STUDIES ON THE EFFICIENT UTILIZATION OF TIGERNUT (*CYPERUS ESCULENTUS*) AS A SOURCE OF VEGETABLE OIL, ETHANOL AND ANIMAL FEED

BY

ABLAKU, BARNABAS ENGA
B.Sc. (HONS) PURE CHEMISTRY, (KEFFI), 2010
NSU/NAS/M.Sc./039/11/12

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE IN MASTER OF SCIENCE (M.Sc.) INDUSTRIAL CHEMISTRY OF THE SCHOOL OF POSTGRADUATE STUDIES, NASARAWA STATE UNIVERSITY, KEFFI.

NOVEMBER, 2016.

DECLARATION

I hereby declare that this dissertation has been written by me and it is a report of my research work. It has not been presented in any previous application for M.Sc. Industrial Chemistry. All quotations are indicated and sources of information specially acknowledged by means of references.

ABLAKU, BARNABAS ENGA

CERTIFICATION

The dissertation (Studies on the Efficient Utilization of Tigernut [*Cyperus esculentus*] As a Source of Vegetable Oil, Ethanol and Animal Feed) meets the regulations governing the award of M.Sc. Industrial Chemistry, Faculty of Natural and Applied Sciences of Nasarawa State University, Keffi.

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DEDICATION

I dedicate this work to God Almighty who gave me the inspiration, knowledge and understanding to do this work to the end. May He alone take all the Glory.

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ABSTRACT

Fresh tigernut tubers (yellow variety) were screened, washed, dried, ground into coarse powder and the powder extracted with hexane. Similarly, fresh tigernut tubers were sprouted, screened, washed, dried, ground into coarse powder and extracted with hexane. Hexane extract was filtered and solvent removed under reduced pressure affording tigernut oil in 32.63±1.31 and 36.67±0.63% yield, for un-sprouted and sprouted samples respectively. Results of physicochemical analyses of the oil confirmed that tigernut tuber is a good source of edible (vegetable) oil. Following oil extraction as above, the residue obtained was saccharified and then fermented. Three saccharification methods were investigated: enzyme, acid and enzyme/acid, respectively. The yield of ethanol produced from un-sprouted tigernut residue after oil extraction is as follows: 112±0.95 (no saccharification), 128±1.08 (acid), 176±1.26 (enzyme) and 252±1.00 (enzyme/acid) cm^3 per Kg dry matter. The yield of ethanol produced from sprouted tigernut residue, after oil extraction, is as follows: 148±1.39 (acid), 184±1.25 (enzyme) and 284±1.12 (enzyme/acid) cm^3 per Kg dry matter respectively. Direct fermentation of sprouted tigernut without saccharification yielded 128±1.21 cm^3 per Kg dry matter. The results show that sprouting followed by enzyme/acid saccharification gave the highest yield of ethyl alcohol. The fermentation residues were analyzed for nutrients composition. There is increase in crude protein (18%), crude fibre (17%) and ash (39%), between un-sprouted tigernut flour and fermentation residue. Crude fat and carbohydrate contents of fermentation residues decreased by 28% and 42% respectively, from the un-sprouted tigernut flour. The elements copper, phosphorus, potassium, magnesium, zinc and iron showed increase whereas manganese, calcium and sodium showed decrease. The amino acids lysine, histidine, arginine, aspartic acid, glutamic acid, proline, glycine, alanine, cystine,

valine, isoleucine, leucine tyrosine, threonine, serine and phenylalanine increased on per cent dry matter bases.

CHAPTER ONE

INTRODUCTION

1.1. Background of the Study

Cyperus esculentus (tigernut) is one of the oldest root crops in use before modern civilization. It was discovered about 2000BC (Okafor *et al.*, 2003). Tigernut has other names such as yellow nut sedge, chufa, flat sedge, rushnut, water grass, earth almond, northern nut grass and nut grass. In Nigeria, it is known by names such as "Ofio" in Yoruba, "Aya" in Hausa and "Aki Hausa" in Igbo. The plant is cultivated mainly in the Northern regions of Nigeria. It is also cultivated in few areas of North-Central region of Nigeria, most especially Southern Kaduna and Niger States. There are three varieties of tigernut: black, brown and yellow (Okafor *et al.*, 2003; Umerie & Enebeli, 1997). Among these three varieties, only yellow and brown varieties are cultivated in Nigeria. The yellow variety is most preferred to all other varieties for the reasons like tuber size, attractive colour and fleshier look. Tigernut tuber can be eaten un-sprouted, roasted, dried, baked or made into a refreshing beverage called *kunun aya* (Belewu & Abodurin, 2006; Oladele & Aina, 2007).

Tigernut is cultivated and used in other parts of the world. In Spain, tigernut tubers are used to make a milk-like drink called "*horchata de chufa*." This drink is also called *orgeat* in English-speaking countries. The drink is a non-alcoholic refreshing drink that is mostly consumed in summer (Mosquera *et al*., 1996). The beverage was first made by Muslims during the annual fasting (Ramadan), but it is now widespread in Spain and several other European and Latin-American countries (Cortés *et al*., 2005). In Spain, the "*horchata*" industry is of economic importance (Rubert *et al*., 2011). The acceptance of "*horchata*" has recently been extended to other countries (United

Kingdom, France, Portugal, Argentina, and United States of America) (Pascual *et al*., 2000; Rubert *et al*., 2011).

In Nigeria, tigernut is not cultivated in large amount. Farmers engage themselves in farming little plots of land around their homes to control erosion and to keep away reptiles. Other common practice among the northerners in Nigeria is the use of tigernut as purgative (if taken in large amount). Beverages from tigernut are served as weaning food. The residue after preparation of the beverage drink is usually dried and used as animal feed or fuel for starting fire locally. There is no report so far where tigernut oil was used as vegetable oil in Nigeria. The major reason for this limitation is lack of local extraction technique. Tigernut oil has been extracted in little amounts from to time for research purposes only.

In spite of the availability of tigernut, it has little commercial and economic values at present. The tubers are normally eaten raw, to suck the juice and sugar present only. The chaff or residue after chewing is spat out. This becomes waste.

1.2. Statement of the Problem

Tigernut is a root crop rich in fat, glucose, sucrose and starch. Physicochemical properties of the oil were determined and reported by different groups (Adejuyitan *et al*., 2009; Muhammad *et al*., 2011; Barninas *et al*., 2001; Zhang *et al*., 1996). The quality index evaluation and composition of fatty acids present in tigernut oil are dominated by oleic acid. These show that, tigernut oil can be used as vegetable oil (Sidohoundé *et al*., 2014). Tigernut oil has been transesterified, and preliminary studies into biofuel characteristics of the oil have also been undertaken and reported. It was found that tigernut oil could be used as a source of biofuel (Adejuyitan *et al*., 2009; Muhammad *et al*., 2011; Barninas *et al*., 2001; Zhang *et al*., 1996).

Non-alcoholic fermentation of un-sprouted tigernut tuber for the production of local beverage drink "*kunun aya*" was explored. It was discovered that the beverage drink is very rich in nutrients (protein, fat, fibre, mineral etc.) for both children and adults. The high lauric acid content of tigernut makes it an added advantage to the user of the product (Belewu & Abodunrin, 2006). Alcoholic fermentation of tigernut tuber has not been reported. Since tigernut has been reported to be very rich in glucose, sucrose and starch, it can be fermented to produce the chemical ethanol.

Utilization of tigernut in animal feed has been investigated. It was found that; tigernut is deficient in essential amino acid and hence, it cannot be consumed as animal feed (Bamgbose *et al*., 2003; Agbabiaka *et al*., 2012). Without supplementation, there is no information on any investigation where tigernut is first fermented to produce ethanol, then using the residue from fermentation for animal feed.

Macro mineral, amino acid, protein, fiber, fat and carbohydrate (glucose, sucrose, starch) contents of un-sprouted tigernut tuber have been investigated (Ekeanyanwu & Ononogbu, 2010; Okafor *et al*., 2003; Shaker *et al*., 2009; Oladele & Aina, 2007; Emmanuel- Ikpeme *et al*., 2012).

To the best of our knowledge, research carried out so far on tigernut has focused, in each case, one aspect at a time. For example, when the oil has been of interest, the residue arising from oil extraction is simply discarded, thereby ignoring the potential use of the residue. Therefore, a detailed investigation of the crop is warranted where tigernut oil is recovered, the residue after oil extraction is fermented to yield ethanol and the fermentation residue processed into animal feed. This would engender full value utilization of the crop where no part is wasted.

1.3. Aim and Objectives of the Study

The aim of this study is to investigate processing of the same sample of tigernut tuber flour to obtain vegetable oil, Ethanol and fermentation residue that is processed into animal feed.

The objectives of the study are to:

a) analyse tigernut for its nutritional, mineral and amino acid content.
b) extract tigernut oil by solvent extraction using hexane and carryout physicochemical characterization of the tigernut oil obtained.
c) saccharify the residue obtained after solvent extraction under acid, enzyme and enzyme/acid conditions.
d) ferment the products of saccharification and to obtain ethanol by distillation, and
e) analyse the fermentation residue for its nutritional, mineral and amino acid content.

1.4. Justification of the Study

Cyperus esculentus is a crop that is readily available in Nigeria but has remained underutilized. At this time, it is only used for production of a local beverage drink, *kunun aya* or it is simply eaten raw for its sweet taste. Finding additional uses for the crop is recommended especially in industry where it can be processed into materials for industrial use. Tigernut is not a primary food source in Nigeria. Therefore, channelling the crop into production of vegetable oil, ethanol or animal feed would not upset food supply, rather, it would provide an additional source of revenue for the farmers.

1.5. Scope of the Study

The study is limited to:

a) solvent extraction of oil from un-sprouted and sprouted tigernut tubers
b) physicochemical characterization of the extracted oil
c) saccharification of the residue after oil extraction. Saccharification under acid, enzyme and enzyme/acid conditions.
d) fermentation of the saccharified slurry from (c) above.
e) recovery of ethanol from (d) above.
f) determination of nutritional, mineral, amino acid and anti- nutritional composition of the fermentation residue from (d).

1.6. Definition of Terms

Crude Fermentation: this refers to the fermentation of tigernut sample without saccharification.

Processed Tigernut: this refers to tigernut flour/residue after sprouting, saccharification using different techniques or fermentation.

Sprouted Tigernut: this refers to fresh tigernut tuber (yellow variety), that was sprouted, screened, washed, dried and blended into coarse powder.

Un-sprouted Tigernut: this refers to fresh tigernut tuber (yellow variety), that was screened, washed, dried and blended into coarse powder.

CHAPTER TWO

LITERATURE REVIEW

2.1. Tigernut Oil

The tuber of tigernut is high in oil content ranging from 16.0±1.36 to 35.4±2.11 % (Emmanuel-Ikpeme *et al.*, 2012; Adejuyitan *et al.*, 2009). The oil was found to be lauric acid graded oil which is non-acidic, stable and non-drying. The oil was found to be resistant to peroxidation (Zhang *et al.*, 1996; Barninas, 2001).

Major fatty acids present in tigernut oil are myristoleic acid (0.2%), stearic acid (3.2%), arachidiic acid (0.4%), palmitoleic acid (0.3%), oleic/elaidic acid (72.6%), linoleic/linoelaidic acid (8.9%), and α-linolenic acid (0.4%). Tigernut oil has a monounsaturated profile [>60% monounsaturated fatty acids (MUFA)]. Its fatty acid (FA) profile is similar to that of olive, hazelnut, macadamia nut, avocado, and apricot kernel oils (Dubois *et al.*, 2007). Vegetable oils are mainly triacylglycerols, made by esterification of three FAs on the glycerol skeleton. The three positions are not equivalent when it comes to bioavailability and human health. Indeed, the 2-position is conserved during the entire digestive process, which explains why in natural fats and oils the most physiologically important FAs are esterified to the 2-position (Hunter 2001). Linssen *et al.* (1988) have also analysed and compared the fatty acid and triglyceride compositions of tigernut and olive oils. The fatty acid compositions of both oils showed a great similarity despite the oils having different origins; tigernut is a tuber and olive is a fruit. They also studied the fatty acid composition of tigernut oil and olive oil with regard to the 2-position of the triglyceride and observed slight differences. The palmitic and linoleic acid content was slightly higher in tigernut oil than in olive oil and there were also small differences in the oleic and linoleic acid

contents of the oils. Although there were some differences in the fatty acid composition in the 2-position, the two oils show great similarity. These results indicated that the total positional fatty acid distribution in the triglyceride might be similar. Although the fatty acid composition and distribution of the oils are nearly the same, the oils are different in colour and taste, caused by accompanying components. Olive oil has a green colour while tigernut oil has a golden yellow colour. The taste of olive oil is very characteristic, but the taste of tigernut oil is neutral. Tigernut oil could be used in the same manner as olive oil.

Yeboah *et al.* (2011) have studied the phytosterols and the vitamin E compounds (tocopherols and tocotrienol) present in tigernut oil. The total vitamin E content was 120.1 μg/g of oil and the phytosterol content in tigernut was dominated by β-sitosterol at 50.37%, which translated into 517.25 μg/g of oil. The other common vegetable oil phytosterols like stigmasterol (20.62%, 225.25 μg/g of oil) and campesterol (15.33%, 161.35 μg/g of oil) were found to be present in larger amounts. Other minor phytosterols in tigernut oil were δ5-avenasterol (3.75%, 37.57 μg/g of oil) and δ7-avenasterol (1.73%, 17.04 μg/g of oil), which together made a total phytosterol content of 986.49 μg/g of tigernut oil. This moderately high content of phytosterols further enriches the quality and value of tigernut oil as a food source. In fact, tigernut oil is much richer in phytosterols than olive oil (100 μg/g of oil), and hence it is the phytosterol profile of tigernut oil that truly distinguishes it from olive oil.

