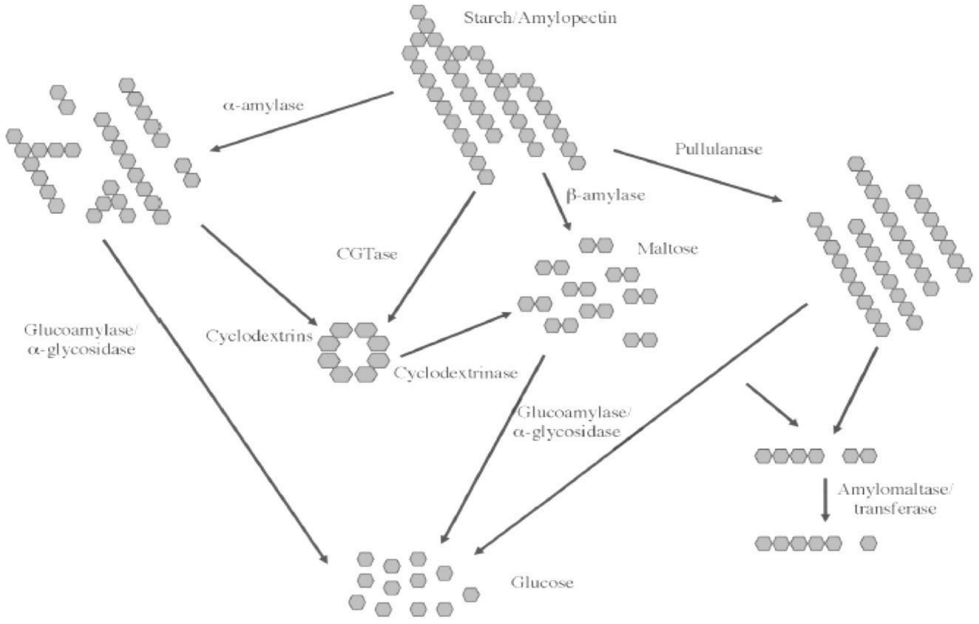
Starch, a carbohydrate polymer of glucose is found in plants and plant materials. The utilization of starch and its derivatives in industrial processes, particularly food processing, has led to the development of numerous methods of starch processing and breakdown.

2.7.1 Chemical hydrolysis of starch

The discovery that starch could be transformed into a sweet substance by heating with dilute acid (chloric acid) was made in 1811 by the Russian chemist Kirchoff (BeMiller and Whistler, 2009). It requires acidic medium (pH: 1-2), high temperatures (150-230°C) and high pressure (Yankov *et al.*, 1986). In recent years, enzymatic methods have largely replaced the use of chemicals.

2.7.2 Enzymatic starch hydrolysis

The use of enzymes in starch hydrolysis is healthier and safer for the environment and the consumer than the use of chemicals. Enzymes also perform more specific hydrolysis reactions (Eliasson, 2004). Compared with acid hydrolysis, the enzymatic hydrolysis requires mild pH (68), lower temperatures (up to 100°C) and normal pressure. More often α-amylase (from different sources) are used rather than β-amylase (Kolusheva and Marinova, 2007).



CGTase= Cyclodextrin GlycosylTransferase

Plate 2.3: Enzymatic hydrolysis of starch and its main derivatives (Kolusheva and Marinova, 2007)

2.7.2.1 Alpha-amylases

Alpha-amylase is usually obtained from *Aspergillus orizae*, *Bacillus amyloliquefaciens* and *Bacillus licheniformis.* They are endo-α-1,4-glucanases (Nigam and Singh, 1995) and hydrolyze starch into oligomers (Veen *et al.*, 2006). Nigam and Singh (1995) mentioned that the end products from α-amylase action are mainly glucose, maltose and maltodextrins.

During enzymatic hydrolysis, dextrins are obtained and if the enzyme acts continuously, maltose accumulates. These maltose molecules have free glycoside group and hence reducing properties (Kolusheva and Marinova, 2007). The inhibitors of α-amylase are primary proteins and hydrolysis products (Nigam and Singh, 1995).

The main food applications of α-amylase as stated by Aiyer (2005) are: liquefaction of starch, maltose manufacturing, manufacturing of high fructose syrups, manufacturing of

oligosaccharides mixture, maltotetraose/G4 syrup manufacture, manufacturing of dextrins, direct fermentation of starch to ethanol.

2.7.2.2 Glucoamylases

Glucoamylase, also called amyloglucosidase is obtained from *Aspergillus niger* and *Rhizopus* species. It is an exo-α-1, 4-glucanase. It hydrolyses starch α-1, 4 bonds efficiently into glucose (Nigam and Singh, 1995; Veen *et al*., 2006).

It breaks down starch by addition of water between glucose units, liberating single glucose sugars. Glucoamylase is industrially significant in the conversion of biomass to refined sugars and sweeteners. It is used in glucose syrup production from liquefied starch (under the action of α-amylase) (Nigam and Singh, 1995).

2.7.2.3 Factors affecting enzymatic hydrolysis of starch

Temperature, pH of the medium, substrate concentration and enzyme concentration play significant roles on the efficiency of enzyme action on starch degradation. Other factors which affect the enzyme activity during starch hydrolysis include the stirring speed, size and shape of starch granule, amylose content, lipid content as well as phosphate content (Yankov *et al*. 1986; Tester *et al.*, 2006).

Depending on the source of the enzyme and/or manufacturer, optimum temperature for enzymatic hydrolysis of starch ranges from 50-55°C for thermolabile enzymes and 9-100°C for thermo-resistant enzymes. The optimum pH for α-amylase activity ranges from 6 to 8 while substrate concentration of between 20 to 35% is ideal for optimum hydrolysis. According to Yankov *et al*. (1986), the recommended enzyme concentration ranges from 0.03% to 1%.

According to Tester *et al.*, (2006), the larger the starch granules, the smaller the surface to be attached to and hydrolyzed by enzymes. The shape of the starch molecule which varies from spherical to polyhedral also affects the surface area. The lower the amylose content of the starch, the greater the amount of native starch that can be hydrolyzed by amylases. The presence of lipid, especially lipid-amylose complexes confers some resistance to hydrolysis while phosphorylation (phosphate monoesters, phospholipids and inorganic phosphates) restricts hydrolysis of starch granules. Absar *et al*. (2009) concluded that high-phosphorus starches are more resistant to enzymatic hydrolysis than the medium-phosphorus starch.

2.8 Sensory evaluation of foods

Sensory evaluation is ―a scientific discipline used to evoke, measure, analyse and interpret reactions to those characteristics of foods and materials as they are perceived by senses of sight, smell, taste, touch and hearing‖ according to the Sensory Evaluation Division of the Institute of Food Technologists (Stone and Sidel, 2004). The sensory test used for an analysis depends on the objectives of the analysis. There are two types of sensory tests being objective and subjective tests.

2.8.1 Descriptive tests

Descriptive test, a type objective testing method, aims at identifying the nature of sensory difference and/or the magnitude of the difference.

There are different descriptive tests, namely Flavour Profile Method, Texture Profile Method, Quantitative Flavour Profiling, Spectrum Method, Generic Descriptive Analysis, Free-Choice Profiling and the Quantitative Descriptive Analysis (Murray *et al.*, 2001).

The Quantitative Descriptive Analysis (QDA) is a method which uses 10-12 panellists and requires the presence of at least 10 of the panellists during the vocabulary generation with training period of 6 up to 10 hours (Lawless and Heymann, 1998; Stone and Sidel, 2004; ChambersIV and Wolf, 2005). Murray *et al*. (2001) stipulates that QDA is the only test that allows the use of reference standards when problems with a particular term arise. Generally the line scale is used for QDA test, on which the assessors indicate perceived intensity by a mark.

2.8.2 Hedonic rating scale

Of all scales and tests methods, the nine-point hedonic scale occupies a unique niche in terms of its general applicability to the measurement of product acceptance/preference (Stone and Sidel, 2004). The method relies on the naive or untrained respondent's capacity to report, directly and reliably, their feelings of like or dislike within the context of the test. An important aspect of the method is that it is used with untrained people, although a minimum level of verbal ability is required for adequate performance. No attempt is made to direct the actual response (ChambersIV and Wolf, 2005).

2.9 Control of chalkiness in plant-based milks

Chalky mouthfeel or chalkiness is a defect used to describe a food which coats the mouth and throat with fine, grainy particles (Kuntz *et al*., 1978). This sensorial defect is detected mostly in plant-based milks such as tigernut milk and soymilk. Different approaches have been explored to control chalkiness in plant-based milks. Kuntz *et al*. (1978) observed that increasing the pH of the milk decreases chalkiness while decreasing solids concentration through centrifugation removes the particles responsible for chalkiness.

The removal of starch granules to achieve a reasonably good shelf-stable tigernut milk has been investigated but such treatment was found to lead to the loss of the natural organoleptic properties of the tigernut milk (Corrales *et al*., 2012). This gives indication that the removal of starch granules cannot be used to effectively control chalkiness in tigernut milk. Rosenthal *et al*. (2003) used enzymes to improve soymilk whole quality, including the reduction of chalky mouthfeel. Thus treating plant-based milks with enzymes could help solve their associated sensory challenges.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sources of materials

Rhizomes of tigernut were collected in September 2013, from a farmer in *Mmaa baasa* village (at Twifo-Praso) in the Central region of Ghana. The nuts were harvested in August 2013 and stored as it was with the sand in a plastic drum.

3.2 Preliminary sample preparation

3.2.1 Sorting

Soil particles were removed from the nuts by constantly rubbing the nuts against the inner surface of a traditional basket. The brown tigernut was then manually separated from the other two varieties (black and yellow) and weighed.

Tigernuts with mechanical injuries and/or those with more than three holes were separated as damaged tubers and weighed and the percentage loss calculated as,

$\%Loss = (W_d/W_i) \times 100$

Where W_d: weight of damaged tubers

W_i: initial weight before sorting.

3.2.2 Washing and Drying

The tigernut sample was divided into four batches of 3 kg each. Each batch was washed in 12 l of water. The washed tigernut sample was spread on the floor of a low-ventilated room and allowed to dry overnight. The fresh tigernut sample was then dried in an oven (Genlab, model MINO/50) at 55°C for 92 h in order to get a final moisture content of at most 18%. The dried tigernut sample was then stored in a closed bucket at room temperature.

3.3 Moisture content determination of tigernut samples

The moisture content was determined on the fresh tigernut and on the dried tigernut samples. All moisture content determination was done using the protocol described by the Association of Official Analytical Chemists (AOAC) (1997). Approximately 2 g of each sample was crushed, weighed into a previously dried and weighed petri-dish and dried in an oven (Genlab, model MINO/50) at 105 °C for 8 h. The petri dishes were transferred into a desiccator for cooling after which they were weighed. Moisture content was determined by difference and expressed as a percentage.