Some characteristic functions of tigernut oil include reducing of the levels of triglycerides in blood (Temple *et al*., 1989). It produces dilatation in veins, preventing arteriosclerosis (Ros, 2010). It causes positive effects on digestive secretions (gastric, pancreatic and biliar), due to high content of oleic acid, the most powerful stimulator

of production of Colecistokine (responsible for digestive secretions) (Yeboah *et al*., 2011). It also causes the stimulation of the absorption of calcium in bones and the production of new bony material, due to short and medium chain fatty acids, oleic acid and essential fatty acids (Christian, 2006). It is very effective against skin alterations and aging process due to its elevated contents of Vitamin E, which activates microcirculation of skin (Kim *et al*., 2007). It is highly recommended for infants and the elderly because of its content of Vitamin E and antioxidant effect in cell membrane (Linssen *et al*., 1989). It inhibits oxidation of lipoproteins, also called transporters of cholesterol in blood (related to the development of cardiovascular illness), and other substances (related to the development of some kinds of cancer) (Onwuka, 2005).

2.2. Fermentation of Tigernut Seed Flour

Non-alcoholic fermentation (*kunu*) of tigernut has been reported by different groups. It was discovered that the fermented product (*kunu*) is very rich in simple sugar. The energy content of the fermented product residue is also very high (Belewu & Belewu, 2007). The residue after fermentation was found to be rich in amino acid and crude fiber. To the best of our knowledge, there is no literature report on fermentation of tigernut to produce ethanol.

2.3. Nutritional Food Value of Tigernut

Tigernut is rich in nutrient content (starch, fat, sugars and protein), mineral (phosphorus, potassium) with vitamins E and C (Belewu & Belewu, 2007). Tigernut was reported to be high in dietary fibre content, which could be effective in the treatment/prevention of gastrointestinal diseases. Tigernut flour is also rich in protein (7%) (Temple *et al.,* 1990). The protein in tigernut is of high biological value considering the many essential amino acids it contains. These amino acids are higher

than those proposed by Food and Agriculture Organization (2002). The amino acid content of raw tigernut meets up with the daily recommended intake for adults. Carbohydrates such as reducing sugar (7.4%), soluble polysaccharide (7.4%) and starch (86.4%) were also reported in some study (Temple *et al.,* 1990).

2.4. Animal Feed from Tigernut Seed Flour

Due to inconsistent pricing system on cereals and skyrocketing price on the available ones on demand in Nigeria, researchers are looking into substitutes for some of these cereals in animal diet. Some of these cereals include maize, wheat, sorghum and millet. Utilization of fermentation residues has found a place in animal nutrition. It not only reduces the cost of feeding but also creates an outlet for disposing this material. Thus, brewery spent grains have been utilized as feed for animals for many years (Szponar *et al*., 2003).

To the best of our knowledge, there has not been any report as at the time of this study, where oil was extracted from tigernut, the residue after oil extraction used for fermentation and then residue after fermentation used for animal feed. Agbabiaka *et al*. (2012) investigated the use of un-sprouted tigernut tuber (without oil extraction and fermentation) as animal feed. They discovered that tigernut is low in some essential nutrients especially amino acids, and therefore do not meet the recommended daily intake for animal feed. Similarly, a quantity (33.33%) of tigernut was included in the diet of cockerel starters in another investigation by Ade-Omowaye *et al.* (2008). They recorded an extra weight gain in the chickens. The use of tigernut tuber as animal feed has also been reported by Fathia *et al*. (2013). It was found that goats feed with tigernut tubers have quality milk and labneh prepared from the goat's milk.

Lignocellulosic substrates, being cheap and readily available from brewery spent grains, have recently gained considerable interest, because of their possible use in secondary fermentation.

2.5. Mineral Rich Feed as Source of Nutrients

Mineral concentrates are sources of nutrients that should be taken into account when formulating diets. These include the major minerals, Ca, P, Na, K, Cl, vitamins, including B_{12}, choline and vitamin D and essential fatty acids. Considerations are given to these nutrients because they are beneficial and in some cases high composition can be detrimental and limit the inclusion level or even growth.

Mineral compositions of fermented residue from tigernut have not been reported. Raw tigernut was found to contain low composition of micro elements such as Fe, Zn, Cu and Mg. whereas, high composition of macro elements were observed from un-sprouted tigernut tubers (Ekeanyanwu and Ononogbu, 2010; Adejuyitan, 2011). It is generally observed that fermentation residue is usually abundant in mineral composition.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Materials

3.1.1. Sample Collection

Fresh tigernut tubers (yellow variety) were used for this study. The fresh tubers were obtained from Keffi Main Market in Keffi, Nasarawa State, Nigeria.

3.1.2. Reagents

a) *Saccharomyces cerevisiae* (5g per 50cm^3 of water).

b) Amylase Enzyme (1g per 100cm^3).

c) Fermax yeast nutrient (1g per 100cm^3).

d) Citric acid.

e) Potassium metabisulphite (1 tablet per litre).

f) Normal laboratory bench reagents.

3.1.3. Apparatus/equipments

a) Simple distillation setup with condenser.

b) Fermentation flask (anaerobic flask).

c) Water bath.

d) Rotary evaporator.

e) Atomic Absorption Spectrophotometer (AAS).

f) Flame Photometer.

g) Technicon Sequential Multisample Amino Acid Analyzer (TSM).

h) Oven.

i) Muffle furnace.

j) Soxhlet extractor.

k) pH meter.

3.2. Sample Preparation

3.2.1. Preparation of Un-sprouted Tigernut Flour

Process preparation of un-sprouted tigernut is as outlined in Figure 3.1. Fresh tubers of tigernut were thoroughly screened to remove bad tubers, stones and debris. Fresh tigernut tubers (1kg) were shade dried. The "dry" tubers were ground with a laboratory electric blender to a coarse powder and stored in amber bottles for subsequent use.

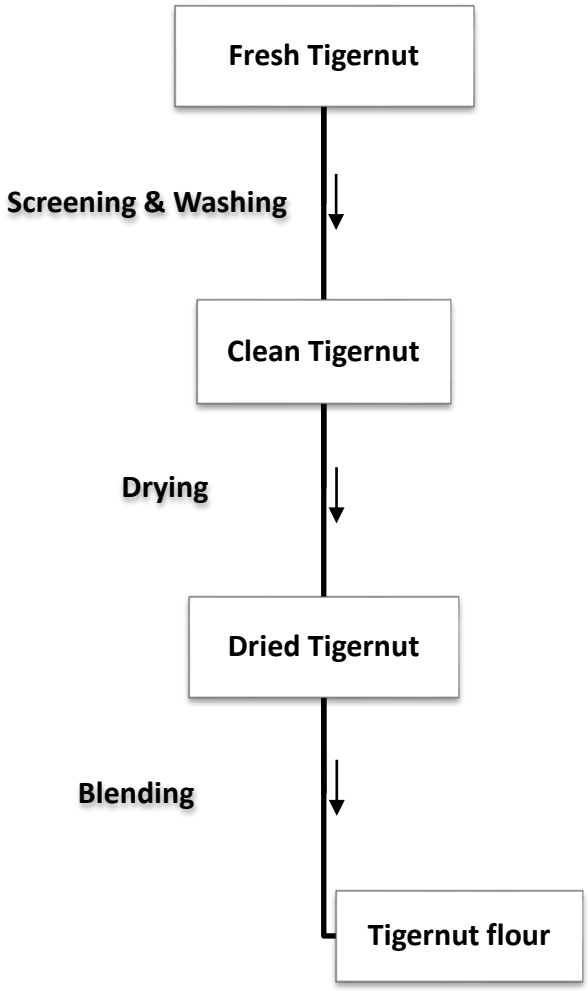


Figure 3.1: Preparation of un-sprouted tigernut flour

3.2.2. Preparation of Sprouted Tigernut Flour

Process preparation of un-sprouted tigernut is as outlined in Figure 3.2. Fresh tigernut tubers were thoroughly screened to remove bad tubers, stones and debris. Fresh tigernut tubers (1kg) were sprouted at room temperature (being the optimum temperature for sprouting tubers) for 7 days followed by shade drying. The dry sample was ground with laboratory electric blender to a coarse powder and stored in amber bottles for subsequent use.

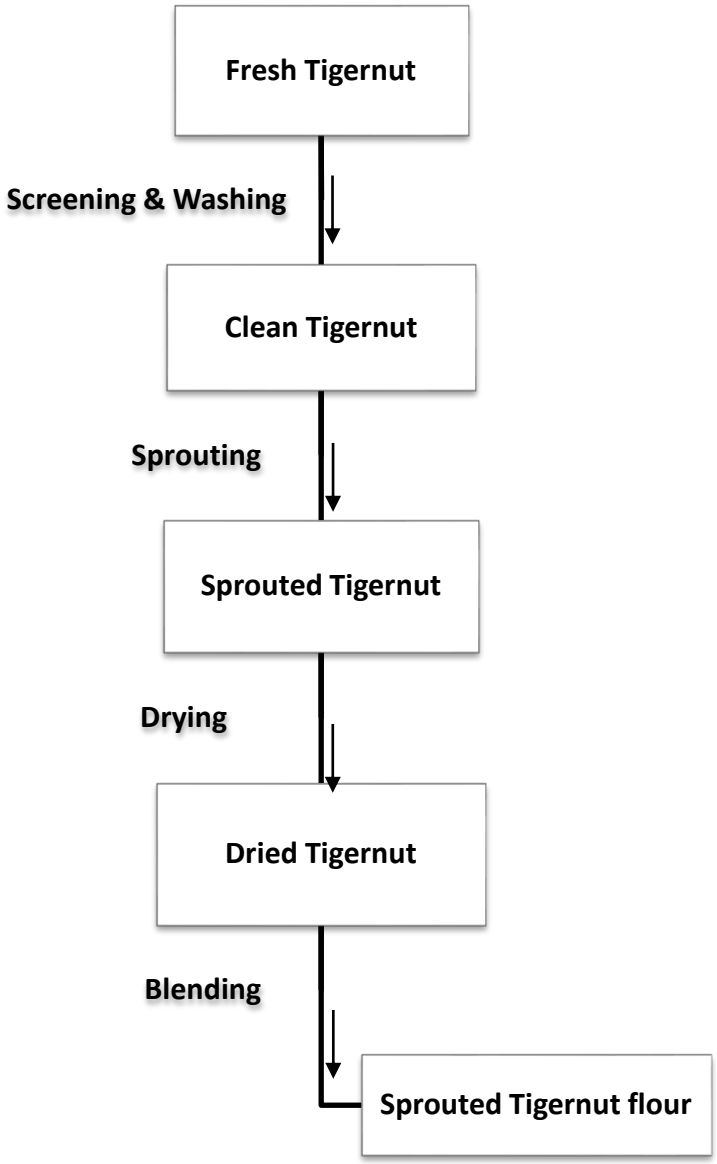


Figure 3.2: Preparation of sprouted tigernut flour

3.3. Oil Extraction

Tigernut oil was extracted with n-hexane according to methods described in Association of Official Analytical Chemists (AOAC, 1995).

3.4. Physicochemical Properties of Tigernut Oil

Colour, refractive index, specific gravity, acid value, saponification value, iodine value, peroxide value, free fatty acids, ester value, moisture content were determined according to method of analysis described in AOAC (1995).

3.4.1. Determination of Saponification Value

Tigernut oil (2.0g) was added to 20 cm^3 of 0.2M ethanolic (95%) potassium hydroxide in 500 cm^3 round bottom flask. The flask with its content was refluxed for 30 minutes on a water bath. Few drops of phenolphthalein indicator were added and the hot solution was allowed to cool. Excess KOH in the solution was titrated against 0.5M HCl. A blank titration was carried out using the same procedure (Pearson, 1976; IUPAC, 1979; Ogungbenle, 2003). Saponification value was calculated using the relationship in equation 3.1.

$$\text{Saponification value} = \frac{56.1\text{M}\ (\text{V}_2 - \text{V}_1)}{\text{Weight of sample oil}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.1$$

Where M = Molarity of hydrochloric acid. V_1 = volume of HCl used in the titration. V_2 = volume of HCl used in the blank.

3.4.2. Determination of Peroxide Value

The oil sample (2.0g) was weighed into 200 cm^3 conical flask containing 20cm^3 of petroleum ether and allowed to boil for 30 seconds in a water bath. Aqueous potassium iodide solution (50%, 25cm^3) and distilled water (25cm^3) were added. The resulting

mixture was titrated with sodium thiosulphate solution (0.002M). During the titration a milky white precipitate was observed and the total disappearance of the precipitate indicated the end-point of the titration. The peroxide value of the sample oil was calculated using equation 3.2. The same procedure was repeated for the blank (Ogungbenle, 2014).

$$\text{Peroxide value} = \frac{100(T_B - T_S)M}{\text{Weight of sample oil}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.2$$

Where N = molarity of thiosulphate. T_S = volume of thosulphate used in the sample test. T_B = volume of thiosulphate used in the blank.

3.4.3. Determination of Acid Value

The sample oil (10g) was weighed into a 250 cm^3 conical flask. Ethanol (50 cm^3) was measured into the flask. The content of the flask was boiled on a water bath for 5 minutes. Few drops of phenolphthalein indicator were added to the content in the flask. The mixture was then titrated with potassium hydroxide (0.1M) until a pink colour was observed, indicating the end-point (Ogungbenle, 2014). The acid value of the sample oil was calculated using equation 3.3.

$$\text{Acid value} = \frac{56.1 \text{ x } M \text{ x } (T_S - T_B)}{\text{Weight of sample oil}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.3$$

Where; M = molarity of potassium hydroxide. T_S = Titre value of the sample. T_B = Titre value of the blank. 56.1= molecular weight of KOH.

3.4.4. Determination of Iodine Value

The oil sample (2.0g) was transferred into a flask containing carbon tetrachloride (10 cm^3). Wijs' solution (25cm^3) was pipetted into the flask containing the sample (Wijs' solution consists of iodine monochloride in glacial acetic acid). The blank was

prepared. The mixture was stored in a cupboard (dark place) for 30 minutes at room temperature. After that, 15 cm^3 potassium iodide solution (0.1 M) was added along with fresh distilled water (100 cm^3). The resulting mixture was titrated with sodium thiosulphate solution (0.1 M) using starch indicator (2 cm^3). The titration was continued until the blue colour just disappeared after thorough shaking with the stopper on, indicating the end point (Pearson, 1976; AOAC, 2000; Ogungbenle, 2014). The iodine value was calculated using equation 3.4.

$$\text{Iodine value} = \frac{12.692\ (T_B - T_S)\ M}{\text{Weight of sample oil}} \ldots\ldots\ldots\ldots\ldots 3.4$$

Where M = molarity of sodium thiosulphate solution. T_S = Titre value of the sample. T_B = Titre value of the blank.

3.4.5. Determination of Specific Gravity

The tigernut oil sample (40 cm^3) was homogenized and poured into a measuring cylinder (500 cm^3) gently to avoid air bubbles. The temperature was controlled to avoid drifting in the temperature value. Hydrometer was dipped into the oil carefully to avoid resting on the wall of the cylinder and the reading was then taken in at 25^oC (g/cm^3) (Ogungbenle, 2014).

3.4.6. Determination of Refractive Index

The oil was dried to make it free of moisture. Two drops of the oil were put on the lower prism of the equipment and the prism was closed up. The water was passed through the jacket at about 45℃, the jacket was adjusted until the equipment read temperature of 40℃. The light was adjusted and the compensator was moved until a dark border line was observed on the cross wire. The reading on the equipment was recorded (Ogungbenle, 2014).

3.5. Saccharification

3.5.1. Acid Saccharification

The sample (30g) was slurried with 150 cm^3 of distilled water in a 250 cm^3 Erlenmeyer flask. The slurry was treated with citric acid solution (0.2 $mol.dm^{-3}$) to adjust the pH to 6.5. The must was autoclaved at 121^oC for 30 minutes. The acid hydrolysed slurry was stirred at constant rate for about 1h and allowed to cool to room temperature. The slurry was ready for fermentation after 24h.

3.5.2. Enzyme Saccharification

The sample (30g) was slurried with 150 cm^3 of distilled water in an Erlenmeyer flask. The slurry was treated with potassium metabisulphite (chemical sterilization). The slurry was saccharified using amyloglucosidase at a composition of 0.5% V/V and allowed to stand. The slurry was ready for fermentation after 24h.

3.5.3. Enzyme/Acid Saccharification

The sample (30g) was slurried with 150 cm^3 of distilled water and treated with amyloglucosidase at a composition of 0.5% V/V and allowed to stand for 24h. After 24h, the must was treated with 0.2 $mol.dm^{-3}$ of citric acid and autoclaved at 121^oC for 30min. The hydrolysed sample was stirred at constant rate for about 1h. After liquefaction, the pH of the hydrolysed slurry was adjusted to 6.5 using 10% ammonium hydroxide (NH_4OH) or citric acid. The slurry was ready for fermentation after 24h.

3.6. Fermentation

The sample (30g) was slurried with 150 cm^3 of distilled water. The slurry was saccharified using different approaches, that is, acid saccharification, enzyme

saccharification and acid/enzyme saccharification. 10 cm^3 starter broth solution containing 0.03% v/v yeast was added to the must collected above after saccharification to convert the soluble sugars to ethanol under fermentation conditions, that is, anaerobic circumstance, pH of 6.5 and at room temperature. Fermentation was allowed to proceed for 2-4 days. After fermentation, the fermentation broth was filtered. The filtrate was distilled for ethanol recovery. The residue obtained from the filtration of the fermented broth was dried and analysed for the following parameters: proximate analysis, mineral content and amino acid.

The procedure in section 3.6, (the preceding paragraph) was repeated for all sprouted and non-sprouted tigernut tuber flour. Fermentation of tigernut without saccharification was done to serve as control.

3.7. Proximate Analysis

Analysis of the samples for moisture, ash and crude fibre were carried out in triplicate using the methods described in AOAC (1990). The nitrogen was determined by the micro Kjedahl method described by Pearson (1976) and nitrogen content converted to protein by multiplying by a factor of 6.25. Carbohydrate was determined by subtracting the sum of all other parameters (moisture, ash, fibre and crude protein) from 100%.

3.7.1. Moisture Content Determination

Porcelain crucible was washed and dried in the oven at 105^oC and cooled in desiccator. The weight of porcelain crucible was noted as W_1. A known mass of the sample (2g) was taken in the crucible as W_2. The oven temperature was set to 105^oC, little above boiling point of water for total moisture removal. The sample was placed in the oven and removed after a period of 1hr and cooled in a desiccator and re-weighed. This step

was repeated until constant weight was noted and recorded as the final weight (W_3). The moisture content was calculated in per cent as shown in equation 3.5.

$$\% \text{ Moisture } = \frac{W_2 - W_3}{W_2 - W_1} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.5$$

3.7.2. Ash Content

Porcelain crucible was washed thoroughly, dried and weighed as W_1. A known mass of the sample (2g) was taken in the crucible as W_2. The sample was placed in a muffle furnace at a temperature of 550^{o}C for 5-8hrs. The sample was removed after incineration and cooled in the desiccator. After cooling, the weight was noted and recorded as W_3. Percentage ash was determined using equation 3.6.

$$\% \text{ Ash } = \frac{W_3 - W_1}{W_2 - W_1} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.6$$

3.7.3. Crude Fibre Determination

A dilute acid (100 cm^3 of 0.25M tetraoxosulphate (VI) acid) was added to 2g of the sample in a round bottom flask and boiled under reflux for 30mins. The hot solution was filtered and the residue washed severally with warm water until acid-free. Sodium hydroxide (0.25 M, 100 cm^3) was added to the residue which was quantitatively transferred back into the flask and boiled for 30mins. The hot solution was again filtered and the residue was washed with warm water until base-free. A dry aluminium crucible was weighed and its weight noted as W_1. The sample was added to the aluminium crucible, and the crucible and sample weighed as W_2. It was dried in the oven at 105^{o}C for 2hrs, cooled in a desiccator and weighed as W_3. The percentage crude fibre was calculated using equation 3.7.

$$\% \text{ Crude Fibre } = \frac{W_2 - W_3}{W_2 - W_1} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.7$$

3.7.4. Crude Fat Determination

A moisture free sample (2g) was transferred into a thimble. Using soxhlet extraction, the sample was allowed to reflux for about 6hrs using hexane. The thimble was removed with care and dried in the oven at 105^{o}C for 1hr. The sample was allowed to cool in desiccator and weighed (W). Fat was calculated using equation 3.8.

$$\% \text{ Fat } = \frac{\text{Weight of Fat}}{\text{Weight of Sample}} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.8.$$

$$\text{Weight of Fat } = \text{Weight of sample before extraction (2g)} - \text{Weight of sample after extraction}$$

3.7.5. Crude Protein (Nitrogen) Determination

Concentrated tetraoxosulphate (VI) acid (25cm^3) was added to 1.5g sample with 10g potassium sulphate and 0.7g mercury (as catalyst) in a Pyrex Kjedahl flask. The flask was inclined at an angle approximately 60^{o} and was covered with a glass funnel. The flask was heated gently until foaming stops. When foaming ceased, the heat was increased for 90 - 120mins until the solution became colourless. The solution was allowed to cool. Little at a time and with frequent shaking, 100cm^3 of distilled water was carefully added with 25cm^3 of 0.5M sodium thiosulphate and in excess 50% sodium hydroxide (70cm^3). Ammonia was distilled off into tetraoxosulphate (VI) acid (0.5 M, 100cm^3). A blank determination exactly as above was done with the nitrogen-containing sample omitted using sodium hydroxide with 2 drops of methyl red. Protein was determined by first calculating the percentage nitrogen which is given using equation 3.9. M = molarity of acid (0.5), V = end point of titration.

$$\% \text{ Nitrogen} = \frac{0.014 \text{ x M x V}}{\text{Weight of Sample}} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots 3.9.$$

Before calculating crude protein using equation 3.10.

$$\text{Crude Protein} = \% \text{ Nitrogen x } 6.25 \ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.10$$

3.7.6. Carbohydrate Determination

This is determined by summing all the various components (% moisture, % ash, % fibre, % fat, and % protein) and subtracting from 100% as shown in equation 3.11.

$$\text{Carbohydrate} = 100 - (a + b + c + d) \ldots\ldots\ldots\ldots\ldots 3.11$$

Where a = % moisture, b = % ash, c = fibre and d = crude protein.

3.8. Mineral Analysis

The method described in AOAC (1990) was used for mineral analysis.

Each sample (2.00g) was digested with 15cm^3 of concentrated trioxonitrate (IV) acid in a fume hood until brown fume stopped and white fume was given out. The resulting mixture was filtered and made up to 100 cm^3 in a volumetric flask. Sodium and potassium were determined using a flame photometer (Model 405, corning UK) (Pearson, 1976). All other metals were determined using Atomic Absorption Spectrophotometer (Perkin-Elmer model 403, Norwalk Ct; USA) (Techtron, 1976). The minerals were reported in mg/100g sample.

3.9. Amino Acid

The amino acid profile in the known sample was determined using methods described by Benitez (1989). The sample was dried to constant weight, defatted, hydrolyzed,

evaporated in a rotary evaporator and loaded into the Technicon Sequential Multi-Sample Amino Acid Analyser (TSM) for analysis.

3.9.1. Defatting Sample

The sample was defatted using chloroform/methanol mixture of ratio 2:1. About 4g of the sample was put in extraction thimble and extracted for 15 hours in soxhlet extraction apparatus (AOAC, 2006).

3.9.2. Nitrogen Determination

A small amount (200mg) of ground sample was weighed, wrapped in whatman filter paper (No.1) and put in the Kjeldhal digestion flask. Concentrated sulphuric acid ($10cm^3$) was added. Catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Pieces of anti-bumping granules were added.

The flask was then put in Kjeldhal digestion apparatus and digested for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100 cm^3 in standard volumetric flask. Aliquot ($10cm^3$) of the diluted solution with 10 cm^3 of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10 cm^3 of 2% boric acid containing 4 drops of methyl red indicator.

The distillate was then titrated with standard 0.01 M hydrochloric acid until the solution changed to grey colour. % Nitrogen was calculated using equation 3.12.

$$\text{Percentage Nitrogen} = \frac{(a - b) \text{ x } 0.01 \text{ x } 14 \text{ x } v}{W \text{ x } C} \text{ x } 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.12$$

Where:

a = Titer value of the digested sample

b = Titer value of blank sample

v = Volume after dilution ($100cm^3$)

W = Weight of dried sample (mg)

C = Aliquot of the sample used ($10cm^3$)

14 = Nitrogen constant in mg.

3.9.3. Hydrolysis of the Sample

A known weight of the defatted sample was weighed into a borouke. Hydrochloric acid (6 N, 7 cm^3) was added and oxygen was expelled by passing nitrogen into the borouke (this is to avoid possible oxidation of some amino acids during hydrolysis e.g methionine and cystine). The borouke was then put in an oven pre-set at $105^0C \pm 5^0C$ for 22 hours. The borouke was allowed to cool before opening. The content was filtered to remove humins. It should be noted that tryptophan is destroyed by hydrochloric acid (6 N) during hydrolysis.

The filtrate was then evaporated to dryness in hot air oven. The residue was dissolved with 5 cm^3 to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

3.9.4. Loading of the Hydrolysate into TSM Analyser

Aliquot amount of the hydrolysate (5 to 10µL) obtained as in section 3.9.3 was dispensed into the cartridge of the analyser. The TSM analyser is designed to separate and analyse free acidic, neutral and basic amino acids present in the hydrolysate. The period of analysis lasted for 76 minutes.

3.9.5. Method of Calculating Amino Acid Values from the Chromatogram Peaks

An integrator attached to the Analyser calculates the peak area proportional to the concentration of each of the amino acids. Approximate area of each peak was then obtained by multiplying the height with the width at half-height.

The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using equation 3.13.

$$\mathrm{NE} = \frac{\text{Area of Norleucin Peak}}{\text{Area of Each Amino Acid}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.13$$

A constant, S, was calculated for each amino acid in the standard mixture:

$$\mathrm{S_{std}} = \mathrm{NE_{std}} \text{ x Molecular weight x } \mu\mathrm{MAA_{std}}$$

Finally, the amount of each amino acid present in the sample was calculated in g/16gN or g/100g protein using equation 3.14 and 3.15.

$$\text{Concentration}\left(\frac{\mathrm{g}}{100\mathrm{g}}\text{protein}\right) = \mathrm{NH} \text{ x } \mathrm{W@}\frac{\mathrm{NH}}{2} \text{ x } \mathrm{S_{std}} \text{ x C} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.14$$

$$\text{But,} \quad \mathrm{C} = \frac{\text{Dilution x 16}}{\text{Sample Wt (g) x N\% x 10 x Vol. loaded.}} \div \mathrm{NH} \text{ x W (nleu)} \ldots\ldots\ldots 3.15$$

where NH = Net height, W = Width @ half height, nleu = Norleucine

3.9.6. Determination of Isoelectric Point (pI)

The predicted isoelectric point was evaluated using equation 3.16. (Olaofe & Akintayo, 2000).

$$pIm = \sum_{i=1}^{n=1} pI_i X_i \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.16$$

where, pIm is the isoelectric point of the mixture of amino acids, pI_i is the isoelectric point of the i^{th} amino acids in the mixture and X_i is the mass or mole fraction of the amino acids in the mixture.

3.9.7. Determination of Amino Acid Scores (AAS)

In this method, only essential amino acids was scored. Amino acid score was calculated using equation 3.17. (WHO/FAO, 1973).

$$\text{AAS} = \frac{\text{Amount of amino acid per test protein } (\text{mg}^{-1})}{\text{Amount of amino acid per protein in reference pattern } (\text{mg/g})} \ldots\ldots\ldots\ldots .3.17$$

Amino Acid Score was also calculated based on the composition of the amino acids obtained in the samples compared with the suggested pattern of requirements for fingerlings, chicks, piglets and rats.