3.4 Extraction of tigernut milk

The method described by Belewu and Abodunrin (2006) was used for the extraction of the tigernut milk. A preliminary extraction was done to determine the average milk yield of the brown variety of tigernut. Sieves with pores sizes 300 and 100 µm were used simultaneously; the smaller size mesh being under the bigger. A flow chart of the extraction of tigernut milk is presented in Figure 3.1. Bottled tigernut milk was then subjected to enzymatic hydrolysis.

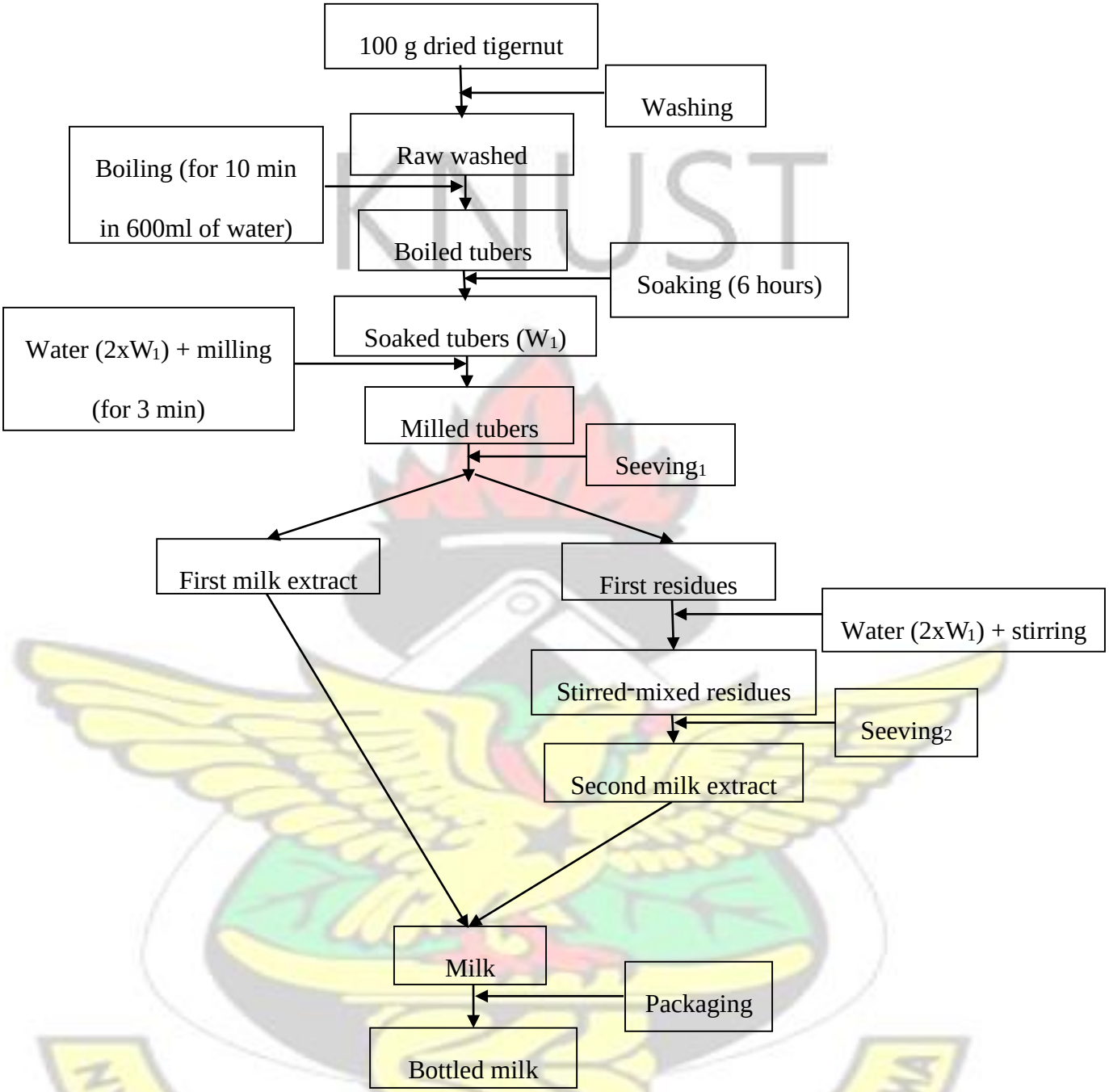


Figure 3.1: Flow chart of tigernut milk extraction

W_1: weight of the soaked tubers

3.5 Starch hydrolysis of the milk extract

The hydrolysis of the starch contained in the tigernut milk was done using α-amylase from *Aspergillus orizae* (product number 10065, Sigma-Aldrich) and glucoamylase from *Aspergillus*

niger (product number 10115, Sigma-Aldrich) in five different proportions. The properties of the enzymes used are presented in Appendices 12 and 13 respectively. The hydrolysis was conducted in two phases. The total enzyme concentration in the tigernut milk was 1% in all cases (Table 3.1). The enzyme mixtures were added to the tigernut milk (130 ml) and hydrolysis allowed for 4 h at 47- 50 °C in a water bath. The samples were manually shaken every 20 min and aliquots taken after every 1 h for analysis. Tigernut milk which had not been treated with enzymes, was subjected to the same heat and sampling treatment to serve as control. Bottled hydrolyzed aliquots, control and raw tigernut milks were stored in a freezer (-27 °C) for further analysis.

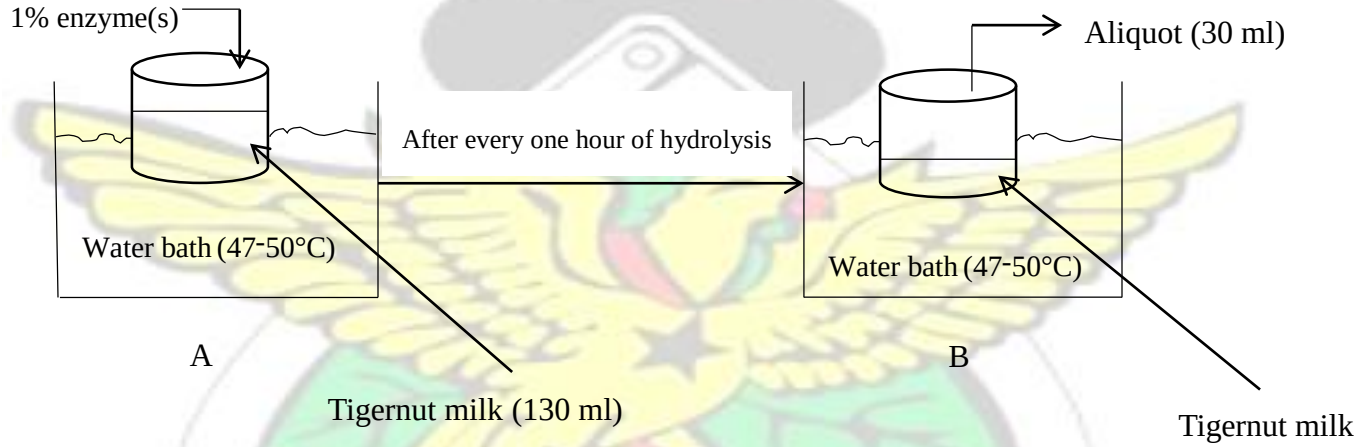


Figure 3.2: Hydrolysis procedure of starch in tigernut milk

A is the initial stage of the hydrolysis process and B is the stage after every 1 h when 30 ml aliquot is taken

Table 3.1: Ratios of enzymes used per sample of tigernut milk and aliquots from each

Samples with enzymes ratios	**Raw** (0α:0gl)	**C1** (0.8 α:0.2gl)	**C2** (1 α:0gl)	**C3** (0.5 α:0.5gl)	**C4** (0 α:1gl)	**C5** (0.2 α:0.8gl)	**Control** (0 α:0gl)

Samples Code	**R**	**T1**	**T2**	**T3**	**T4**	**T5**	**C**
Number of aliquots	0	4	4	4	4	4	4

α: α-amylase, gl: glucoamylase

The raw sample was not subjected to the hydrolysis temperature and time but the control went through the process of hydrolysis.

The preliminary study (phase 1), embodied T1 and T2, meanwhile phase 2 took care of T3, T4, T5 and the raw was part of both phases.

3.6 Total starch determination

The total starch concentration of the samples was determined according to the method described by Kiliç and Özbek (2004). Standard starch solutions from concentration 0.05 to 1 mg/ml were prepared in beakers. About 1.33 ml of iodine solution (0.05% KI and 0.015% I_2) was added to 1 ml of each standard starch solution and the volume topped up to 15 ml with distilled water. The resultant solution was then mixed (with a vortex for 2 min**)** and its absorbance was read at 550 nm against that of a blank containing 1.33 ml of iodine solution using a spectrophotometer (JACTERMAC SM23A, Germany). Absorbance of each sample was determined by using 1 ml of the 10^{-3} serial dilution of the milk samples, to which 1.33ml of iodine solution was added and the volume topped up to 15 ml with distilled water. The calibration chart equation (Appendix 1) was used to convert the absorbance of the samples into the corresponding starch concentration (%).

3.7 Glucose determination

The anthrone assay was used for glucose content determination. About 2 g of anthrone was dissolved in 100 ml conc. H_2SO_4. Standard glucose solutions from concentrations of 5 to 100 g/ml were prepared in beakers. About 4 ml anthrone reagent was added to 0.8 ml of each standard glucose solution and mixed. The mixture was incubated for 10 min in boiling water, cooled and the absorbance read at 620 nm using a spectrophotometer (JACTERMAC SM23A, Germany). The standard calibration curve was plotted from the absorbance readings (Appendix 2). Serial dilutions of the tigernut milk samples were made. One (1) ml of the 10^{-3} dilution was centrifuged at 2000 tr/s for 5 min and aproximately 0.8 ml of the supernatant was mixed with 4 ml of the anthrone reagent. The mixture was then incubated in a boiling water bath for 10 min and allowed to cool at room temperature. The absorbance was measured at 620 nm against a blank containing 4 ml of the anthrone reagent using spectrophotometer (JACTERMAC SM23A, Germany).

3.8 Determination of pH

The pH was measured using a pH-meter from HANNA instruments (model Piccolo2). The pHmeter was calibrated using buffers of pH 7 and pH 4. Three (3) ml of the samples were put in test-tubes and the pH was measured. The readings were recorded directly from the pH-meter.

3.9 Total soluble solids (°Brix) measurement

The °Brix was measured using a digital refractometer from Reichert (model AR200 ver1.0). The sample was poured on the dried and clean prism of the refractometer. The results were directly read on the numerical part of the refractometer.

3.10 Sensory analysis

Selection and training of the Quantitative Descriptive Analysis (QDA) panel

Recruitment: Twelve panellists were selected from the staff of Crop Research/Fumesua and from students of the department of Food Science and Technology/KNUST, based on their participation in similar studies and willingness to participate.