3.9.8. Determination of the Predicted Protein Efficiency Ratio

The predicted protein efficiency ratio (P-PER) was determined using equation 3.18. (Nieman *et al*., 1992).

$$\text{P} - \text{PER} = 0.468 + 0.454(\text{Leu}) - 0.105(\text{Tyr}) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.18$$

P-PER = Predicted Protein efficiency ratio, Leu = Leucin, Tyr = Tyrosin.

3.9.9. Determination of Essential Amino Acid Scores for Fingerlings, Chicks, Piglets and Rat

In this method, only essential amino acid were scored. Amino acid score was calculated using equation 3.17. (WHO/FAO, 1973).

3.10. Anti-nutrient Composition of Un-sprouted and Processed Tigernut Tubers

The anti-nutrient content (Tannins, Phytic Acid and Oxalate) of the un-sprouted and processed tigernut was determined according to methods described by AOAC, (2006), Abeza *et al*. (1968), McCance, and Widdowson, (1935) and Harbone, (1973).

3.10.1. Tannins

Tannins were determined using methods described by AOAC (2006). This method was however slightly modified. About 2g of ground plant were defatted for 2 hours using Soxhlet extractor. The residue was dried in oven for 12 hours at 100°C, boiled with 300 cm^3 of distilled water, diluted to 500 cm^3 in standard volumetric flask and filtered through non -absorbent cotton wool. A volume of 25 cm^3 of the infusion was measured into 2 litter porcelain dish and titrated with 0.1N potassium permanganate (0.1N potassium permanganate was standardized against 0.lN oxalic acid) until blue solution changed to green. Few drops of 0.lN potassium permanganate were added. The difference between the two titrations was multiplied by 0.006235 to obtain the amount of tannin in the sample.

3.10.2. Phytic Acid

Phytic acid content of each sample was determined by the modified method of McCance & Widdowson (1935). About 10g aliquot of the powdered sample was extracted (hydrochloric acid, 0.5 M, 20 cm^3) with vigorous shaking in an MSE orbital shaker at 200 rpm for 1 hour to ensure homogeneity and then neutralized by adding 20 cm^3 of 20% sodium hydroxide. Feric chloride (1%, 50 cm^3) in hydrochloric acid (1 N) was added. The mixture brought just to a boil. This resultant mixture was filtered through an ash-less whatman No. 40 filter paper. The resultant residue was dissolved in about 10 cm^3 of 2% sodium hydroxide. This procedure dissolves phytic acid while

precipitating ferric hydroxide which was removed by filtration. The filtrate (containing phytic acid) was digested with a mixture of concentrated sulphuric acid and perchloric acid in the ratio 5:1 (v/v) in a Kjeldahl flask. The pH of the digest was adjusted to 4.5 and phosphate determined colorimetrically at 710nm.

3.10.3. Oxalate

The total acid of the powdered samples was determined by the modified method of Abeza *et al*. (1968). About 2g aliquot of the plant materials was weighted into a 250 cm^3 flask. Distilled water (190cm^3) and hydrochloric acid (6 M, 10 cm^3) were added. The mixture was digested for 1 hour on boiling water bath, cooled, transferred into a 250 cm^3 volumetric flask and made up to mark. The solution from the volumetric flak was filtered. Four drops of methyl red indicator were added followed by concentrated ammonia until the solution turned faint yellow. It was then heated to 100 ^oC, allowed to cool and filtered to remove precipitate containing ferric ions. The filtrate was boiled; 10 cm^3 of 5% calcium chloride added with constant stirring and was allowed to stand overnight. The mixture was filtered through whatman No. 40 filter paper. The precipitate was washed several times with distilled water. The precipitate was transferred quantitatively to a beaker and 5cm^3 of 25% sulphuric acid was added to dissolve the precipitate. The resultant solution was maintained at 80 ^OC and titrated against 0.5% potassium permanganate until the pink colour persisted for approximately one minute. A blank was also run for the test sample. From the amount of potassium permanganate used, the oxalate content of the unknown sample was calculated using the relationship: 1cm^3 of $KMnO_4$ = 2.2mg oxalate.

CHAPTER FOUR

RESULTS

4.1. Yield of Tigernut Oil

The yield of oil extracted from un-sprouted tigernut flour is 32.63%, whereas the yield of oil extracted from sprouted tigernut flour is 36.67%.

Table 4.1: Physicochemical Properties of Tigernut Oil

	VIII	**IX**
Colour	Golden Yellow	Golden Yellow
Refractive index @ 30°	1.48±0.12	1.41±0.22
Iodine number (mg KOH/g)	80.20±0.11	73.10±0.51
Saponification value (mg KOH/g)	165.23±0.43	193.31±0.49
Specific gravity @ 25°C (g/cm^3)	0.80±0.01	0.73±0.05
Acid value (mg KOH/g)	0.80±0.57	0.90±0.55
Peroxide value ($mEqO_2/kg$)	6.90±1.74	5.30±0.12

VIII = Oil from un-sprouted tigernut tuber flour; IX = Oil from sprouted tigernut; VIII-IX = Difference

Table 4.2: Ethanol Recovered from Fermentation of Un-sprouted and Sprouted Tigernut after Oil Extraction (cm^3 per Kg dry matter)

Parameter	**X** **(cm^3 per Kg)**	**XI** **(cm^3 per Kg)**	**XII** **(cm^3 per Kg)**	**XIII** **(cm^3 per Kg)**
Crude Fermentation	84.0±1.65	96.0±1.55	112.0±0.95	128.0±1.21
Enzyme Hydrolysis & Fermentation	164.0±1.33	192.0±0.98	176.0±1.26	184.0±1.25
Acid Hydrolysis & Fermentation	92.0±1.41	108.0±1.21	128.0±1.08	148.0±1.39
Enzyme-Acid Hydrolysis & Fermentation	188.0±1.90	220.0±1.05	252.0±1.00	284.0±1.12

X = Un-sprouted (un-extracted) tigernut; XI = Sprouted (un-extracted) tigernut; XII = Residue of un-sprouted tigernut after oil extraction; XIII = Residue of sprouted tigernut after oil extraction.

Table 4.3: Proximate Composition of Un-sprouted and Processed Tigernut Tuber Flour (g/100g dry matter)

	Before oil extraction and fermentation		After fermentation				
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**
Moisture (%)	0.67±0.10	1.00±0.11	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.01±1.42
Ash (%)	2.83±0.62	2.43±1.02	42.31±0.88	45.38±1.01	46.41±0.86	44.21±1.21	41.01±1.52
Crude fat (%)	32.63±1.31	36.67±0.63	2.83±0.81	4.65±0.99	4.42±0.43	5.87±1.32	6.21±1.42
Crude fibre (%)	7.63±1.37	6.10±0.89	17.56±1.34	19.89±1.02	21.91±0.89	24.49±1.83	23.93±1.74
Crude protein (%)	7.27±1.01	5.86±1.02	20.63±0.79	23.91±1.22	19.54±1.56	24.37±0.67	23.21±1.43
Carbohydrate (%)	48.97±2.01	47.94±1.19	16.67±1.75	6.17±1.31	7.72±1.32	1.06±1.02	4.63±0.96
[a]Fatty acid (%)	26.10	29.34	2.26	3.72	3.54	4.70	4.97
[b]Energy (KJ/100g)	2163.39	2271.22	738.81	683.41	626.96	649.50	703.05

[a] Fatty acid = Crude fat x 0.8 (Pearson, 1976). [b] Energy = (Carbohydrate x 17KJ) + (Crude protein x 17KJ) + (Crude fat x 37KJ) (Pearson, 1976). I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting.

Table 4.4: Difference in Proximate Composition of Un-sprouted and Processed Tigernut Tuber Flour (g/100g dry matter)

Component	I	I-II (%)	I-III (%)	I-IV (%)	I-V (%)	I-VI (%)	I-VII (%)
Moisture (%)	0.67	-0.33	0.67	0.67	0.67	0.67	-0.34
Ash (%)	2.83	0.40	-39.48	-42.55	-43.55	-41.38	-38.18
Crude fat (%)	32.63	-3.47	29.80	27.98	28.21	26.76	26.42
Crude fibre (%)	7.63	1.53	-9.93	-12.26	-14.28	-16.86	-16.30
Crude protein (%)	7.27	1.41	-13.36	-16.64	-12.27	-17.10	-16.66
Carbohydrate (%)	48.97	1.04	32.30	42.80	41.25	47.91	44.34
[a]Fatty acid (%)	26.10	-3.24	23.84	22.38	22.56	21.40	21.13
[b]Energy (KJ/100g)	2163.39	-107.83	1424.58	1479.98	1536.43	1513.89	1460.30

I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting.

Table 4.5: Mineral Composition of Un-sprouted and Processed Tigernut Flour (mg/100g dry matter)

	Before oil extraction and fermentation		After oil extraction and fermentation				
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**
Na	239.75	113.54	151.33	153.53	141.31	135.20	168.53
Ca	161.35	145.22	158.28	157.61	155.21	171.28	161.24
Fe	0.42	0.31	1.11	1.22	1.38	1.31	2.12
Zn	0.02	0.01	1.18	1.74	1.89	0.88	0.92
K	28.60	34.51	43.37	42.17	40.15	32.91	32.28
Mg	49.88	51.54	58.91	60.11	60.87	57.38	58.16
Cu	0.02	0.03	1.10	1.35	1.21	0.96	1.10
Mn	5.43	1.54	4.82	8.69	5.16	8.36	3.62
P	132.97	122.87	163.21	160.81	155.19	154.21	189.25
Na/K	8.38	3.29	3.49	3.64	3.52	4.11	5.22
Ca/P	1.21	1.18	0.97	0.98	1.00	1.11	0.85

I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting.

Table 4.6: Difference in Mineral Composition of Un-sprouted and Processed Tigernut Tuber Flour (mg/100g dry matter)

	I	I-II (%)	I-III (%)	I-IV (%)	I-V (%)	I-VI (%)	I-VII (%)
Na	239.75	126.21	88.42	86.22	98.44	104.55	71.22
Ca	161.35	16.13	3.07	3.74	6.14	-9.93	0.11
Fe	0.42	0.11	-0.69	-0.80	-0.96	-0.89	-1.70
Zn	0.02	0.01	-1.16	-1.72	-1.87	-0.86	-0.90
K	28.60	-5.91	-14.77	-13.57	-11.55	-4.31	-3.68
Mg	49.88	-1.66	-9.03	-10.23	-10.99	-7.50	-8.28
Cu	0.02	-0.01	-1.08	-1.33	-1.19	-0.94	-1.08
Mn	5.43	3.89	0.61	-3.26	0.27	-2.93	1.81
P	132.97	10.10	-30.24	-27.84	-22.22	-21.24	-56.28
Na/K	8.38	5.09	4.89	4.74	4.86	4.27	3.16
Ca/P	1.21	0.03	0.24	0.23	0.21	0.10	0.36

I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting.

Table 4.7: Amino Acid Composition of Un-sprouted and Processed Tigernut Flour (mg/100g crude protein)

	Before Fermentation		After Fermentation				
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**
Ser	3.21	3.14	5.80	4.47	3.00	5.21	4.20
Glu	7.69	7.31	13.60	10.94	10.09	11.18	8.31
Pro	2.61	2.73	4.64	4.18	3.60	4.51	3.82
Gly	4.08	4.12	6.86	3.99	4.30	4.31	4.76
Ala	5.89	5.63	10.27	6.38	7.37	7.86	7.09
Cys	0.53	0.44	0.97	0.69	0.55	0.65	0.41
Tyr	2.18	2.20	2.93	3.81	3.47	3.62	2.91
Lys*	4.17	4.11	7.09	6.60	5.61	6.88	5.31
His*	2.88	2.62	4.32	3.81	2.98	4.10	4.01
Arg*	3.73	3.91	5.44	6.64	5.62	6.72	5.44
Asp	9.35	10.41	16.85	15.72	15.02	15.95	12.39
Thr*	2.35	3.01	4.09	3.58	3.18	3.87	3.42
Val*	3.71	4.10	6.51	4.99	4.14	4.81	5.31
Met*	7.39	6.31	3.00	2.36	2.15	2.41	2.16
Ile*	3.14	3.18	5.02	3.39	3.20	3.89	4.22
Leu*	5.13	6.31	9.74	8.39	7.98	8.63	8.14
Phe*	2.78	3.52	5.28	4.93	4.58	5.10	4.69

* Essential amino acids; I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting.

Table 4.8: Difference in Amino Acid Composition of Un-sprouted and Processed Tigernut Flour (mg/100g dry matter)

	I	I-II	I-III	I-IV	I-V	I-VI	I-VII
Ser	3.21	0.07	-2.59	-1.26	0.21	-2.00	-0.99
Glu	7.69	0.38	-5.91	-3.25	-2.40	-3.49	-0.62
Pro	2.61	-0.12	-2.03	-1.57	-0.99	-1.90	-1.21
Gly	4.08	-0.04	-2.78	0.09	-0.22	-0.23	-0.68
Ala	5.89	0.26	-4.38	-0.49	-1.48	-1.97	-1.20
Cys	0.53	0.09	-0.44	-0.16	-0.02	-0.12	0.12
Tyr	2.18	-0.02	-0.75	-1.63	-1.29	-1.44	-0.73
Lys*	4.17	0.06	-2.92	-2.43	-1.44	-2.71	-1.14
His*	2.88	0.26	-1.44	-0.93	-0.10	-1.22	-1.13
Arg*	3.73	-0.18	-1.71	-2.91	-1.89	-2.99	-1.71
Asp	9.35	-1.06	-7.50	-6.37	-5.67	-6.60	-3.04
Thr*	2.35	-0.66	-1.74	-1.23	-0.83	-1.52	-1.07
Val*	3.71	-0.39	-2.80	-1.28	-0.43	-1.10	-1.60
Met*	7.39	1.08	4.39	5.03	5.24	4.98	5.23
Ile*	3.14	-0.04	-1.88	-0.25	-0.06	-0.75	-1.08
Leu*	5.13	-1.18	-4.61	-3.26	-2.85	-3.50	-3.01
Phe*	2.78	-0.74	-2.50	-2.15	-1.80	-2.32	-1.91

* Essential amino acids; I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting.