Screening and training: Recruited panellists ability: to recognise the basic taste (sweet, bitter, salt) and to discriminate different scent and colour were tested. Training sessions were held for a period of two weeks at the rate of two hours, twice in a week. In a group discussion, panellists were made to list quality attributes of vegetable milks: commercial soymilk (Vitamilk®), and tigernut milk. The listed quality attributes were discussed at large using local descriptors for all panellists to have a common understanding. The following sensory attributes were finally considered for the product evaluation: creamy colour, sweet taste, tigernut-like flavour and chalky mouthfeel.

The ability to describe and use the attributes in scoring samples was tested using difference test and descriptive test. This was done until panellists were familiar with the quality attributes and the sensory test procedure to be used. The trained panellists contributed in developing the questionnaires used for the real study. Ten out of the twelve panellists were selected for the sensory evaluation based on their ability to score the sensory attributes as taught.

Samples preparation

Three samples (Table 3.2) of tigernut milk were subjected to both consumer test and descriptive test. The three samples were chosen based on the starch reduction and the glucose increase. The tigernut milks were warmed (as usually is the case in chocolate beverages).

Table 3.2: Randomized-coded tigernut samples for sensory analyses

Samples	Enzyme ratios	Samples code

Control/Raw	0α:0gl	509
Sample 1	1α:0gl	145
Sample 2	0.8 α:0.2gl	281

α: α-amylase, gl: glucoamylase

Descriptive test

The descriptive test used to quantify the variation in terms of chalkiness, sweetness, flavour and colour was the Quantitative Descriptive Analysis (QDA). In the QDA, 10 trained-panellists were asked to rate the intensities of each samples independently from one another.

Affective test

To assess consumer acceptability of the tigernut milk products, 50 semi-trained panellists performed the 9-point hedonic rating test. The affective panel was asked to identify the most appropriate phrase for the tested samples.

Questionnaires for QDA and affective test are presented in appendices 3 and 4 respectively.

3.11 Statistical analysis

Results obtained are averages of triplicate determinations. Analysis of Variance (ANOVA) was performed on both the physico-chemical and sensory analysis data. Pearson test was used to correlate physico-chemical parameters (starch content, glucose content, pH, °Brix). Correlations between physico-chemical and sensory characteristics were evaluated using Spearman test. The significance level was 5% for all. The statistical package used was IBM SPSS Statistics version 20 (IBM Corp., 2011).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Findings of preliminary investigations

4.1.1 Sorting

Sorting of good tigernuts resulted in a significant loss in weight due to high amounts of damaged tubers. The debris and unwholesome tubers constituted 11.11% of the entire weight of the sample. Damages to the tubers could be attributed to the action of termites and manual harvesting method. Manually pulling the tubers during harvest could have cause cuts. Such damages on the tubers could have been avoided if the cultivation was industrial, with use of mechanical harvesters and suitable pesticides as mentioned by Kay (1987).

4.1.2 Moisture content of tigernut tubers sample

The moisture content of the fresh sample and that of the dried sample was found to be 37.99 ± 0.27% and 16.02 ± 0.06% respectively. Though the moisture content of the dried sample was below the acceptable level of moisture content (18%) stated by Practical Action (2014), it will enhance the shelf-life of the product. Less moisture content means less free water used for bacterial growth. The dried sample had 21.97% moisture loss after drying at 55°C for 92 hours.

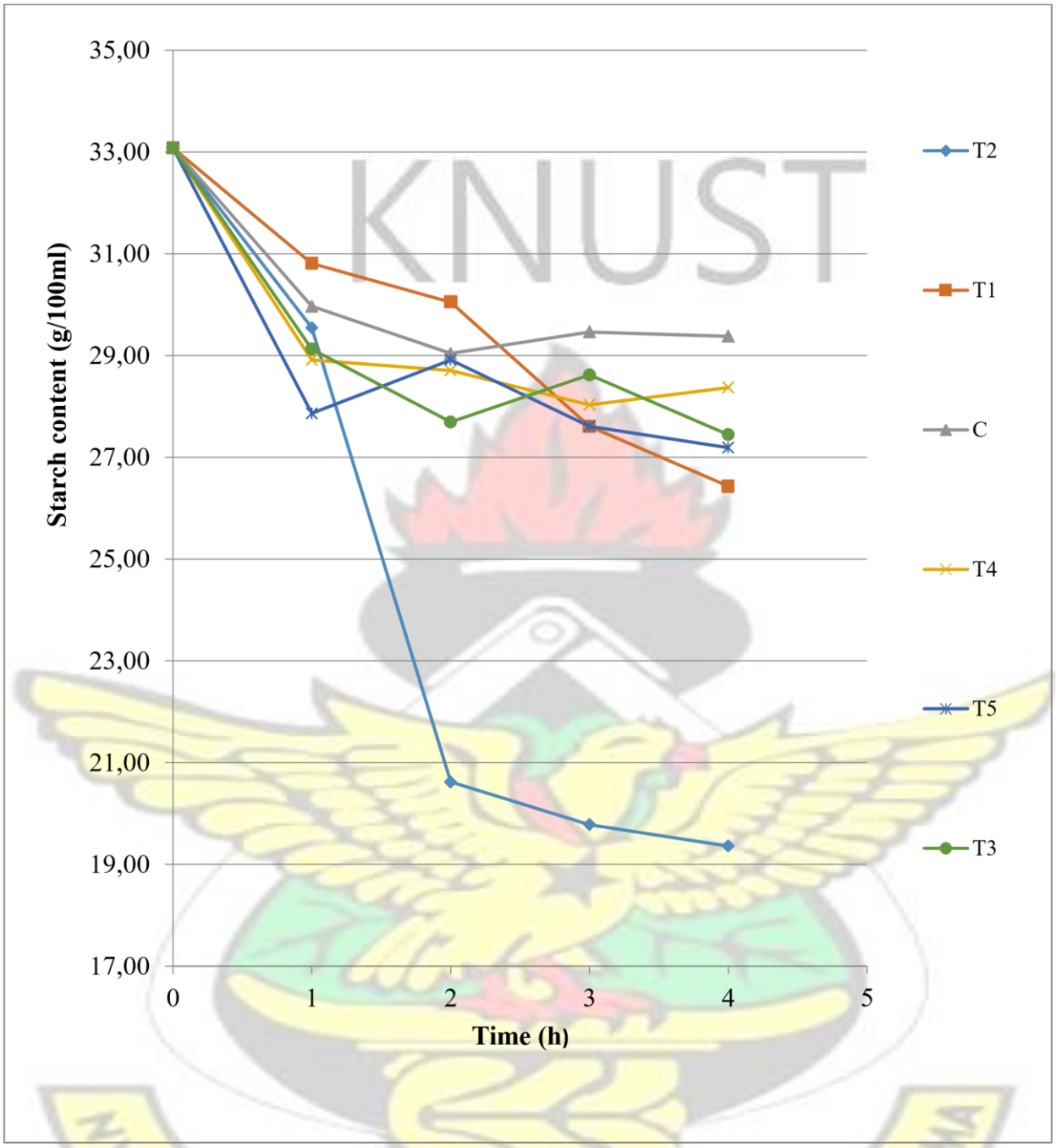
4.1.3 Milk yield

The preliminary milk extraction revealed that the use of 100g of dried tigernut tubers yields an average of 594.33 ± 12.50 ml of milk (Appendix 14). The ratio of 4:1 (w/w) water to boiledsoaked tigernut was determined for the milk extraction as shown in Figure 3.1. This agrees with reports from Cantalejo (1996).

4.2 Starch content of the tigernut milk samples at different hydrolysis time and enzymes concentrations

The analysis of the starch content in the raw tigernut milk samples revealed an initial starch content of 33.08 ± 3.82%, way greater than what has been reported in literature. Cortes *et al*. (2004) and Pascual *et al*. (2000) reported that starch content of tigernut milk is within the range of 2.2-4.3%. These results might be due to cultivar and environmental differences in where they are obtained. The high starch content could also be due to the type of mesh used in the milk extraction. However, the high starch content of tigernut milk found in this study compares well with that found in the tubers as reported by Turesson *et al*. (2010). It was realized that there was a decrease in starch content of the samples regardless of the enzyme(s) composition (Figure 4.1) during the process of hydrolysis. However, the decrement was dependent on enzyme (s) concentration. It was observed that hydrolysis resulted in the reduction of starch.

After 4 hours of hydrolysis, sample T2 had the lowest starch content (19.36 ± 0.39%), followed by sample T1 which had a starch content of 26.43 ± 1.02%. This agrees with reports that amylases target starch upon hydrolysis to yield smaller molecules such as maltodextrins (Kolusheva and Marinova, 2007). The control showed some decrease in starch content and this could be attributed to the presence of enzymes such as catalase and amylase which are intrinsic to the raw tigernut (Adejuyitan, 2011).



T1=0.8%α-amylase+0.2%glucoamylase T2=1%α-amylase
T3=0.5%α-amylase+0.5%glucoamylase T4=1%glucoamylase T5=0.2%α-amylase+0.8%glucoamylase
C=Control

Figure 4.1: Starch content (g/100ml) of tigernut milk samples at different hydrolysis time with different enzymatic composition.

In terms of enzymatic degradation effect on starch, there was a significant difference between sample T1 and the rest of the samples (T2, T3, T4, T5 and C). However, samples T2, T3, T4, T5 and C showed no significant difference among them (Appendix 5).

On face value (Appendix 5) at the end of the hydrolysis time, though the difference was not significant at 95% confidence level, T1 recorded a higher decrease in starch content than T5 and the control, due to the amount of α-amylase present.

In terms of time of hydrolysis, significant differences in starch content decrease was observed at different times, but generally there was no significant difference between two consecutive hours at the confidence level of 95% due to the enzyme digestibility rate of the starch (Appendix 6). The kinetic of the hydrolysis could be considered as relatively slow since it takes two hours to observe a significant difference in starch decrease.

Plate 4.1: Raw tigernut milk

Plate 4.2: Hydrolysis of starch in tigernut milk at 49.1°C

4.3 Glucose content of the tigernut milk samples after hydrolysis

The glucose content increased in the milk during hydrolysis. The highest increase in glucose content was in sample T1; followed by sample T5 (Figure 4.2). The lowest glucose content was observed in sample T4. The results showed that glucoamylase yields more glucose when used in combination with α-amylase.

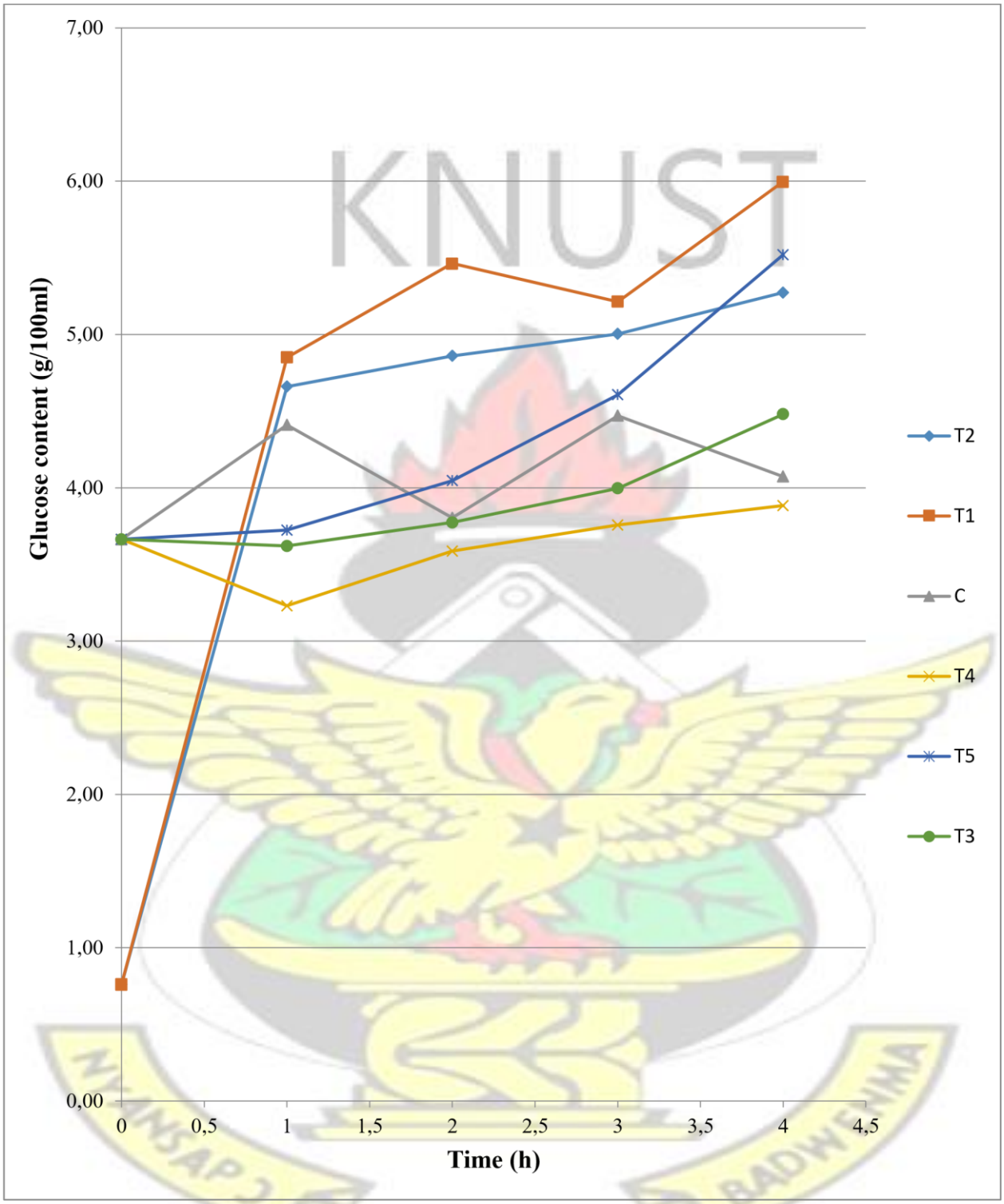
The initial glucose contents of tigernut milk samples T1 and T2 in relation to other samples were lower probably because they were tested 11 weeks earlier. The glucose content of the tigernut tubers may have increased during storage through some processes related to residual enzymes inherent in the tigernut (Adejuyitan, 2011). The test was conducted at different periods due to the structure of the research as well as challenges with obtaining reagents for the work.

Even though the glucose content, after four hours of hydrolysis, of sample T1 was more than that of sample T2, the difference was not significant ($p>0.05$). It is noteworthy that the glucose content of the control was statistically not different from T3 and T4.

On the graph, the glucose content of the control increased and decreased consecutively over time during hydrolysis. There might have been some chemical and/or enzymatic reactions that took place in the control during that period.

Effect of time on glucose content throughout hydrolysis period showed a significant difference between 0 hour and 1 hour. As a general observation, the difference in glucose content between two consecutive hours was not significant at 95% confidence level (Appendix 6).

Partial conclusion: There was no significant difference between the sole use of α-amylase and the combination of α-amylase and glucoamylase at the ratio of 0.8:0.2 in terms of glucose yielding capacity.



T1=0.8%α-amylase+0.2%glucoamylase T2=1%α-amylase
T3=0.5%α-amylase+0.5%glucoamylase T4=1%glucoamylase
T5=0.2%α-amylase+0.8%glucoamylase C=Control

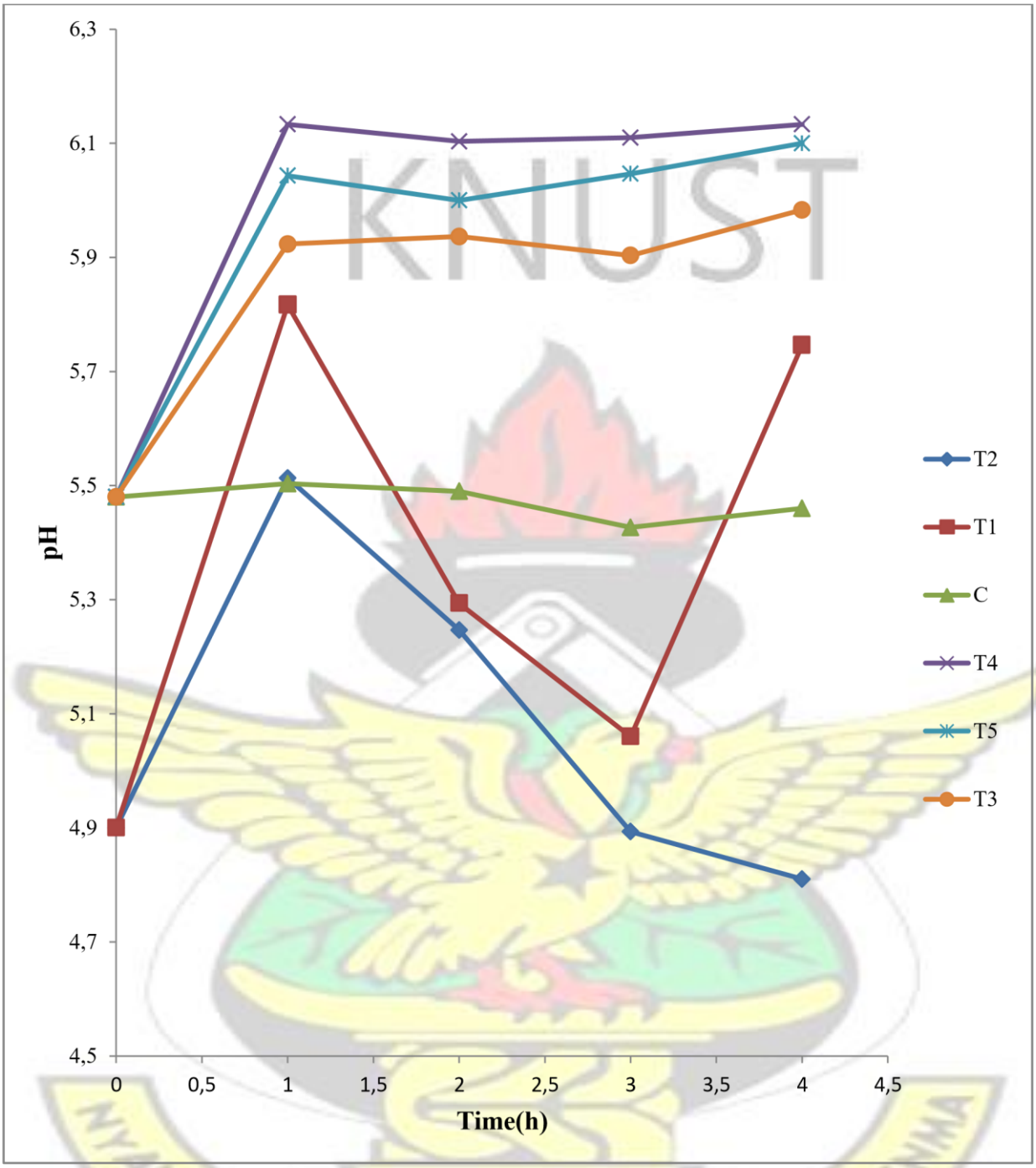
Figure 4.2: Glucose content (g/100ml) of tigernut milk samples at different hydrolysis time with different enzymatic composition

4.4 pH of the tigernut milks

The pH of the tigernut milks under study was monitored throughout the experimental process. The pH of the control was more or less stable during the hydrolysis period, varying from 5.43 to 5.50 with time (Appendix 9). This stability is more visible in Figure 4.3 below. Samples T1 and T2 had an initial pH around 4.9 while the rest recorded a pH of 5.48.

A number of authors have reported that, the pH of the fresh tigernut milk is about 6.3 (Cortes *et al*., 2004; Cortés *et al*., 2005; Adejuyitan, 2011; Asante *et al*., 2014). However, the pH of the fresh samples under study was 5.48 ± 0.02, indicating they were acidic. Similar pH of tigernut milk was reported by Musa and Hamza (2013) in Nigeria. Akoma *et al.* (2006) attributed this to the presence of certain species of lactic acid bacteria namely *Lactobacillus leichmanni* and *Lactobacillus fermentum* that could have caused the pH to decrease during the lapse of time between extraction and analysis.

The general trend of the pH as represented in Figure 4.3 shows a slight increase over hydrolysis period with T4 having the highest pH observed at the end of the experiment.