Table 4.9: Compositions of essential, non-essential, acidic, neutral, sulphur, aromatic amino acid of Un-sprouted and Processed Tigernut Flour

	I	II	III	IV	V	VI	VII
TAA	70.82	78.05	125.41	94.87	86.84	99.70	86.59
TEAA							
With His	47.48	67.34	60.41	54.46	62.36	55.09	47.48
Without His	44.86	63.02	56.60	51.48	58.26	51.08	44.86
% TEAA							
With His	67.04	86.27	48.17	57.40	71.81	55.26	54.83
Without His	63.34	80.74	45.13	54.26	67.09	51.23	51.80
TNEAA	26.19	30.57	58.07	34.46	32.38	37.34	31.50
% TNEAA	36.98	39.16	46.30	36.32	37.29	37.45	36.38
EAAA	21.95	23.34	38.40	27.14	26.99	29.50	29.52
EArAA	5.72	21.21	8.74	8.05	8.72	7.60	5.72
TNAA	39.79	46.55	72.31	46.69	44.52	49.66	46.93
%TNAA	56.18	59.64	57.65	49.21	51.27	49.81	53.80
TAAA	17.04	17.72	30.45	26.66	25.11	27.13	20.70
%TAAA	24.06	22.70	24.28	28.10	28.91	27.21	23.91
TBAA	10.78	10.64	16.85	17.05	14.21	17.70	14.76
%TBAA	15.22	13.63	13.43	17.97	16.36	17.75	17.04
TSAA	7.92	11.75	3.97	3.05	2.70	3.06	2.57
% Cys in TSAA	6.69	3.74	24.43	22.62	20.37	21.24	15.95
pI**	24.17	26.34	42.17	32.46	29.32	34.20	29.99
P – PER***	2.57	3.10	4.58	3.88	3.73	3.71	3.86
Leu/Ile (ratio)	1.00	1.00	1.00	2.00	2.00	2.00	1.00
Leu-Ile (diff.)	1.99	3.13	4.72	5.00	4.78	4.74	3.92
%Leu-Ile	2.81	4.01	3.76	5.27	5.50	4.75	4.52

pI** Isoelectric point; P-PER*** Calculated predicted protein efficiency ratio; TAA= Total Amino Acid; TEAA= Total Essential Amino Acid; TNEAA= Total Non-Essential Amino Acid; EAAA= Essential Aliphatic Amino Acid; EArAA= Essential Aromatic Amino Acid; TNAA= Total Neutral Amino Acid; TAAA= Total Acidic Amino Acid; TBAA= Total Basic Amino Acid; Total Sulphur Amino Acid; I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting.

Table 4.10: Essential Amino Acid Scores for Fingerlings

	[a]IEAA for Fingerlings	I	II	III	IV	V	VI	VII
Arg	**3.90**	0.96	1	1.39	1.70	1.44	1.72	1.39
His	**1.90**	1.52	1.38	2.27	2.01	1.57	2.16	2.11
Ile	**3.60**	0.87	0.88	1.39	0.94	0.89	1.08	1.17
Leu	**4.10**	1.25	1.54	2.38	2.05	1.95	2.10	1.99
Lys	**4.80**	0.87	0.86	1.48	1.38	1.17	1.43	1.10
Met	**4.50**	1.64	1.40	0.67	0.52	0.48	0.54	0.48
Thr	**3.60**	0.65	0.84	1.14	0.99	0.88	1.08	0.95
Val	**3.60**	1.03	1.14	1.81	1.39	1.15	1.34	1.48

I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting; [a]IEAA for fingerlings, Source (NRC, 1977; WHO/FAO/UNU, 1985; WHO/FAO/UNU, 2007).

Table 4.11: Essential Amino Acid Scores for Chicks

	[a]IEAA for Chicks	I	II	III	IV	V	VI	VII
Arg	**6.10**	0.61	0.64	0.89	1.09	0.92	1.1	0.89
His	**1.70**	1.69	1.54	2.54	2.24	1.75	2.41	2.35
Ile	**4.40**	0.71	0.72	1.14	0.77	0.72	0.88	0.96
Leu	**6.70**	0.77	0.94	1.45	1.25	1.19	1.29	1.21
Lys	**6.10**	0.68	0.67	1.16	1.08	0.92	1.13	0.87
Met	**4.40**	1.68	1.43	0.68	0.54	0.49	0.55	0.49
Phe	**7.20**	0.39	0.49	0.73	0.68	0.64	0.71	0.65
Thr	**3.30**	0.71	0.91	1.24	1.08	0.96	1.17	1.04
Val	**4.40**	0.84	0.93	1.48	1.13	0.94	1.09	1.21

I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting; [a]IEAA for chicks, Source (NRC, 1977; WHO/FAO/UNU, 1985; WHO/FAO/UNU, 2007).

Table 4.12: Essential Amino Acid Scores for Piglets

	[a]IEAA for Piglets	I	II	III	IV	V	VI	VII
Arg	**1.50**	2.47	2.61	3.63	4.43	3.75	4.48	3.63
His	**1.50**	1.92	1.75	2.88	2.54	1.99	2.73	2.67
Ile	**4.60**	0.68	0.69	1.09	0.74	0.70	0.85	0.92
Leu	**4.60**	1.11	1.37	2.12	1.82	1.73	1.88	1.77
Lys	**4.70**	0.89	0.87	1.51	1.40	1.19	1.46	1.13
Met	**3.00**	2.46	2.10	1.00	0.79	0.72	0.80	0.72
Phe	**3.60**	0.77	0.98	1.47	1.37	1.27	1.42	1.30
Thr	**3.00**	0.78	1.00	1.36	1.19	1.06	1.29	1.14
Val	**3.10**	1.20	1.32	2.10	1.61	1.34	1.55	1.71

I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting; [a]IEAA for piglets, Source (NRC, 1977; WHO/FAO/UNU, 1985; WHO/FAO/UNU, 2007).

Table 4.13: Essential Amino Acid Scores for Rats

	[a]IEAAS for Rats	I	II	III	IV	V	VI	VII
Arg	**1.00**	3.73	3.91	5.44	6.64	5.62	6.72	5.44
His	**2.10**	1.37	1.25	2.06	1.81	1.42	1.95	1.91
Ile	**3.90**	0.81	0.82	1.29	0.87	0.82	1	1.08
Leu	**4.50**	1.14	1.4	2.16	1.86	1.77	1.92	1.81
Lys	**5.40**	0.77	0.76	1.31	1.22	1.04	1.27	0.98
Met	**3.00**	2.46	2.1	1	0.79	0.72	0.8	0.72
Phe	**5.30**	0.52	0.66	1	0.93	0.86	0.96	0.88
Thr	**3.10**	0.76	0.97	1.32	1.15	1.03	1.25	1.1
Val	**3.10**	1.2	1.32	2.1	1.61	1.34	1.55	1.71

I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting; [a]IEAA for rats, Source (NRC, 1977; WHO/FAO/UNU, 1985; WHO/FAO/UNU, 2007).

Table 4.14: Anti-nutrient contents of un-sprouted and processed tigernut tuber flour (mg/100g dry matter)

	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**Mean**
Oxalates	0.35	0.45	0.66	1.32	0.81	0.68	0.93	0.74
Phytates	0.51	0.72	0.37	0.52	0.43	0.63	0.89	0.58
Tannins	ND	ND	ND	ND	ND	ND	ND	-

I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting; ND = Not detected

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

5.1.1. Yield of Tigernut Oil

The yield of oil extracted from un-sprouted tigernut flour is 32.63%, whereas the yield of oil extracted from sprouted tigernut flour is 36.67%. From the result obtained, sprouted tigernut flour has higher yield of oil compared to the un-sprouted tigernut. The difference between the two yields is about 4.04% of the bulk dry matter.

Increase in lipase activity has been reported in nuts by MacLeod & White (1962). Increase in lipolytic activity during germination and sprouting causes hydrolysis of triacylglycerols to glycerol and constituent fatty acids. The increase in lipolytic activity during germination, may account for the increase in oil content of the sprouted tigernut tubers.

5.1.2. Physicochemical Properties of Tigernut

The physicochemical properties of oils extracted from un-sprouted and sprouted tigernut tuber flour are presented in Table 4.1 and Appendix II.

Oil extracted from both un-sprouted and sprouted tigernut flour appears golden yellow in colour. The refractive index of un-sprouted tigernut oil at 30^{o}C is 1.48. The refractive index of olive oil (1.466) is midway between the refractive index of oil from un-sprouted and sprouted tigernut tuber and vegetable oil (Shaker *et al.,* 2009). The refractive index of un-sprouted tigernut oil is the same with 75% glucose in water, glycerol and borosilicate glass. Sprouted tigernut tuber oil has a lower refractive index (1.41) than the oil from un-sprouted tigernut tuber flour. The refractive index is similar

to the refractive index of 40% glucose in water, diamond and polydimethylsiloxane (Zajac & Hecht, 2003; Lide, 2001; Tan *et al.*, 2005).

Iodine number is often used to determine the amount of unsaturation in fatty acids. This unsaturation is in the form of double bonds, which react with iodine compounds. The higher the iodine number, the more C=C bonds are present in the oil (Thomas, 2002). Table 4.1 shows that, tigernut oil (from both un-sprouted and sprouted tigernut tuber flour) are unsaturated. Iodine number of oil (73.10) from sprouted tigernut is lower compared to the oil (80.20) from un-sprouted sample. Iodine value for tigernut oil is higher than the values reported for cashew nut oil (44.4 mg Iodine g^{-1}) (Aremu *et al*., 2006) and *Citrillus vulgaris* with value of 38.1% (Oladimeji *et al*., 2001). In view of the fact that drying oils have an iodine value above 100 (Duel, 1951), tigernut tuber oil could be categorized as non-drying oil. The iodine number of sprouted tigernut indicates that unsaturation of the oil was reduced during sprouting.

Saponification value represents the number of milligrams of potassium hydroxide required to saponify 1g of lipid. It is a measure of the average molecular weight (or chain length) of all the fatty acids present in the oil. The long chain fatty acids found in fats have a low saponification value because they have relatively fewer numbers of carboxylic functional groups per unit mass of the fat as compared to short chain fatty acids. From Table 4.1, only saponification and acid values of oil from sprouted tigernut tend to be greater than the other properties. Oil from sprouted tigernut has saponification value of 193.31 $mgKOHg^{-1}$ compared to oil from un-sprouted tigernut tuber flour which is 165.23 $mgKOHg^{-1}$. However, there are some vegetable oils with higher saponification values such as coconut oil 255 mg KOH g^{-1}, palm kernel oil 247 mg KOH g^{-1} and butter fat 225 mg KOH g^{-1}. It has been reported by Pearson (1976) that, oils with higher saponification values contain high proportion of lower fatty acids.

The low saponification value and low acid value indicate that the oil may be suitable for soap making. The difference in saponification number between the two oils is about 14.5% (28.08 mg $KOHg^{-1}$).

Tigernut oil remained uniformly liquid at refrigeration temperature. This makes the oil suitable for salad making (Umerie & Enebeli, 1997). The low acid value (0.80 and 0.90 mg $KOHg^{-1}$ for un-sprouted and sprouted tigernut) obtained for tigernut oil is an indication that the triacylglycerol present have not been hydrolysed. It is also an indication that the oil is less vulnerable to lipase action than tropical almond (7.6 mg $KOHg^{-1}$), and fluted pumpkin (3.5 mg $KOHg^{-1}$) (Christian, 2006). Acid value is a measure of the free fatty acid in the oil sample and it can also be used as an indicator of the age of the oil (Onwuka, 2005). The acid number is a measure of the amount of carboxylic acid groups present in an oil sample. Examples of such acids include fatty acids, carboxylic acids, and others. The mass of potassium hydroxide (KOH) in milligrams that is required to neutralize one gram of the oil is referred to as acid value (or "neutralization number" or "acid number" or "acidity").

Detection of peroxide gives the initial evidence of rancidity in unsaturated fats and oils. Other methods are available, but peroxide value is the most widely used. It gives a measure of the extent to which an oil sample has undergone primary oxidation (Christian, 2006). Tigernut oil will become rancid on exposure to air over time. Unsaturation in oils plays a role in autoxidation. Oils with a high degree of unsaturation are most susceptible to autoxidation. Peroxides are intermediates in the autoxidation reaction. Autoxidation is a free radical reaction involving oxygen that leads to deterioration of fats and oils which form off-flavours and off-odours. Peroxide value, is useful for assessing the extent to which spoilage has advanced. Peroxide values of fresh

oils are less than 10 milliequivalents /Kg. When the peroxide value is between 30 and 40 milliequivalents/Kg, a rancid taste is noticeable (Umerie & Enebeli, 1997).

The difference in parameters tested between oil from un-sprouted and sprouted tigernut tuber flour is low, for both refractive index and acid value as shown in Appendix II. The values are 0.07 and -0.10 for refractive index and acid value, respectively. There is little increase in all other parameters of the oil from sprouted tigernut tuber flour, as shown in Appendix II.

5.1.3. Fermentation of Tigernut Flour

The yields of Ethanol recovered from un-sprouted and sprouted tigernut tuber after fermentation, using different techniques of saccharification, are presented in Table 4.2 and Appendix III.

The lowest yield of Ethanol was observed in un-sprouted (un-extracted) tigernut after crude fermentation. The highest yield of ethanol was from sprouted (extracted) tigernut, after enzyme/acid hydrolysis.

Sprouting of tigernut tubers improves the yield of ethanol in all treatments (see Table 4.2). The yield of ethanol in un-extracted tigernut tuber after crude fermentation increases from 84 (un-sprouted) to 96 (sprouted) cm^3 per Kg of dry matter. This justifies the positive effect of sprouting in ethanol production. Sprouting of tigernut tubers increase the amount of simple sugars present in sprouted tigernut, by the action of diastase. Diastase breaks down starch into maltose and sucrose. Enzymes added to the mash break down maltose and sucrose into glucose and fructose units available for fermentation.