T1=0.8%α-amylase+0.2%glucoamylase T2=1%α-amylase T3=0.5%α-amylase+0.5%glucoamylase
T4=1%glucoamylase T5=0.2%α-amylase+0.8%glucoamylase C=Control

Figure 4.3: pH of tigernut milk samples at different hydrolysis time with different enzymatic composition

4.5 Effect of the enzymatic hydrolysis on the total soluble solids content

The total soluble solids also known as °Brix was measured throughout the experiment. It varied from 3.17 ± 0.06 to 6.27 ± 0.35% (Appendix 6). The initial total soluble solids of 3.17-4.27% is

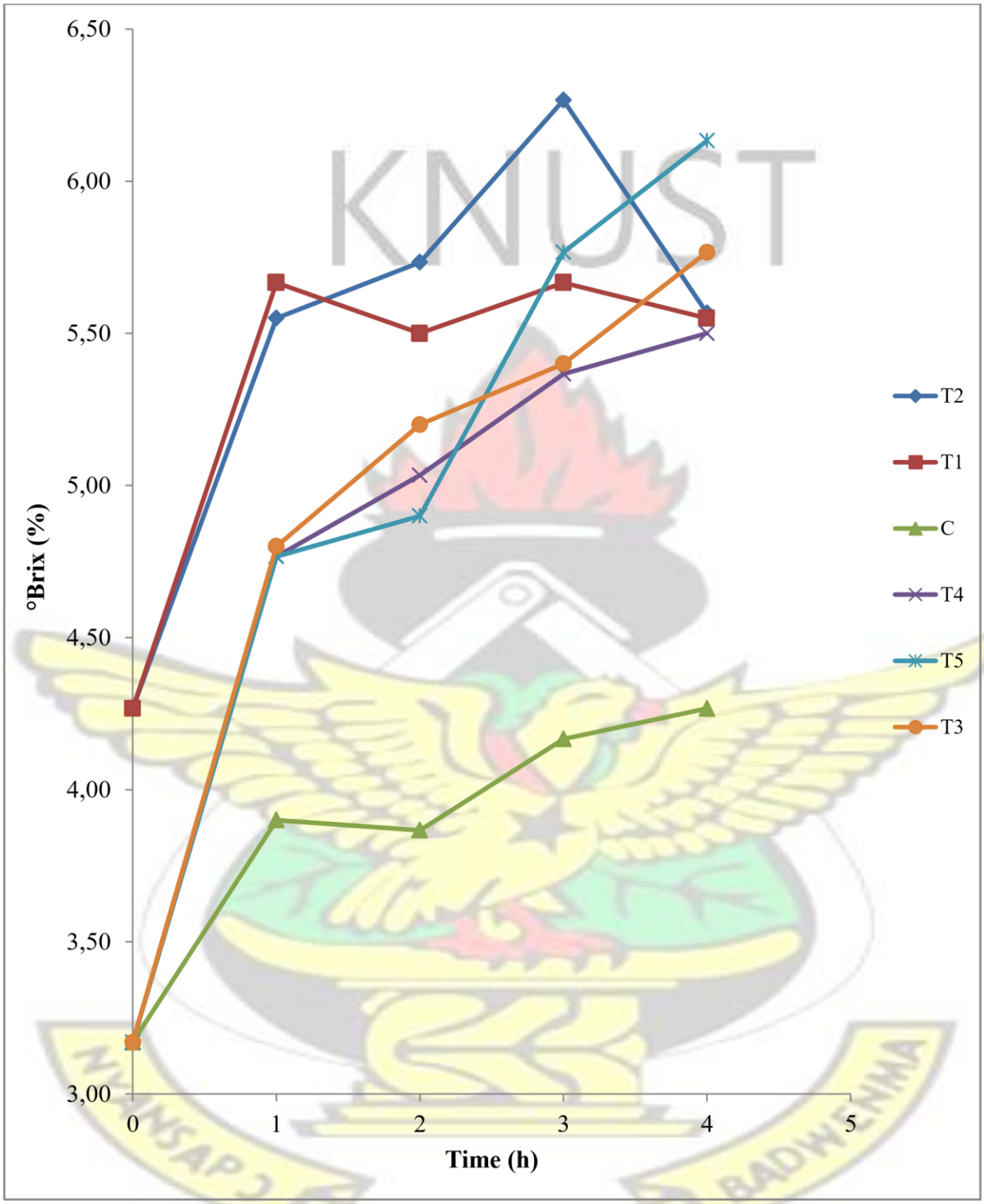
consistent with standards in Spain that stipulates the unsweetened tigernut milk total soluble solids should be about 4% (Cortes *et al*., 2004). At the end of the experiment, the highest total soluble solids was 6.13 ± 0.06%, recorded for sample T5 and the lowest was 4.27 ± 0.12% in the control. The second highest total soluble solids was observed in T3, followed by T2 (Figure 4.4).

This is due to the fact that there were more soluble solids in the milk T5 than in the others. The synergy of action between α-amylase and glucoamylase explains this. The first one hydrolyzes the insoluble starch into smaller soluble compounds such as maltodextrins and the second one completes the action of the first one by yielding glucose, the smallest (and soluble) unit of starch.

When it came to the effect of enzymes on the total soluble solids, it was established statistically that milk with 1% α-amylase and that with 0.8% α-amylase + 0.2% glucoamylase gave almost the same results. These two samples were significantly different from the others (Appendix 5).

The effect of time on total soluble solids over hydrolysis showed no statistical difference between 1 hour and 2 hours as well as between 3 hours and 4 hours (Appendix 6).

Partial conclusion: Just like enzymes effect on glucose yield, C1 and C2 had almost the same effect on total soluble solids. It means that the use of one or the other makes no significant difference. The most efficient enzyme combination in terms of total soluble solids was 0.2% αamylase+0.8% glucoamylase. There was no significant difference in soluble solids after hydrolysis for 3 and 4 hours.



T1=0.8%α-amylase+0.2%glucoamylase T2=1%α-amylase
T3=0.5%α-amylase+0.5%glucoamylase T4=1%glucoamylase
T5=0.2%α-amylase+0.8%glucoamylase C=Control

Figure 4.4: °Brix of tigernut milk samples at different hydrolysis time with different enzymatic composition

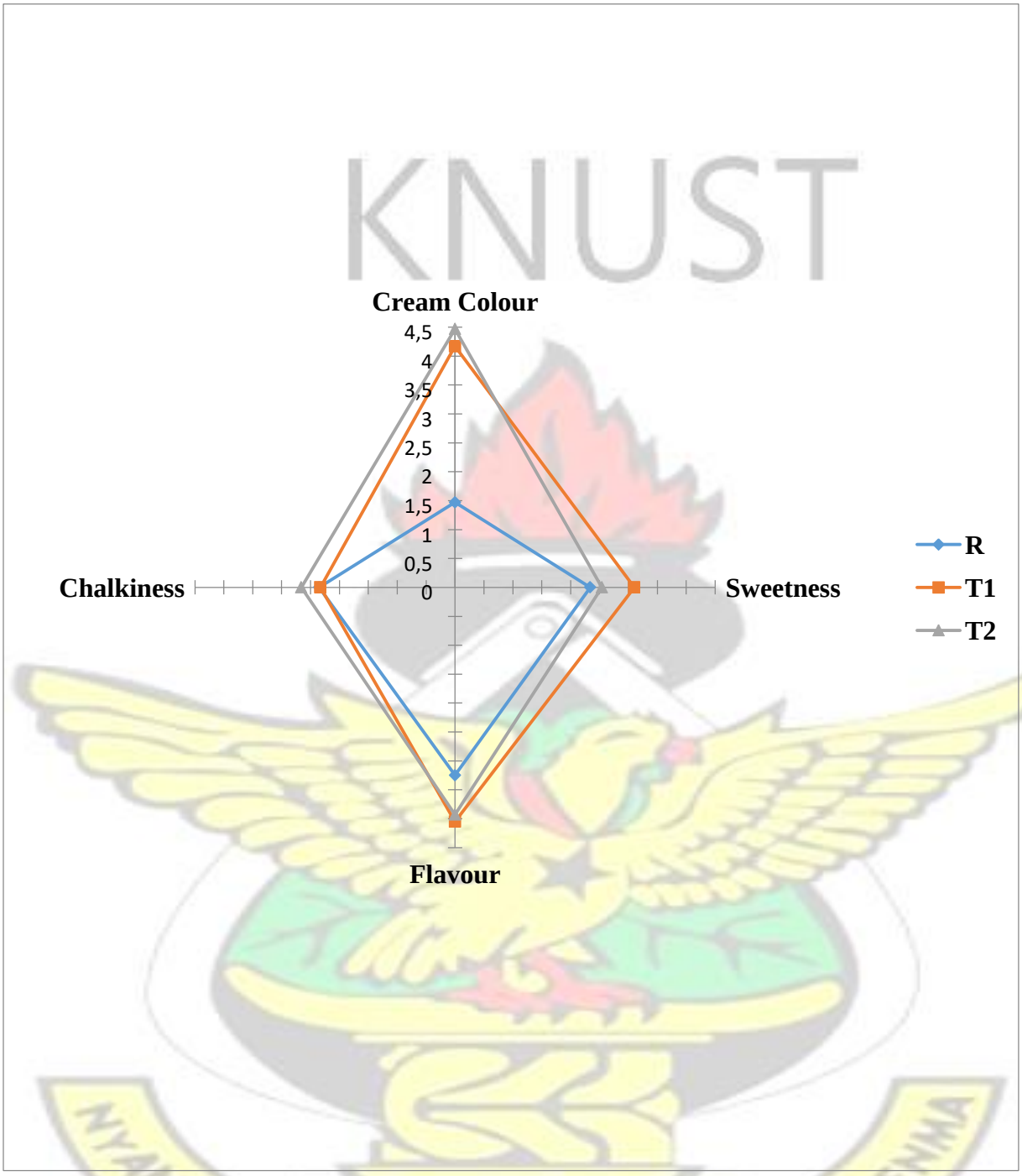
4.6 Quantitative Descriptive Analysis (QDA)

The hydrolyzed sample with 0.8% α-amylase+0.2% glucoamylase and that with 1% α-amylase showed greater decrease of starch as well as increase of glucose, making them eligible for sensory testing.

Results of QDA (Appendix 10) showed a difference between the raw and the hydrolyzed samples in terms of colour. The raw sample had a lighter creamish colour than the hydrolyzed samples. Hydrolysis, done at 50 °C for 4 hours surely darkened the colour of the milk and this may be due to browning reactions. These reactions also affected the moisten earth-like flavour of the hydrolyzed samples (Pascual *et al*., 2000). As presented in Figure 4.5 below, the flavour of T1 was stronger than that of the raw and that of T2. This is because it contained more glucose for these reactions

Sample T1 was sweeter than the other two, which could be attributed to the fact that glucoamylase contributed to the yield of more glucose. This sample had approximately the same level of chalkiness as the raw, and T2 had the strongest chalky mouthfeel; even though the starch was hydrolyzed to diminish the level of chalkiness in the hydrolyzed samples.

A study conducted by Rosenthal *et al*. (2003) led to the improvement of soymilk quality by also using enzymes. The enzymes used in that study were to hydrolyze pectin and cellulose rather than starch. This could explain the efficiency of their study on chalkiness reduction.



T1=0.8%α-amylase+0.2%glucoamylase T2=1%α-amylase R=Raw
0 cm= light cream colour 15 cm= dark cream colour
0 cm= not sweet 15 cm=extremely sweet
0 cm= low flavour 15 cm= strong flavour
0 cm= not chalky 15 cm= extremely chalky

Figure 4.5: Quantitative Descriptive Analysis of colour, sweetness, chalkiness and flavour of three tigernut milk samples

The sweetness of the two hydrolyzed samples as compared to that of the raw did not show a significant difference at the confidence level of 95%. This result confirmed results from

physicochemical analysis where there was no significant difference between the hydrolyzed samples and the control (Appendix 5).

Statistics showed no significant difference amongst the three tested samples (Appendix 7) or between the hydrolyzed ones and the raw (Appendix 10) in terms of flavour and chalkiness. It is true that the starch content of the samples decreased with hydrolysis to a certain extent, but this did not affect the sensory perception of the respondents.

Partial conclusion: The colour of the hydrolyzed samples was brown compared to that of the raw but in terms of sweetness, flavour and chalkiness; there was no significant difference between the three tested samples. The hydrolysis of starch in tigernut milk did not significantly diminish the chalky mouthfeel.

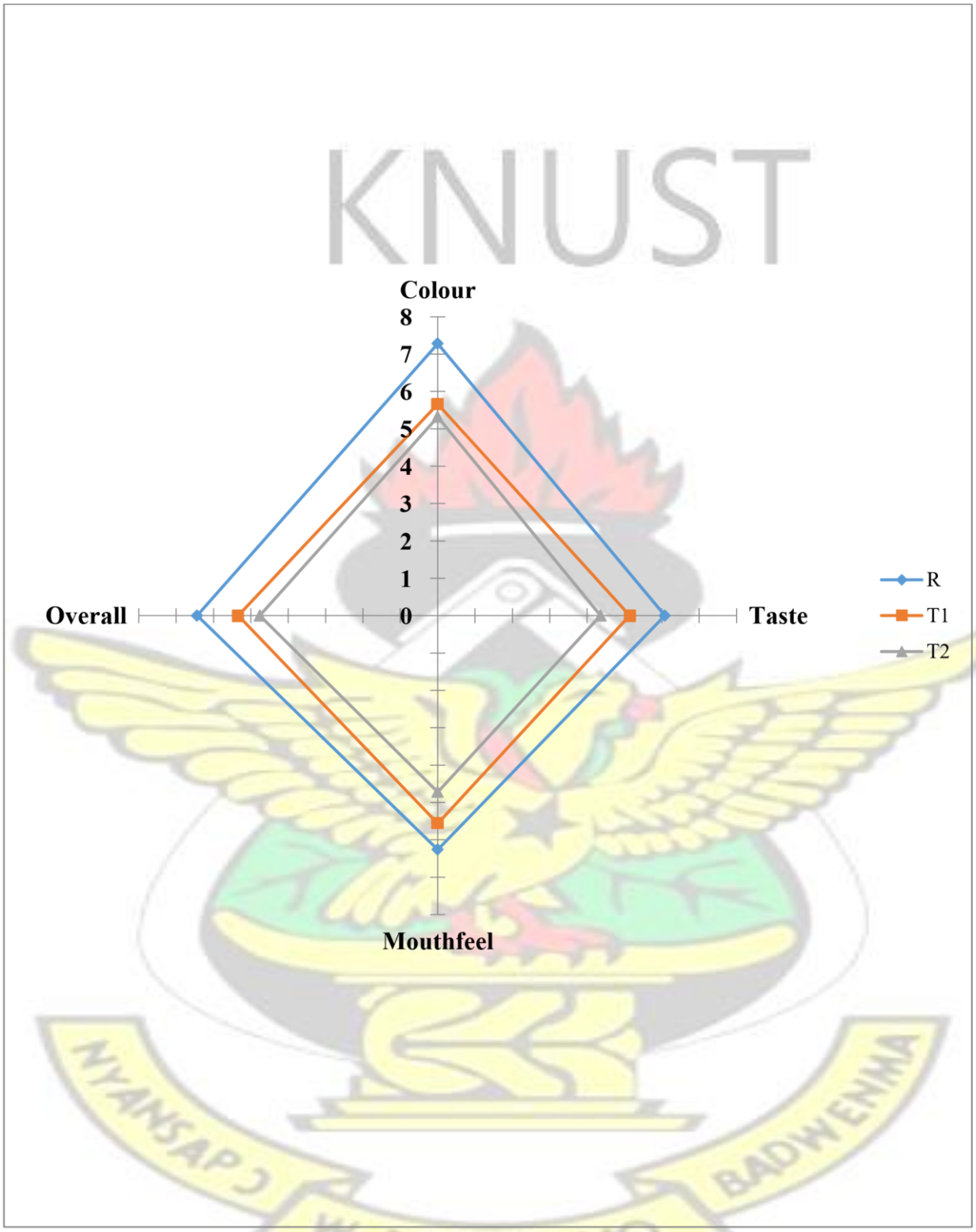
4.7 Affective sensory test

The hedonic rating test showed significant difference between the two hydrolyzed samples and the raw in terms of colour and overall acceptability as presented in Appendix 11. However, there was no significant difference between T1 and T2 (Appendix 8). The colour of the raw sample was the most preferred, probably because it was not affected by hydrolysis and corresponded more to the general ―milky‖ colour.

When it came to sweet taste and chalky mouthfeel, the raw showed no significant difference with T1. The level of likeness was the same. This was unexpected knowing that T1 had more glucose after hydrolysis and was expected to be sweeter than the others. This could be because the respondents were not able to differentiate between the products.

Figure 4.6 below shows that in all attributes, the raw was the most preferred, followed by T1 and T2 was the least preferred.

Partial conclusion: The raw tigernut milk was the most accepted by the respondents on the hedonic test. Since the level of likeness between the raw sample and the hydrolyzed sample T1 were the same in terms of taste and mouthfeel, the overall preference of the respondents could have been mainly driven by the colour. This implies that the starch hydrolysis to reduce chalkiness during this study was not efficient.



T1=0.8%α-amylase+0.2%glucoamylase T2=1%α-amylase R=Raw
1= dislike extremely 5=neither like nor dislike 9=like extremely

Figure 4.6: Hedonic rating test for colour, taste, mouthfeel and overall of three tigernut samples

Plate 4.3: Photograph showing various stages of the sensory analysis of the samples

4.8 Correlations between parameters

Results of correlation tests between starch, glucose, pH and °Brix (Table 4. 1) showed that the total soluble solids had a very significant correlation ($p < 0.01$) with starch and glucose contents. It showed a negative but strong correlation for starch and a positive correlation for glucose. A decrease in starch content increased the °Brix and glucose content. Glucose and starch contents

had a negative correlation; starch content decreases with increase in glucose content. The pH had a positive correlation with glucose. This correlation was significant ($p < 0.05$).

Table 4.1: Correlations of starch, glucose, pH and °Brix

		Starch	Glucose	pH	Brix
Starch	Pearson Correlation	1	-.493**	.035	-.567**
	Sig. (2-tailed)		.000	.739	.000
	N	91	91	91	91
Glucose	Pearson Correlation	-.493**	1	.228*	.451**
	Sig. (2-tailed)	.000		.028	.000
	N	91	93	93	93
pH	Pearson Correlation	.035	.228*	1	.185
	Sig. (2-tailed)	.739	.028		.076
	N	91	93	93	93
Brix	Pearson Correlation	-.567**	.451**	.185	1
	Sig. (2-tailed)	.000	.000	.076	
	N	91	93	93	93

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

Correlations between the physico-chemical and affective sensory parameters as shown in Table 4.2 demonstrated weak and positive correlations between starch content and the sensory attributes colour, taste, mouthfeel and overall preference as well as between glucose content and the sensory attributes taste, mouthfeel and overall preference. But there was a weak and negative correlation between glucose content and colour as well as between pH and chalky mouthfeel. As glucose increases, likeness of the colour decreases, confirming the hypothesis of browning reactions that

took place in the hydrolyzed products. And as pH increases, chalkiness decreases; this reflects results found by Kuntz *et al*.(1978). However, these correlations were not significant.

There were strong and positive correlations between colour, taste, mouthfeel and overall preference and perfect correlations between taste, mouthfeel and overall. It means that taste and mouthfeel had significant impact on the overall preference of the product. Thus, liking the taste and/or mouthfeel of the product implies liking the product. The taste and mouthfeel of tigernut milk were important in determining consumer preference. Improving on the taste and/or mouthfeel will positively impact on consumer acceptability of the tigernut milk. These correlations were indeed very significant ($p<0.01$).

The four hours of hydrolysis data of physico-chemical parameters was used for the physico-chemical and sensory correlations.

Table 4.2: Correlations between physico-chemical and affective sensory characteristics of tigernut milk

		Starch	Glucose	pH	Brix	Colour	Taste	Mouth feel	Over all
Spearman's rho	Starch Correlation Coefficient	1.000	-.467	.150	-.603	.367	.250	.250	.250
	Sig. (2tailed)		.205	.700	.086	.332	.516	.516	.516
	N	9	9	9	9	9	9	9	9
	Glucose Correlation Coefficient	-.467	1.000	.517	.728*	-.033	.150	.150	.150
	Sig. (2tailed)	.205		.154	.026	.932	.700	.700	.700
	N	9	9	9	9	9	9	9	9
	pH Correlation Coefficient	.150	.517	1.000	.452	-.183	-.200	-.200	-.200
	Sig. (2tailed)	.700	.154		.222	.637	.606	.606	.606
	N	9	9	9	9	9	9	9	9

Brix	Correlation Coefficient	-.603	.728*	.452	1.000	-.527	-.343	-.343	-.343
	Sig. (2tailed)	.086	.026	.222		.145	.366	.366	.366
	N	9	9	9	9	9	9	9	9
Colour	Correlation Coefficient	.367	-.033	-.183	-.527	1.000	.950**	.950**	.950**
	Sig. (2tailed)	.332	.932	.637	.145		.000	.000	.000
	N	9	9	9	9	9	9	9	9
Taste	Correlation Coefficient	.250	.150	-.200	-.343	.950**	1.000	1.000**	1.000**
	Sig. (2tailed)	.516	.700	.606	.366	.000			
	N	9	9	9	9	9	9	9	9
Mouth feel	Correlation Coefficient	.250	.150	-.200	-.343	.950**	1.000**	1.000	1.000**
	Sig. (2tailed)	.516	.700	.606	.366	.000			
	N	9	9	9	9	9	9	9	9
Overall	Correlation Coefficient	.250	.150	-.200	-.343	.950**	1.000**	1.000**	1.000
	Sig. (2tailed)	.516	.700	.606	.366	.000			
	N	9	9	9	9	9	9	9	9

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

4.9 Limitation of study

Knowing that sensory analyses would be performed and enzymes could not be removed from the samples to ensure safety, this experiment considered a ceiling of 1% enzymes addition. Specific activities of the enzymes used were relatively low. Also, hydrolysis time and interval time of aliquot sampling during the experiment were both relatively short. There is also the possibility that the affective panel were not regular consumers of tigernut milk, making them unfamiliar with the organoleptic properties of tigernuts. All these factors limited a broad outcome of the study. Availability of equipment and enzymes, and hydrolysis of only starch (to reduce chalkiness) were also limitation factors.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Starch content (33%) of the aqueous extract from tigernut cultivated in the Twifo-Praso/Central region- Ghana was about 10 times higher than what has been reported in literature, making it an economically viable commodity for industrial utilization. During enzymatic hydrolysis, starch content decreased to 19%. Statistics showed that the sole use of α-amylase gave the most reduction of starch content. This makes the use of 1% α-amylase ideal for starch content reduction in tigernut milk. Hydrolysis for three and four hours did not give much difference in starch reduction. However, the optimum time was 3 hours.

Glucose content of tigernut milk increased from 3% to 6% after 4 hours of hydrolysis time. The results showed that the use of a combination of α-amylase and glucoamylase at the ratio of 0.8:0.2 or that of sole α-amylase did not affect the increase in glucose content significantly. Thus, glucose increase was inversely correlated to starch decrease.

Initial °Brix of the fresh tigernut milk (3 - 4) conformed to that recommended in Spain. However, 1% α-amylase gave the most significant total soluble solids increase. The °Brix increased with an increase in glucose.