When saccharified with acid, there was increase in the yield of Ethanol produced. The yield varies from 92-128 cm^3 per Kg of dry matter in un-sprouted tigernut, and 108-148cm^3 per Kg of dry matter in sprouted tigernut.

Saccharified with enzyme and acid (Table 4.2.) leads to increase in the yield of Ethanol produced. This technique of saccharification employed accounts for these increases in yields of Ethanol, to about 252cm^3 per Kg of dry matter for un-sprouted tigernut tuber flour, and 284cm^3 per Kg of dry matter for sprouted tigernut.

5.1.4. Proximate Composition of Tigernut

The proximate compositions of un-sprouted and sprouted tigernut tuber flour before and after fermentation are presented in Table 4.3 and Appendix IV. Fermentation residue of both un-sprouted and sprouted tigernut processed by different techniques of saccharification, was found to be abundant in some nutritional components such as ash, crude fiber, and crude protein. There was deficiency in the nutrient values of carbohydrate, fatty acids and energy.

The chemical composition of tigernut is similar to those of tubers and nuts. The ash content of tigernut before fermentation is within the usual range for tubers and nuts, 1.5-3.0 g per 100g dry matter (Addy & Eteshola 1984; Linssen *et al*., 1989; Temple *et al*., 1989; Coşkuner *et al*., 2002). After fermentation with different saccharification techniques, the ash content of the fermentation residue was found to be higher compared to the composition of the un-sprouted and sprouted samples (2.83±0.62 and 2.43±1.02 respectively). Fermentation residue after crude fermentation has value of 42.31±0.88 mg per 100g dry matter. For fermentation residues after enzyme, acid, enzyme/acid saccharification, they have values ranging from 45.38±1.01, 46.41±0.86,

and 44.21±1.21 mg per 100g dry matter respectively. Finally, fermentation residue after sprouting has value of 41.01±1.52 mg per 100g dry matter.

The main constituents of tigernut are carbohydrates and oil. The oil content is 32%. Some authors have reported that the oil content of tigernut varies between 22.8 and 32.8 g/100 g (Mokady & Dolev, 1970; Addy & Eteshola, 1984; Linssen *et al*., 1988; Temple *et al*., 1989; Coşkuner *et al*., 2002). Tigernut lipid, protein, and carbohydrate (fiber and starch included) contents are higher than those in root and tuber crops. In general, tubers have a high content of carbohydrates although their profile and relative content change according to varieties and ripening stage. The starch content of tigernut tubers decreases and the reducing sugar (invert sugar) content increases during storage (Coşkuner *et al*., 2002). The carbohydrates of tigernut tuber are composed, mainly, of starch and dietary fiber. Tigernut tubers contain almost twice the quantity of starch as potato or sweet potato tubers (Coşkuner *et al*., 2002). However, if tigernut is compared to real nuts, it can be observed that its fiber content is within the usual range for nuts (Alegría-Torán & Farré-Rovira 2003; Ros 2010).

The energy content of fermentation residue was found to be low. Fermentation residue from un-sprouted tigernut has higher nutritional value than fermentation residue after sprouting, enzyme, acid, and enzyme/acid saccharification.

The results obtained show that saccharification and sprouting of tigernut tuber before fermentation lead to increase in nutrient value of fermentation residue. Direct fermentation without sprouting or saccharification of tigernut flour did not lead to appreciable increase in nutrient content (See Table 4.3 and Appendix IV).

The percentage of carbohydrate remaining after fermentation comprises of fibre and lignocellulosic materials (Kaura & Saxena, 2004). Because of the rich protein and fibre

content of fermentation residue, it is a good material for animal feed, especially ruminants and poultry (Huige, 1994). The digestibility of the fermentation residue is improved by the presence of microorganisms (Kaura & Saxena, 2004).

The difference in proximate composition between un-sprouted and processed tigernut tuber flour is presented in Table 4.4 and Appendix V. Parameters with negative values indicate increase from the un-sprouted sample (control), whereas a positive value signifies a decrease from the un-sprouted sample (control).

Generally, fermentation is known to improve protein content of many plant materials. Fermentation of processed tigernut shows a corresponding increase (seen Appendix V). Crude fibre and protein also indicate increase in nutritional value of processed tigernut flour. Carbohydrate, which is the major component consumed during fermentation shows decrease of about 32% for crude fermentation, 42%, 41%, and 47 % for enzyme, acid, enzyme/acid respectively; and 44% for sprouted residue.

Fermentation processes do not use oil. The observed decrease in oil content could mean decrease in the energy content of the fermented substrate.

The observed increase in crude protein content of the substrate resulted from protein synthesis by the microorganism using simple breakdown products from the substrate. This increase in protein content is in conformity with the findings of Abu (1997), who reported protein enrichment in sweet potato subjected to solid state fermentation. Similarly, Iyayi and Losel (2001) reported protein enrichment in cassava by-products through solid state fermentation using fungi. The ash content (41.01 to 46.41%, Table4.3) of the fermentation residue is comparable to the ash content (44 to 48%) of soya feed, a widely used source of animal feed (Abu, 1997). The fermentation residue

can therefore be rated high as a protein source capable of meeting the 20-22% protein requirement of chickens (FAO, 1965; Chinke, 2000).

The observed increase in the crude fibre content of fermentation residue is probably due to the fibre-degrading activities of the fungi *S. cerevisiae*. Degradation of fibre by these fungi has been reported (Han, 1978; Ogbonna, 1980). High crude fibre content in diets of animals, including chickens, often pose problems of digestibility and reduced feed intake, which leads to reduced animal performance (Smith, 1990). The crude fibre value of 12.27% is, however, still higher than the maximum 3.5 - 4% requirement for chicken (Han, 1978; Ogbonna 1980; Smith, 1990).

5.1.5. Mineral Composition of Tigernut

Dietary elements (commonly known as dietary minerals or mineral nutrients) are chemical elements required by living organisms, other than the four elements carbon, hydrogen, nitrogen, and oxygen present in common organic molecules. Chemical elements in order of abundance in the human body include the seven major dietary elements calcium, phosphorus, potassium, sulphur, sodium, chlorine, and magnesium. Important "trace" or minor dietary elements, necessary for mammalian life, include iron, cobalt, copper, zinc, manganese, molybdenum, iodine, bromine, and selenium (WHO/FAO/UNU, 2007).

In good health the ratio of calcium to phosphorus in the blood is 10:4. If there is a glandular imbalance, especially in regard to the parathyroid glands, then this ratio will be maintained at a different level, causing long-term health deterioration (WHO/FAO, 1973). In particular, a high ratio of phosphorus to calcium sensitizes the body and increases inflammatory tendencies (Yeboah *et al*., 2011).

Ca/P is required in an ideal ratio of 2:1 in animal feed (WHO/FAO/UNU, 2007). The Ca:P in un-sprouted and processed tigernut is 1:1.

Na/K ratio is a very important factor in human and animal nutrition. The ratio is better kept at minimal level for healthy well-being. 1:2 is the ideal ratio for animal feed (Rudravaram *et al*., 2006; FAO, 2002). The Na/K ratio in tigernut is very high especially in un-sprouted sample. High Na/K ratio would give rise to cardiovascular diseases or eventually cardiac arrest. Sprouted tigernut has the lowest Na/K ratio. After fermentation, different fermented residues contain about 3:1 of Na/K. Potassium needs supplementation to adjust the ratio to 1:2 or Na needs reduction in its concentration to maintain the ideal ratio.

Na, Ca, K, Mg and P show a higher percentage of the minerals respectively, signifying that they are the major minerals. They are abundant in un-sprouted, sprouted and processed tigernut tuber flour and fermentation residue respectively. Micro minerals (Fe, Zn, and Cu) show low concentration in un-sprouted, sprouted and fermentation residue. The concentration of micro minerals is not required in large amount as clearly seen in Table 4.5 and Appendix VI.

Table 4.6 and Appendix VII, show the difference in mineral concentration between the un-sprouted, sprouted and processed tigernut tuber flour. Close observation of the table shows that there is a corresponding decrease in the mineral content of Na and Ca, Na/K and Ca/P. Similarly, a corresponding increase in Ca content of fermentation residue after enzyme/acid saccharification only. All other minerals show an increase, in different amounts.

The increase in phosphorus content is very pronounced except in sprouted samples. As observed in Table 4.6, fermented tigernut flour is also rich in mineral nutrients as suggested by Carpenter and Booth (1973) and FAO (2002).

Shipard (2005) reported that when seeds are sprouted, minerals chelate with protein, in a way that increases their function. This may explain the increase in concentration of mineral in almost all sprouted tigernut samples.

5.1.6. Amino Acid Composition of Tigernut Flour (g/100g)

The amino acid profiles of un-sprouted and processed fermentation residue are presented in Table 4.7 and Appendix VIII.

It was observed that fermentation residues have high amino acid content when compared with unfermented tigernut flour. Only Methionine showed a sharp decrease in concentration after fermentation. Methionine has concentration ranging from 6.31 to 7.39 mg/100g dry matter in sprouted and un-sprouted tigernut tuber flour, respectively. Methionine has concentration of 3.00mg/100g dry matter after crude fermentation. The concentration varies after various saccharification methods, thus 2.36, 2.15, and 2.41mg/100g dry matter in fermentation residue from enzyme, acid and enzyme/acid saccharification, respectively. The concentration of 2.16mg/100g dry matter was observed for fermentation residue from sprouted tigernut. Cysteine did not show any change in its concentration before and after fermentation. Cysteine has concentration ranging from 0.44 to 0.53mg/100g dry matter in sprouted and un-sprouted tigernut tuber flour. Cysteine has concentration of 0.97mg/100g dry matter after crude fermentation. The concentration varies after various saccharification methods, thus 0.69, 0.55, and 0.65mg/100g dry matter in fermentation residue from enzyme, acid and enzyme/acid

saccharification, respectively. The concentration of 0.41mg/100g dry matter was observed for fermentation residue from sprouted tigernut.

The presence of Phenylalanine and Tyrosine in fermentation residue is at the recommended level for animal feed (NRC, 1977; WHO/FAO/UNU, 1985). Phenylalanine and Tyrosine have the ability to reduce the risk of tumour bearing in animals. They (Phenylalanine & Tyrosine) also can reduce the rate of tumour growth and spread in tumour bearing animals (Austic *et al*., 1991).

Phenylalanine has concentration from 2.78 to 3.52mg/100g dry matter in un-sprouted and sprouted tigernut tuber flour. Phenylalanine has concentration of 5.28mg/100g dry matter after crude fermentation. The concentration varies after various saccharification methods, 4.93, 4.58, and 5.10mg/100g dry matter in fermentation residue from enzyme, acid and enzyme/acid saccharification respectively. The concentration of 4.69mg/100g dry matter was obtained for fermentation residue from sprouted tigernut.

Tyrosine did not show any increase or decrease in its concentration before and after fermentation, except for various saccharification methods. Thus 3.81, 3.47, and 3.62mg/100g dry matter in fermentation residue from enzyme, acid and enzyme/acid saccharification respectively. Tyrosine has concentration ranging from 2.18 to 2.20mg/100g dry matter in un-sprouted and sprouted tigernut tuber flour.

Tyrosine has concentration of 2.93mg/100g dry matter after crude fermentation. The concentration of 2.91mg/100g dry matter was observed for fermentation residue from sprouted tigernut.

Arginine reduce/slow tumour growth by 50% (Tsiagbe *et al*., 1987; Austic *et al*., 1991). Arginine shows a marked increase in its concentration before and after fermentation.

Arginine shows concentration from 3.73 to 3.91mg/100g dry matter in un-sprouted and sprouted tigernut tuber flour. Arginine has concentration of 5.44mg/100g dry matter after crude fermentation. The concentration varies after various saccharification methods, consequently 6.64, 5.62, and 6.72mg/100g dry matter in fermentation residue from enzyme, acid and enzyme/acid saccharification respectively. The concentration of 5.44mg/100g dry matter was recorded for fermentation residue from sprouted tigernut.

Glutamic acid and Arginine also have high concentration. The concentration when in excess will help boost the immune system of the animals when fed with spent grains from tigernut tubers. Glutamic acid shows a noticeable increase in its concentration before and after fermentation. Arginine shows concentration from 7.69 to 7.31mg/100g dry matter in un-sprouted and sprouted tigernut tuber flour. Glutamic acid show highest concentration of 13.60mg/100g dry matter after crude fermentation. The concentration shows a discrepancy after various saccharification methods, thus 10.94, 10.09, and 11.18mg/100g dry matter in fermentation residue from enzyme, acid and enzyme/acid saccharification respectively. The concentration of 8.31mg/100g dry matter was recorded for fermentation residue from sprouted tigernut.

Voluntary feed intake is important in all species but especially so in aquaculture, where feeds must first attract fish or crustaceans and then be palatable to be accepted. Amino acids, betaine and inosine appear to act as attractants. Glycine, Proline, Taurine and Valine appear to be preferred by carnivorous fish, while aspartic and glutamic acids are preferred by omvivorous fish (NRC, 1977; WHO/FAO, 1985).

Glycine did not show any marked change in its concentration before and after fermentation except for crude fermentation.

Glycine shows a sharp increase in concentration to 6.86mg/100g dry matter after crude fermentation. Glycine also shows consistent concentration of 4.08 to 4.12mg/100g dry matter in un-sprouted and sprouted tigernut tuber flour. The concentration varies after various saccharification methods, consequently 3.99, 4.30, and 4.31mg/100g dry matter in fermentation residue from enzyme, acid and enzyme/acid saccharification respectively. The concentration of 4.76mg/100g dry matter was detected for fermentation residue from sprouted tigernut. Proline did not show a marked increase or decrease in its concentration before and after fermentation.

Concentrations ranging from 3.73 to 3.91mg/100g dry matter in un-sprouted and sprouted tigernut tuber flour were observed. Concentration of 5.44mg/100g dry matter after crude fermentation was also observed for Proline. The concentration varies after various saccharification methods, consequently 4.18, 3.60, and 4.51mg/100g dry matter in fermentation residue from enzyme, acid and enzyme/acid saccharification respectively. The concentration of 3.82mg/100g dry matter was detected for fermentation residue from sprouted tigernut. Valine shows a marked increase in its concentration before and after fermentation. Valine shows concentration ranging from 3.71 to 4.10mg/100g dry matter in un-sprouted and sprouted tigernut tuber flour. Valine indicates concentration of 6.51mg/100g dry matter after crude fermentation. The concentration varies after various saccharification methods, accordingly 4.99, 4.14, and 4.81mg/100g dry matter in fermentation residue from enzyme, acid and enzyme/acid saccharification respectively. The concentration of 5.31mg/100g dry matter was obtained for fermentation residue from sprouted tigernut.

Glutamic acid at the various concentrations observed, can maintain mucosal cells to improve gut barrier functions against bacterial infection (Fathia *et al.,* 2013). The

concentration compares favourably with the concentration of Glutamic acid, Alanine and Tyrosine in brewery spent grains from maize and sorghum.

Fermentation residue from crude fermentation has the highest mean value of amino acid content of 6.61. This signifies that, crude fermentation favours amino acid content of tigernut tuber flour. Other treatments that show high yield include fermentation residue from enzyme, and enzyme/acid saccharification. Fermentation residue from sprouted and acid saccharified tigernut tuber flour is the least in amino acid content. Aspartic acid is the amino acid with the highest mean value, while Cysteine is the amino acid with least mean value. Leucine is the highest essential amino acid; it has a mean value of 7.76.

Threonine is the least essential amino acid with mean value of 3.36. Tryphtophan is destroyed by 6M mineral acid, hence its presence was not detected.

Chavan *et al.* (1989) reported that complex qualitative changes occur during soaking and sprouting of seeds. The conversion of stored proteins in seeds into albumins and globulins during sprouting may improve the quality of seeds proteins. Many studies have reported increase in the content of some amino acids after sprouting.

An increase in proteolytic activity during sprouting is desirable for nutritional improvement. It leads to hydrolysis of prolamins and the liberated amino acids such as glutamic acid and proline are converted to limiting amino acids such as lysine.

5.1.7. Essential Amino Acid Scores (EAAS)

Essential amino acids are necessary for the growth and maintenance of body balance. They cannot be synthesised by the body; hence ingestion of these amino acids in food is very paramount. Recommended daily intakes of these essential amino acids have been

determined by organisations that regulate feeding in humans and farm animals (NRC, 1977; FAO, 2002).

Essential Amino Acid Score is a simple ratio between the Total Essential Amino Acid (see Table 4.9) and the Ideal Essential Amino Acid. Whenever Essential Amino Acid Score is less than one (EAAS<1), it suggests that the Essential Amino Acid Composition of that individual amino acid in the fermentation residue is below the Ideal Essential Amino Acid hence it needs supplementation. But whenever the Essential Amino Acid Score is greater than one (EAAS>1), this implies that the Essential Amino Acid Composition of that individual amino acid in the fermentation residue is above the Ideal Essential Amino Acid. If the Essential Amino Acid Score is equal to one (EAAS = 1), it indicates that the Essential Amino Acid Composition is equal to the Ideal Essential Amino Acid of that individual amino acid in the fermentation residue.

5.1.8. Essential Amino Acid Scores for Fingerlings

The Essential Amino Acid Score for fingerlings of cart fish is reported in Table 4.10 and Appendix XI.

In un-sprouted tigernut tuber flour, Arginine, Isoleucine, Lysine and Threonine are deficient in Essential Amino Acid Composition when compared with the daily recommended intake. Hence, supplementation of Arginine, Isoleucine, Lysine and Threonine is recommended. Histidine, Leucine, Methionine and Valine are rich in their individual Essential Amino Acid Composition. They satisfy the recommended daily intake as suggested by NRC (1977), WHO/FAO/UNU, (1985), WHO/FAO/UNU (2007).

Sprouting improved the concentration of Arginine only, such that it reaches the required daily intake for fingerlings. Arginine, Histidine, Leucine, Methionine and Valine have Essential Amino Acid Composition greater than the recommended daily intake; and therefore don't need any supplementation.

Fermentation residue from crude fermentation was found to be low in Essential Amino Acid Composition of Methionine, Isoleucine and Lysine. Supplementation of Methionine, Isoleucine and Lysine is necessary since they do not satisfy the recommended daily intake for fingerlings. The Essential Amino Acid Composition of Arginine, Histidine, and Leucine were found to be high, thus supplementation is not required.

Activities of enzyme in tigernut fermented residue reduce the Essential Amino Acid Composition of Methionine, Isoleucine, and Threonine fall below the Ideal Essential Amino Acid recommended daily intake. Hence, supplementation of Methionine, Isoleucine, Methionine and Threonine is recommended. The Essential Amino Acid Score of Arginine, Histidine, Leucine, Lysine, and Valine are very rich and satisfy the daily recommended intake for fingerlings as proposed by NRC (1977), WHO/FAO/UNU, (1985), WHO/FAO/UNU (2007).

Fermentation residue from acid saccharification of tigernut tuber flour was found to be low in Essential Amino Acid Composition of Methionine. However, the Essential Amino Acid Composition of Arginine and Lysine was found to be high in the fermentation residue. Essential Amino Acid Composition of Isoleucine, Methionine and Threonine fall below the Ideal Essential Amino Acid endorsed for daily consumption. Therefore, supplementation of Isoleucine, Methionine and Threonine is recommended.

The Essential Amino Acid Composition of Arginine, Histidine, Leucine, Lysine, and Valine are high and satisfy the daily recommended intake for fingerlings.

Fermentation residue from enzyme/acid saccharification was found to be low in Essential Amino Acid Composition of Methionine but this follow with a corresponding high concentration recorded for Arginine, Isoleucine, Lysine and Threonine. The Essential Amino Acid Composition for only Methionine fell below the Ideal Essential Amino Acid recommended for daily intake, hence it needs supplementation. The Essential Amino Acid Composition of Arginine, Histidine, Isoleucine, Leucine, Lysine, Threonine and Valine are very high and okay for fingerlings.

Fermentation residue from sprouted tigernut was found to be low in the Essential Amino Acid Composition for Methionine and Threonine. However, there was increase in the Essential Amino Acid Composition of Arginine, Isoleucine, and Lysine. The Essential Amino Acid Composition for Methionine and Threonine fell below the Ideal Essential Amino Acid recommended for daily intake. Hence, they need supplementation. The Essential Amino Acid Composition of Arginine, Histidine, Isoleucine, Leucine, Lysine, and Valine are very high and satisfactory for fingerlings.

5.1.9. Essential Amino Acid Scores for Chicks

The Essential Amino Acid Composition for chicks is reported in Table 4.11 and Appendix XII.

In un-sprouted tigernut tuber flour, Arginine, Isoleucine, Leucine, Lysine, Phenylalanine, Threonine and Valine were found to be deficient in Essential Amino Acid Composition when compared with the daily recommended intake (Ideal Essential Amino Acid). However, Arginine, Isoleucine, Leucine, Lysine, Phenylalanine,

Threonine and Valine need supplementation. Histidine, and Methionine are rich in the respective Essential Amino Acid Composition.

Sprouting did not improve the concentration of all the amino acids that were lower than the Ideal Essential Amino Acid (Arginine, Isoleucine, Leucine, Lysine, Phenylalanine, Threonine and Valine). Only Histidine, and Methionine have Essential Amino Acid Composition greater than the recommended daily intake; and therefore don't need any supplementation.

Fermentation residue from crude fermentation was found to be high in Essential Amino Acid Composition of Isoleucine, Leucine, Lysine, Threonine and Valine. Nevertheless, the Essential Amino Acid Composition of Methionine falls below the Ideal Essential Amino Acid. Hence Methionine needs supplementation. Fermentation residue from crude fermentation has Essential Amino Acid Composition of Histidine, Isoleucine, Leucine, Lysine, Threonine and Valine in abundance. Hence, supplementation of these amino acids is not necessary.

Fermented residue after enzyme saccharification was found to be low in Essential Amino Acid Composition of Isoleucine and Methionine. This is as a result of enzyme activities during tigernut fermentation. The Essential Amino Acid Composition fall below the Ideal Essential Amino Acid recommended daily intake. Hence, Isoleucine and Methionine need supplementation. The Essential Amino Acid Composition of Arginine, Histidine, Leucine, Lysine, Threonine and Valine are high and satisfy the daily recommended intake for chicks.

Fermentation residue from acid saccharification of tigernut tuber flour was found to be low in Essential Amino Acid Composition of Methionine, Arginine, Isoleucine, Lysine, Methionine, Phenylalanine, Threonine and Valine. All these Essential Amino Acid

Composition fall below the Ideal Essential Amino Acid permitted for daily consumption. Therefore, supplementation is required. The Essential Amino Acid Composition of Histidine and Leucine were high and satisfy the daily recommended intake for chicks.

When enzyme/acid saccharification was used, there was decrease in the Essential Amino Acid Composition of Methionine, but with increase in the Essential Amino Acid Composition of Arginine, Leucine, Lysine, Threonine and Valine. The Essential Amino Acid Composition for Isoleucine, and Methionine fell below the Ideal Essential Amino Acid recommended daily intake. Hence it needs supplementation. The Essential Amino Acid Composition of Arginine, Histidine, Leucine, Lysine, Threonine and Valine are high and okay for chicks.

Fermentation residue from sprouted tigernut also shows decrease in the Essential Amino Acid Composition for Methionine. However, there was increase in the Essential Amino Acid Composition of Leucine, Threonine, and Valine. The Essential Amino Acid Composition for Arginine, Isoleucine, Lysine, Methionine and Phenylalanine fell below the Ideal Essential Amino Acid recommended daily intake. Hence it needs supplementation. The Essential Amino Acid Score of Histidine, Leucine, Threonine, and Valine are very high and satisfactory for chicks.

5.1.10. Essential Amino Acid Scores for Piglets

The Essential Amino Acid Composition for piglets is reported in Table 4.12 and Appendix XIII. In un-sprouted tigernut tuber flour, Isoleucine, Lysine, Phenylalanine, and Threonine are deficient in Essential Amino Acid Composition when compared with the Ideal Essential Amino Acid recommended daily intake. The Essential Amino Acid Composition needs supplementation with meat meal. Arginine, Histidine, Leucine,

Methionine and Valine are very rich in their respective Essential Amino Acid Composition. Thus supplementation is not required.

Sprouting did not improve the concentration of all the amino acids that are lower than the Ideal Essential Amino Acid except Threonine. Isoleucine, Lysine and Phenylalanine are deficient in the Essential Amino Acid Composition, therefore supplementation is necessary. Only Arginine, Histidine, Leucine, Valine and Methionine have Ideal Essential Amino Acid greater than the recommended daily intake; and therefore don't need any supplementation.

Fermentation residue from crude fermentation was found to be high in Essential Amino Acid Composition of Lysine, Phenylalanine and Threonine. There was no decrease in the Essential Amino Acid Composition of Methionine below the Ideal Essential Amino Acid as observed in other treatment/feeds. Hence Methionine needs no supplementation. Fermentation residue from crude fermentation has Essential Amino Acid Composition of all the EAA in abundance. Hence, supplementation of these amino acids in fermentation residue from crude fermentation is not necessary.

Activities of enzyme in tigernut fermented residue reduce the Essential Amino Acid Composition of Methionine. Essential Amino Acid Composition of Isoleucine and Methionine fall below the Ideal Essential Amino Acid recommended daily intake for piglets (same as that of chicks). Hence they need supplementation. The Essential Amino Acid Composition of Arginine, Histidine, Leucine, Lysine, Phenylalanine, Threonine and Valine are very rich and satisfy the daily recommended intake for piglets (same as for chicks).

Fermentation residue from acid saccharification of tigernut tuber flour was found to be low in Essential Amino Acid Composition of Methionine. Nevertheless, there were

increase in Essential Amino Acid Composition of Lysine, Phenylalanine and Threonine only. Essential Amino Acid Score of Isoleucine and Methionine fall below the Ideal Essential Amino Acid permitted for daily intake. Therefore supplementation is required. The Essential Amino Acid Composition of Arginine, Histidine, Leucine, Lysine, Phenylalanine, Threonine and Valine are very rich and satisfy the daily recommended intake for piglets.

When enzyme/acid saccharification was employed, it was observed that the fermentation residue was low in the Essential Amino Acid Composition of Methionine. However, there was increase in the Essential Amino Acid Composition of Lysine, Threonine and Phenylalanine. The Essential Amino Acid Composition of Isoleucine and Methionine were found to be lower when compared with the Ideal Essential Amino Acid recommended daily intake for piglets. Therefore, supplementation of the diet with Isoleucine and Methionine is recommended. The Essential Amino Acid Composition of Arginine, Histidine, Leucine, Lysine, Phenylalanine, Threonine and Valine are very high and good for piglets.

Fermentation residue from sprouted tigernut was also found to be low in the Essential Amino Acid Composition for Methionine. Nevertheless, there were increase in the Essential Amino Acid Composition of Lysine, Threonine, and Phenylalanine. The Essential Amino Acid Composition for Isoleucine, and Methionine fell below the Ideal Essential Amino Acid recommended daily intake for piglets. Therefore, supplementation of the feed with Methionine is recommended. The Essential Amino Acid Composition of Arginine, Histidine, Leucine, Lysine, Phenylalanine, Threonine, and Valine are high and satisfactory for piglets.

5.1.11. Essential Amino Acid Scores for Rat

The Essential Amino Acid Score for rat are shown in Table 4.13 and Appendix XIV. In un-sprouted tigernut tuber flour, Isoleucine, Lysine, Phenylalanine, and Threonine are deficient in Essential Amino Acid Composition when compared with the daily recommended intake (Ideal Essential Amino Acid). They need supplementation with meat meal. Arginine, Histidine, Leucine, Valine and Methionine are very rich in their respective Essential Amino Acid Composition.