The statistics showed no significant difference between two consecutive hours of hydrolysis. Considering the effect of enzymes on starch, there was a significant difference between 1% αamylase and 0.8% α-amylase+0.2% glucoamylase. In terms of enzymes yielding effect on

glucose, there was no significant difference between 1% α-amylase and 0.8% α-amylase+0.2% glucoamylase.

From the sensory analyses, it was brought to light that the colour of the hydrolyzed samples was brown compared to that of the raw. But sweetness, flavour and chalkiness were not significantly affected by hydrolysis using 1% α-amylase only or 0.8% α-amylase + 0.2% glucoamylase at 95% confidence level.

5.2 Recommendations

Considering the starch content results, industrials interested in the extraction of tigernut starch could find the use of tigernut cultivar found in Mma baasa village, Central region of Ghana more profitable. Manufacturers interested in reducing starch content of the tigernut milks could opt for the sole use of α-amylase. Also, industries interested in increasing glucose content of tigernut milks could use either the combined enzymes or only α-amylase.

The use of enzymes with higher specific activities is highly desirable; moreover, thermo-resistant enzymes that can be removed after the set time of the experiment such as immobilized enzymes. This can permit the addition and removal of higher enzyme(s) concentration to ensure sensory analyses safety for respondents. Since hydrolysis of starch did not efficiently affect the chalkiness, there is a need to vary the types of enzymes used with the target to decrease chalkiness such enzymes include pectinase, cellulase, lyso-phospholipase, et cetera.

In order to better appreciate the effect of time on the hydrolysis, observations and analyses could be conducted at least, after every 3 hours. The use of modeling methods like response surface technique for example could allow prediction of the various hydrolysates, hours after the end of the set hydrolysis time.

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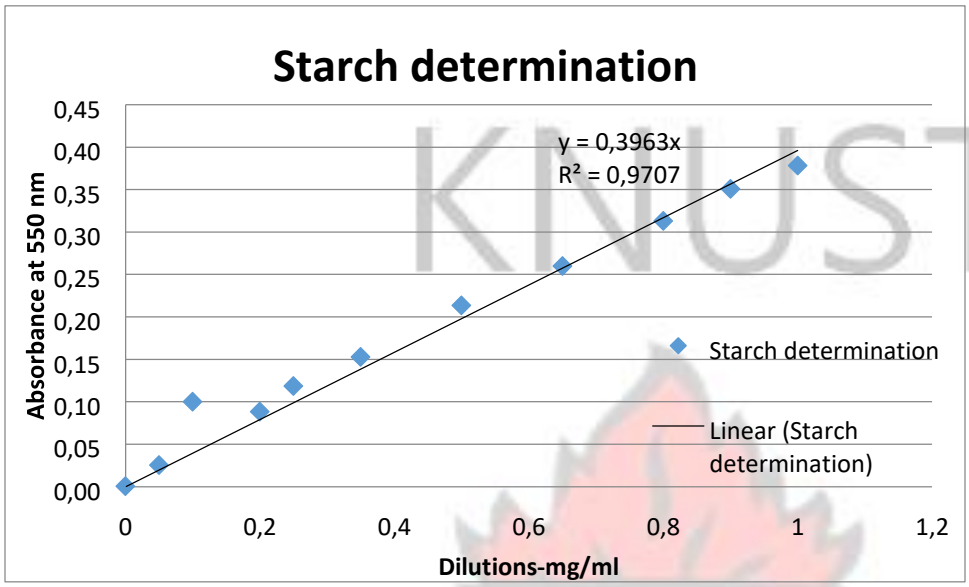
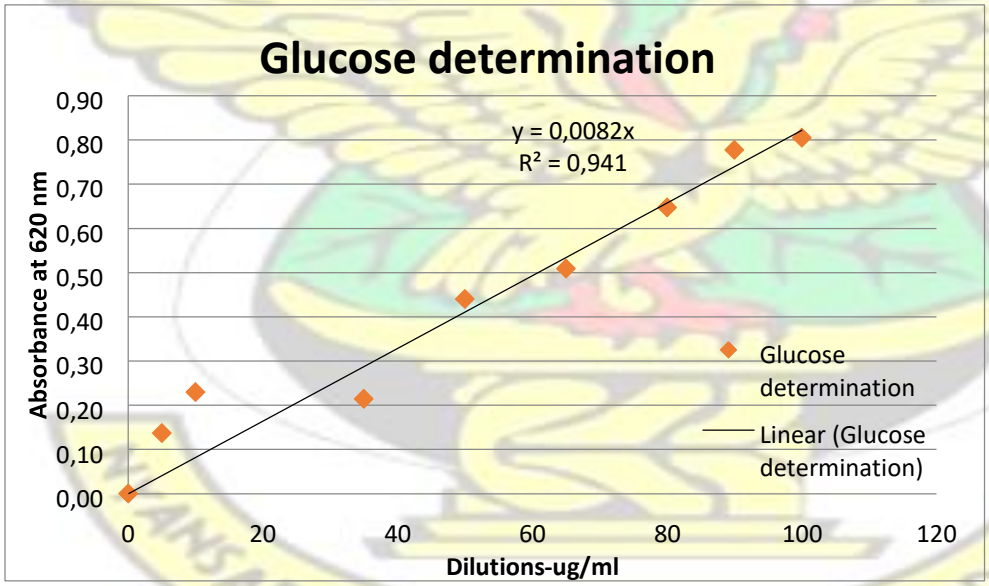
APPENDICES

Appendix 1: Starch standard curve



Appendix 2: Glucose standard curve



Appendix 3: Quantitative Descriptive Analysis of tigernut milk

Definitions

Cream colour: the colour cream is a characteristic colour of milky products. Cream is the pastel colour of yellow, much like as pink is to red. Cream can be produced by mixing white and yellow. Example: the colour of the wall of the sensory room.

_____

Light (Creamish) Dark

Sweet taste: the sweet taste is about sugary taste.

_____

Non (Sugary taste) Strong

Tigernut-like flavour: is a wet earthy-like scent you feel upon eating or drinking. Example: the scent from earth when it is raining or it has rained.

Low (Tigernut like flavour) Strong

Chalkiness mouthfeel: it is the feel of mouth and/ or throat coating upon taking a food product. This coating is like a smooth, residual feeling in the mouth.

Not (Coating mouthfeel) Strong

Descriptive Analysis for milky beverage

Assessor: Date:

You are provided with coded samples of milk. Please evaluate each sample and describe the intensity at which you perceive each product independently from one another in terms of the following attributes as trained:

Colour 509:

Light Dark

145:

cream

Light Dark

281:

Light Dark

Sweetness 509:

Not Highly

145:

Not Highly

281:

Not Highly

Flavour 509:

145: _____

Not Strongly

281: _____

Not Strongly

_____

Not Strongly

Chalkiness 509: _____

Not Highly

145: _____

Not Highly

281: _____

Not Highly

Appendix 4: Hedonic rating test for milky beverage

Assessor: Date:

Please swirl with the provided spoon, observe and taste the beverages in the given order in front of you. Assess one sample at a time. Use the water and biscuits provided to cleanse your palate before tasting each sample:

509 145 281

Indicate how much you like the colour, the taste, the mouthfeel and the overall sample by writing the code in front of the most appropriate phrase below

Colour	Taste	Mouthfeel	Overall
Like extremely	___	___	___
Like very much	___	___	___
Like moderately	___	___	___
Like slightly	___	___	___
Neither like nor dislike	___	___	___
Dislike moderately	___	___	___
Dislike very much	___	___	___
Dislike extremely	___	___	___

Comments:

Appendix 5: Effect of enzymes on starch, glucose, pH and °Brix

Time	Enzymes	Starch	Glucose	pH	°Brix
0	1% α-amylase	33.08±3.82^{a}	3.66±0.16^{a}	5.48±0.02^{a}	4.27±0.21^{a}
	0.8% α-amylase + 0.2 glucoamylase	33.08±3.82^{a}	3.66±0.16^{a}	5.48±0.02^{a}	4.27±0.21^{a}
	0.5% α-amylase + 0.5% glucoamylase	33.08±3.82^{a}	3.66±0.16^{a}	5.48±0.02^{a}	3.17±0.06^{b}
	0.2% α-amylase + 0.8% glucoamylase	33.08±3.82^{a}	3.66±0.16^{a}	5.48±0.02^{a}	3.17±0.06^{b}
	1% glucoamylase	33.08±3.82^{a}	3.66±0.16^{a}	5.48±0.02^{a}	3.17±0.06^{b}
	Control	33.08±3.82^{a}	3.66±0.16^{a}	5.48±0.02^{a}	3.17±0.06^{b}
1	1% α-amylase	29.55±4.01^{a}	4.66±0.23^{a}	5.51±0.21^{a}	5.55±0.07^{a}
	0.8% α-amylase + 0.2 glucoamylase	30.81±1.10^{b}	4.85±0.06^{ab}	5.82±0.08^{b}	5.67±0.15^{a}
	0.5% α-amylase + 0.5% glucoamylase	29.13±0.39^{b}	3.62±0.07^{abc}	5.92±0.08^{c}	4.80±0.26^{b}
	0.2% α-amylase + 0.8% glucoamylase	27.86±0.64^{b}	3.72±0.40$_{abcd}$	6.04±0.06^{c}	4.77±0.25^{b}
	1% glucoamylase	28.92±0.90^{b}	3.23±0.06^{ace}	6.13±0.04^{c}	4.77±0.15^{b}

	Control	29.97±1.14[b]	4.41±0.26[abcde]	5.50±0.12[b]	3.90±0.26[c]
2	1% α-amylase	20.62±0.53[a]	4.86±0.63[a]	5.25±0.48[a]	5.73±0.21[a]
	0.8% α-amylase + 0.2 glucoamylase	30.05±0.25[b]	5.46±0.06[ab]	5.29±0.22[b]	5.50±0.35[a]
	0.5% α-amylase + 0.5% glucoamylase	27.69±0.63[b]	3.77±0.08[abc]	5.94±0.06[c]	5.20±0.17[b]
	0.2% α-amylase + 0.8% glucoamylase	28.92±3.39[b]	4.05±0.40[abcd]	6.00±0.01[c]	4.90±0.17[b]
	1% glucoamylase	28.71±0.89[b]	3.59±0.05[ace]	6.10±0.02[c]	5.03±0.06[b]
	Control	29.04±2.02[b]	3.80±0.29[abcde]	5.49±0.04[b]	3.87±0.12[c]
3	1% α-amylase	19.78±0.73[a]	5.00±0.22[a]	4.89±0.20[a]	6.27±0.35[a]
	0.8% α-amylase + 0.2 glucoamylase	27.61±0.29[b]	5.21±0.17[ab]	5.06±0.19[b]	5.67±0.15[a]
	0.5% α-amylase + 0.5% glucoamylase	28.62±1.52[b]	4.00±0.34[abc]	5.90±0.02[c]	5.40±0.26[b]
	0.2% α-amylase + 0.8% glucoamylase	27.61±0.95[b]	4.61±0.43[abcd]	6.05±0.03[c]	5.77±0.21[b]
	1% glucoamylase	28.03±0.76[b]	3.76±1.25[ace]	6.11±0.01[c]	5.37±0.25[b]
	Control	29.46±0.96[b]	4.47±0.13[abcde]	5.43±0.01[b]	4.17±0.15[c]
4	1% α-amylase	19.36±0.39[a]	5.27±0.20[a]	4.81±0.54[a]	5.57±0.21[a]
	0.8% α-amylase + 0.2 glucoamylase	26.43±1.02[b]	6.00±0.15[ab]	5.75±0.22[b]	5.55±0.07[a]
	0.5% α-amylase + 0.5% glucoamylase	27.44±0.39[b]	4.48±1.19[abc]	5.98±0.04[c]	5.77±0.29[b]
	0.2% α-amylase + 0.8% glucoamylase	27.19±0.77[b]	5.52±1.99[abcd]	6.10±0.03[c]	6.13±0.06[b]
	1% glucoamylase	28.37±2.43[b]	3.88±0.50[ace]	6.13±0.01[c]	5.50±0.26[b]
	Control	29.38±0.39[b]	4.07±0.30[abcde]	5.46±0.08[b]	4.27±0.12[c]

Tukey HSD test compares the samples to one another
Values are mean ± standard deviation
Values with different superscripts on the same column at the same time are significantly different at 95% confidence interval

Appendix 6: Effect of time on starch, glucose, pH and °Brix

Enzymes	Time	Starch	Glucose	pH	°Brix
Control	0	33.08±3.82[a]	3.66±0.16[a]	5.48±0.02[a]	3.17±0.06[a]
	1	29.97±1.14[b]	4.41±0.26[b]	5.50±0.12[b]	3.90±0.26[b]
	2	29.04±2.02[c]	3.80±0.29[b]	5.49±0.04[c]	3.87±0.12[bc]
	3	29.46±0.96[c,d]	4.47±0.13[bc]	5.43±0.01[c]	4.17±0.15[d]
	4	29.38±0.39[c,d]	4.07±0.30[c]	5.46±0.08[bc]	4.27±0.12[d]
1% α-amylase + 0% glucoamylase	0	33.08±3.82[a]	3.66±0.16[a]	4.90±0.07[a]	4.27±0.21[a]
	1	29.55±4.01[b]	4.66±0.23[b]	5.51±0.21[b]	5.55±0.07[b]
	2	20.62±0.53[c]	4.86±0.63[b]	5.25±0.48[c]	5.73±0.21[bc]
	3	19.78±0.73[c,d]	5.00±0.22[bc]	4.89±0.20[c]	6.27±0.35[d]
	4	19.36±0.39[c,d]	5.27±0.20[c]	4.81±0.54[bc]	5.57±0.21[d]
0.8% α-amylase +	0	33.08±3.82[a]	3.66±0.16[a]	4.90±0.07[a]	4.27±0.21[a]

0.2% glucoamylase	1	30.81±1.10^{b}	4.85±0.06^{b}	5.82±0.08^{b}	5.67±0.15^{b}
	2	30.05±0.25^{c}	5.46±0.06^{b}	5.29±0.22^{c}	5.50±0.35^{bc}
	3	27.61±0.29^{c,d}	5.21±0.17^{bc}	5.06±0.19^{c}	5.67±0.15^{d}
	4	26.43±1.02^{c,d}	6.00±0.15^{c}	5.75±0.22^{bc}	5.55±0.07^{d}
0.5% α-amylase + 0.5% glucoamylase	0	33.08±3.82^{a}	3.66±0.16^{a}	5.48±0.02^{a}	3.17±0.06^{a}
	1	29.13±0.39^{b}	3.62±0.07^{b}	5.92±0.08^{b}	4.80±0.26^{b}
	2	27.69±0.63^{c}	3.77±0.08^{b}	5.94±0.06^{c}	5.20±0.17^{bc}
	3	28.62±1.52^{c,d}	4.00±0.34^{bc}	5.90±0.02^{c}	5.40±0.26^{d}
	4	27.44±0.39^{c,d}	4.48±1.19^{c}	5.98±0.04^{bc}	5.77±0.29^{d}
0.2% α-amylase + 0.8% glucoamylase	0	33.08±3.82^{a}	3.66±0.16^{a}	5.48±0.02^{a}	3.17±0.06^{a}
	1	27.86±0.64^{b}	3.72±0.40^{b}	6.04±0.06^{b}	4.77±0.25^{b}
	2	28.92±3.39^{c}	4.05±0.40^{b}	6.00±0.01^{c}	4.90±0.17^{bc}
	3	27.61±0.95^{c,d}	4.61±0.43^{bc}	6.05±0.03^{c}	5.77±0.21^{d}
	4	27.19±0.77^{c,d}	5.52±1.99^{c}	6.10±0.03^{bc}	6.13±0.06^{d}
0% α-amylase + 1% glucoamylase	0	33.08±3.82^{a}	3.66±0.16^{a}	5.48±0.02^{a}	3.17±0.06^{a}
	1	28.92±0.90^{b}	3.23±0.06^{b}	6.13±0.04^{b}	4.77±0.15^{b}
	2	28.71±0.89^{c}	3.59±0.05^{b}	6.10±0.02^{c}	5.03±0.06^{bc}
	3	28.03±0.76^{c,d}	3.76±1.25^{bc}	6.11±0.01^{c}	5.37±0.25^{d}
	4	28.37±2.43^{c,d}	3.88± 0.50^{c}	6.13±0.01^{bc}	5.50±0.26^{d}

Tukey HSD test compares the samples to one another
Values are mean ± standard deviation
Values with different superscripts on the same column at the same enzymatic composition are significantly different at 95% confidence interval

Appendix 7: Tukey HSD results for the Quantitative Descriptive Test

	Raw	0.8% α-amylase + 0.2% glucoamylase	1% α-amylase
Colour cream	1.47±1.18^{a}	4.17±0.94^{b}	4.47±1.36^{b}
Sweetness	2.34±1.33^{a}	3.1±1.00^{a}	2.54±1.28^{a}
Flavour	3.25±1.33^{a}	4.05±1.11^{a}	3.93±1.13^{a}
Chalkiness	2.35±1.51^{a}	2.33±1.72^{a}	2.66±1.83^{a}

Tukey HSD test compares the samples to one another

Values are mean ± standard deviation
Values with different superscripts on the same column at the same enzymatic composition are significantly different at 95% confidence interval

0=light/not 15=dark/highly/strongly

Appendix 8: Tukey HSD results for the affective test

	Raw	0.8% α-amylase + 0.2% glucoamylase	1% α-amylase
Colour	7.28±1.40[a]	5.66±2.41[b]	5.32±2.12[b]
Taste	6.08±1.96[a]	5.14±2.45[a]	4.36±2.50[b]
Mouthfeel	6.26±2.03[a]	5.56±2.31[a]	4.72±2.34[b]
Overall	6.44±1.90[a]	5.34±2.37[b]	4.76±2.50[b]

Tukey HSD test compares the samples to one another
Values are mean ± standard deviation
Values with different superscripts on the same column at the same enzymatic composition are significantly different at 95% confidence interval

1=dislike extremely 5=neither like nor dislike 9=like extremely

Appendix 9: Potential of hydrogen of tigernut milk throughout hydrolysis

	1% αamylase	0.8% αamylase+0.2% glucoamylase	Control	1% glucoamylase	0.2% αamylase+ 0.8% glucoamylase	0.5% α-amylase + 0.5%glucoamylase
0 hour	4.9±0.07	4.9±0.07	5.48±0.02	5.48±0.02	5.48±0.02	5.48±0.02
1 hour	5.51±0.21	5.82±0.08	5.50±0.12	6.13±0.04	6.04±0.06	5.92±0.08
2 hours	5.25±0.48	5.29±0.22	5.49±0.04	6.10±0.02	6.00±0.01	5.94±0.06
3 hours	4.89±0.20	5.06±0.19	5.43±0.01	6.11±0.01	6.05±0.03	5.90±0.02
4 hours	4.81±0.54	5.75±0.22	5.46±0.08	6.13±0.01	6.10±0.03	5.98±0.04

Values are mean ± standard deviation

Appendix 10: Results of three tigernut milk subjected to a QDA

	Raw	0.8% α-amylase+0.2% glucoamylase	1% α-amylase
Colour cream	1.47±1.18[a]	4.17±0.94[b]	4.47±1.36[c]
Sweetness	2.34±1.33[a]	3.1±1.00[a]	2.54±1.28[a]
Flavour	3.25±1.33[a]	4.05±1.11[a]	3.93±1.13[a]
Chalkiness	2.35±1.51[a]	2.33±1.72[a]	2.66±1.83[a]

Dunnett t-tests treat ―raw‖ as a control, and compare all other groups against it.
Values are mean ± standard deviation
Values with different superscripts on the same row are significantly different at 95% confidence interval

0=light/not 15=dark/highly/strongly

Appendix 11: Dunnett's t-test results for the affective test

	Raw	0.8% αamylase+0.2% glucoamylase	1% α-amylase
Colour	7.28±1.40[a]	5.66±2.41[b]	5.32±2.12[c]
Taste	6.08±1.96[a]	5.14±2.45[a]	4.36±2.50[b]
Mouthfeel	6.26±2.03[a]	5.56±2.31[a]	4.72±2.34[b]

Overall	6.44±1.90[a]	5.34±2.37[b]	4.76±2.50[c]

Dunnett t-tests treat ―rawǁ as a control, and compare all other groups against it.
Values are mean ± standard deviation
Values with different superscripts on the same row are significantly different at 95% confidence interval

1=dislike extremely 5=neither like nor dislike 9=like extremely

Appendix 12: Properties and description of α-amylase from *Aspergillus orzae*

Synonym	1,4-α-D-Glucan-glucanohydrolase
Form	Powder
Specific activity	~30 U/mg
Product code	10065
Storage temperature	2-8°C
Unit (U) definition	1 U corresponds to the amount of enzyme which liberates 1 µmol maltose per minute at pH 6.0 and 25°C

Source: https://www.sigmaaldrich.com/catalog/product/sigma/10065?lang=en&region=GH

Appendix 13: Properties and description of amyloglucosidase from *Aspergillus niger*

Synonym	Exo-1,4-α-glucosidase ; Glucoamylase
Form	Powder
Specific activity	~70 U/mg
Optimum temperature	50°C
Product code	10115
Storage temperature	2-8°C
Unit (U) definition	One unit corresponds to the amount of enzyme which liberates 1 µmole of glucose per minute at pH 4.8 and 60 °C

Source: https://www.sigmaaldrich.com/catalog/product/sigma/10115?lang=en&region=GH

Appendix 14: Quantity of tigernut milk obtained per 100g of dried tigernut

Dried tigernut (g)	Tigernut milk (ml)
107	580
100.2	600
103	603