Sprouting did not improve the concentration of all the amino acids that were lower than the Ideal Essential Amino Acid (Isoleucine, Lysine, Phenylalanine, and Threonine). Only Arginine, Histidine, Leucine, Methionine and Valine have Essential Amino Acid Composition greater than the recommended daily intake; and therefore don't need any supplementation.

Fermentation residue from crude fermentation was found to be high in the Essential Amino Acid Composition of Isoleucine, Lysine, Phenylalanine and Threonine. Crude fermentation did not lower the Essential Amino Acid Composition of any amino acid. Fermentation residue from crude fermentation has Essential Amino Acid Composition of all EAA in great quantity. Hence, supplementation of these amino acids in fermentation residue from crude fermentation is not necessary.

Activities of enzyme in tigernut fermented residue reduce the Essential Amino Acid Composition of Methionine. Essential Amino Acid Composition of Isoleucine, Methionine and Phenylalanine were found to be low compared to the Ideal Essential Amino Acid recommended for daily intake. Hence they need supplementation. The Essential Amino Acid Composition of Arginine, Histidine, Leucine, Lysine, Threonine and Valine are very rich and satisfy the daily recommended intake for rat.

Fermentation residue from acid saccharification of tigernut tuber flour were found to be deficient in Essential Amino Acid Composition of Methionine but were found to be abundant in Lysine and Threonine only.

Essential Amino Acid Composition of Isoleucine, Methionine, and Phenylalanine, fell below the Ideal Essential Amino Acid accepted for daily consumption. Therefore, they need supplementation. The Essential Amino Acid Score of Arginine, Histidine, Leucine, Lysine, Threonine and Valine are very rich and satisfy the daily recommended intake for rat.

When enzyme/acid saccharification was employed, there was decrease in the Essential Amino Acid Composition of Methionine but there was increase in the Essential Amino Acid Score of Isoleucine, Lysine, and Threonine. The Essential Amino Acid Score for Phenylalanine, and Methionine fell below the Ideal Essential Amino Acid recommended for daily intake. However, supplementation may be required. The Essential Amino Acid Composition of Arginine, Histidine, Isoleucine, Leucine, Lysine, Threonine and Valine were found to be high and precise for rat.

Fermentation residues from sprouted tigernut also experience decrease in Essential Amino Acid Score for Methionine. Nevertheless, this follows with a corresponding increase in the Essential Amino Acid Composition of Isoleucine, and Threonine. The Essential Amino Acid Composition for Lysine, Methionine and Phenylalanine were found to be lower than the Ideal Essential Amino Acid recommended for daily intake. Hence it needs supplementation. The Essential Amino Acid Score of Arginine, Histidine, Isoleucine, Leucine, Threonine, and Valine are very high and in acceptable range for rat.

5.1.12. Anti-nutritional Contents of Un-sprouted and Processed Tigernut Tuber Flour

Table 4.14 and Appendix XV present the anti-nutrient content of tigernut tuber flour from un-sprouted, sprouted and fermentation residue.

The levels of anti-nutrients determined were very low compared to those reported for peanuts (Ejigui *et al.,* 2005). Phytates in biological systems may chelate divalent metals like calcium and magnesium, or block the absorption of essential minerals in the intestinal tract, thus decreasing their bioavailability (Chukwuma *et al.,* 2010). Phytates chelate with mineral elements, thereby having an effect on the utilization of minerals. They also react with basic remains of protein.

Tannins, and to some extent oxalates, bind to proteins, thereby making them difficult to digest in the body. Tannins were not detected in both un-sprouted and sprouted tigernut tuber flour.

Oxalates can remove calcium in the form of calcium oxalate in the blood and thus may result in kidney damage (Akintonwa & Tunwashe 1992). Odumudu (1992) and Okafor *et al.* (2003) had earlier reported low contents of these anti-nutrients in tigernut tuber flour compared with other fruits, nuts, tubers, and vegetables.

Preliminary investigations on the acute toxicity of the tuber extract of tigernut in mice showed that the aqueous extract of tigernut tuber was not toxic to mice at the administered concentrations (Chukwuma *et al.,* 2010).

Phytic acid, an antinutritional factor, occurs primarily in the seed coats and germ tissue of plant seeds. It forms insoluble or nearly insoluble compounds with many metal ions, including those of calcium, iron, magnesium and zinc, reducing their dietary

availability. Diets high in phytic acid content and poor in these minerals produce mineral deficiency in experimental animals (Chukwuma *et al.,* 2010). It is also found that sprouting decrease levels of phytic acid. Similarly, Shipard (2005) stated that enzymes of germination and sprouting can help eliminate detrimental antinutritional substances. However, reduction in amount of antinutrients as a result of soaking is very marginal. Therefore, soaking of tigernut is not enough to counteract antinutrient effects (Egli *et al*., 2002).

5.2 Conclusion

Tigernut is found to be rich in essential minerals, oil and carbohydrate. The physicochemical parameters of tigernut oil are comparable with the physicochemical parameters of olive and avocado oil. The difference between them is colour and taste. Tigernut oil is tasteless and appears golden yellow. Higher yield of ethanol is recorded for all samples that were saccharified prior to fermentation. The fermentation residue is found to be rich in protein, fibre and essential amino acids. Some essential amino acids (Phenylalanine, Methionine, Arginine & Lysine) need supplementation, before they can be used as animal feed. The fermentation residue has minimum level of antinutrients. Hence, it is a potential source of animal feed.

5.3 Recommendation

Tigernut is a crop that grows well in the Northern part of Nigeria. It is a crop with huge potentials for its exploitation for production of high quality vegetable oil, ethanol and animal feed. Further investigation of the crop with a view to expanding its uses in vegetable oil, ethanol and animal feed is warranted.

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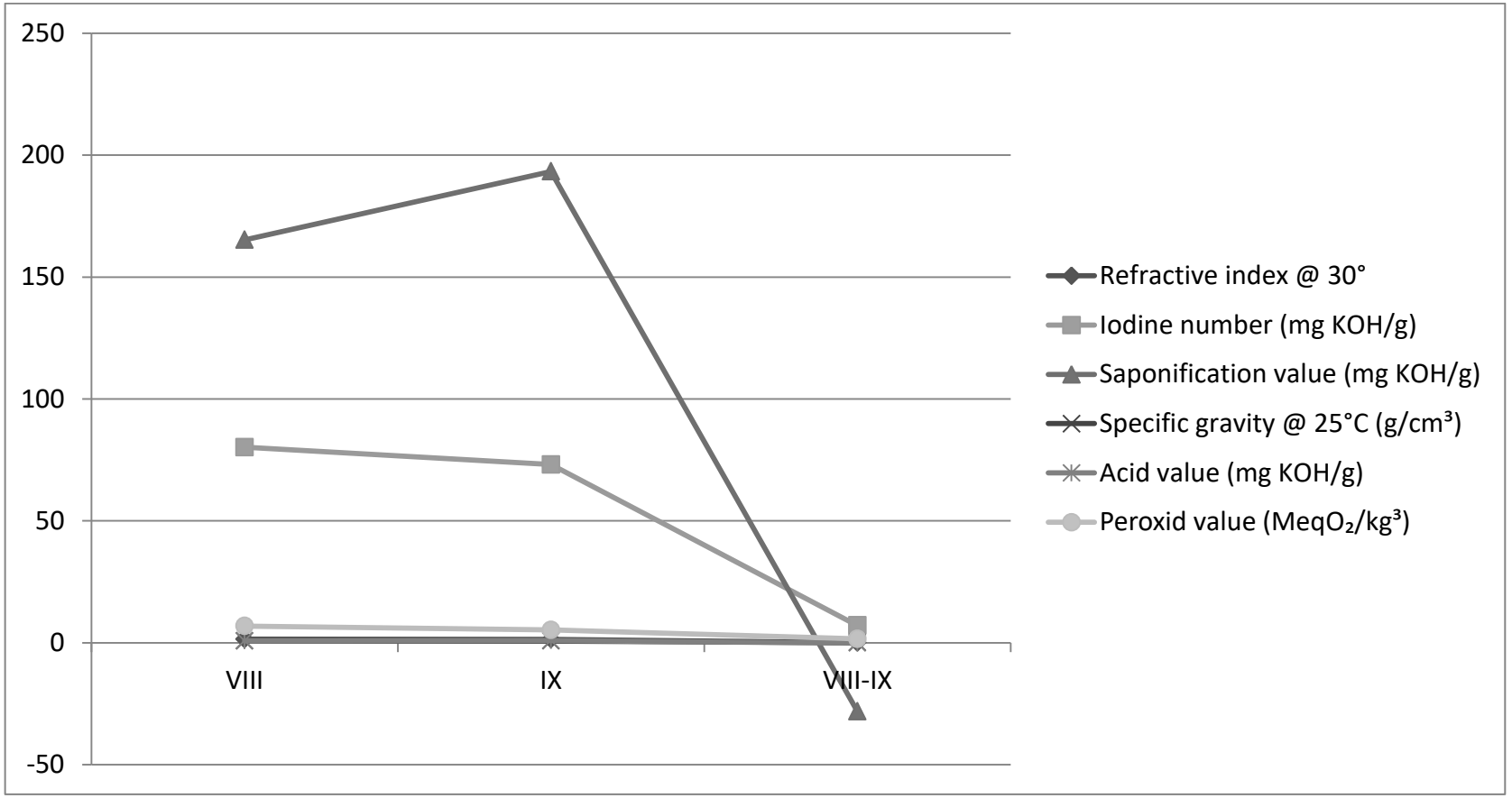
APPENDIX I

ABBREVIATIONS OF AMINO ACIDS AND MEANINGS

Ser	Serine
Glu	Glutamic acid
Pro	Proline
Gly	Glycine
Ala	Alanine
Cys	Cysteine
Tyr	Tyrosine
Lys*	Lysine
His*	Histidine
Arg*	Arginine
Asp	Aspartic acid
Thr*	Threonine
Val*	Valine
Met*	Methionine
Ile*	Isoleucine
Leu*	Leucine
Phe*	Phenylalanine

APPENDIX II

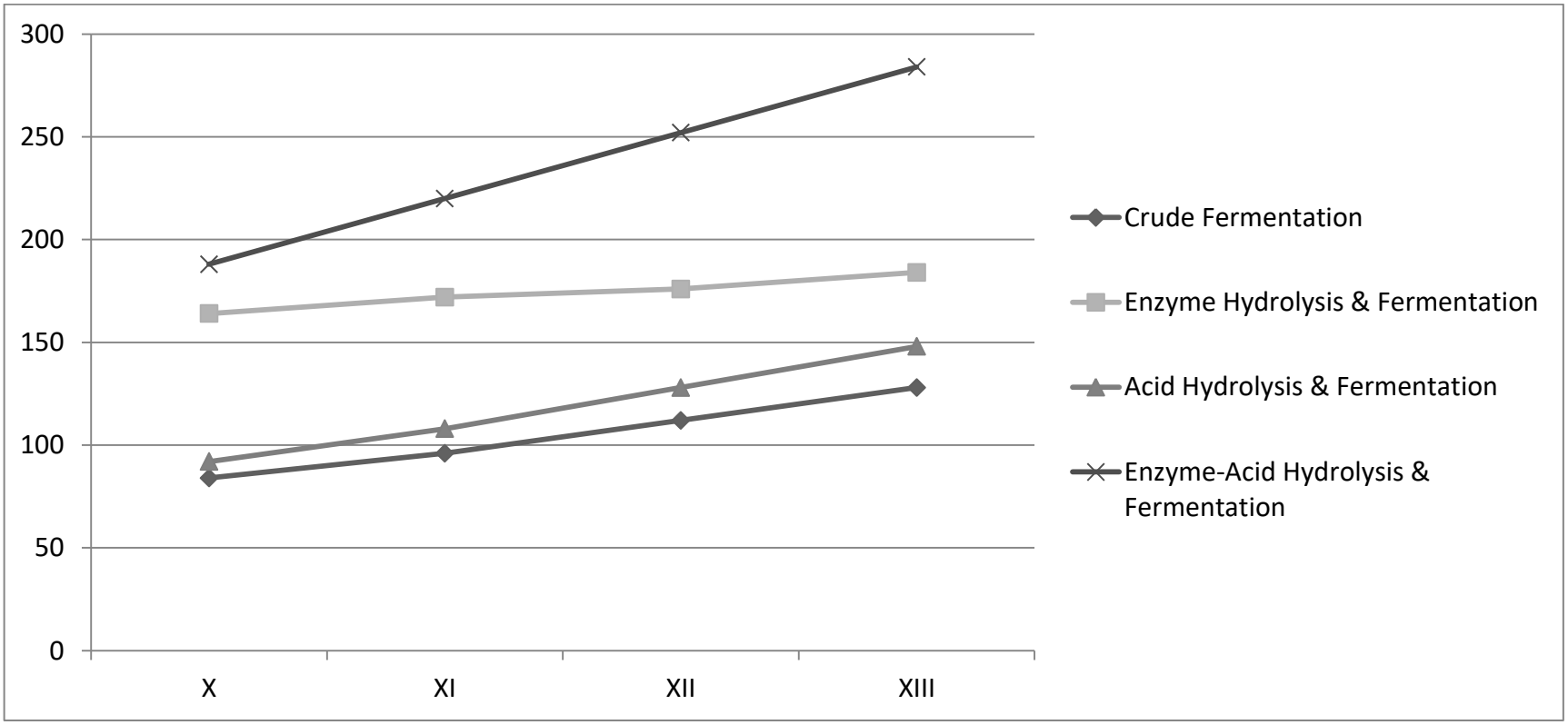
PLOT OF PHYSICOCHEMICAL PROPERTIES OF TIGERNUT OIL



VIII = Oil from un-sprouted tigernut tuber flour; IX = Oil from sprouted tigernut; VIII-IX = Difference.

APPENDIX III

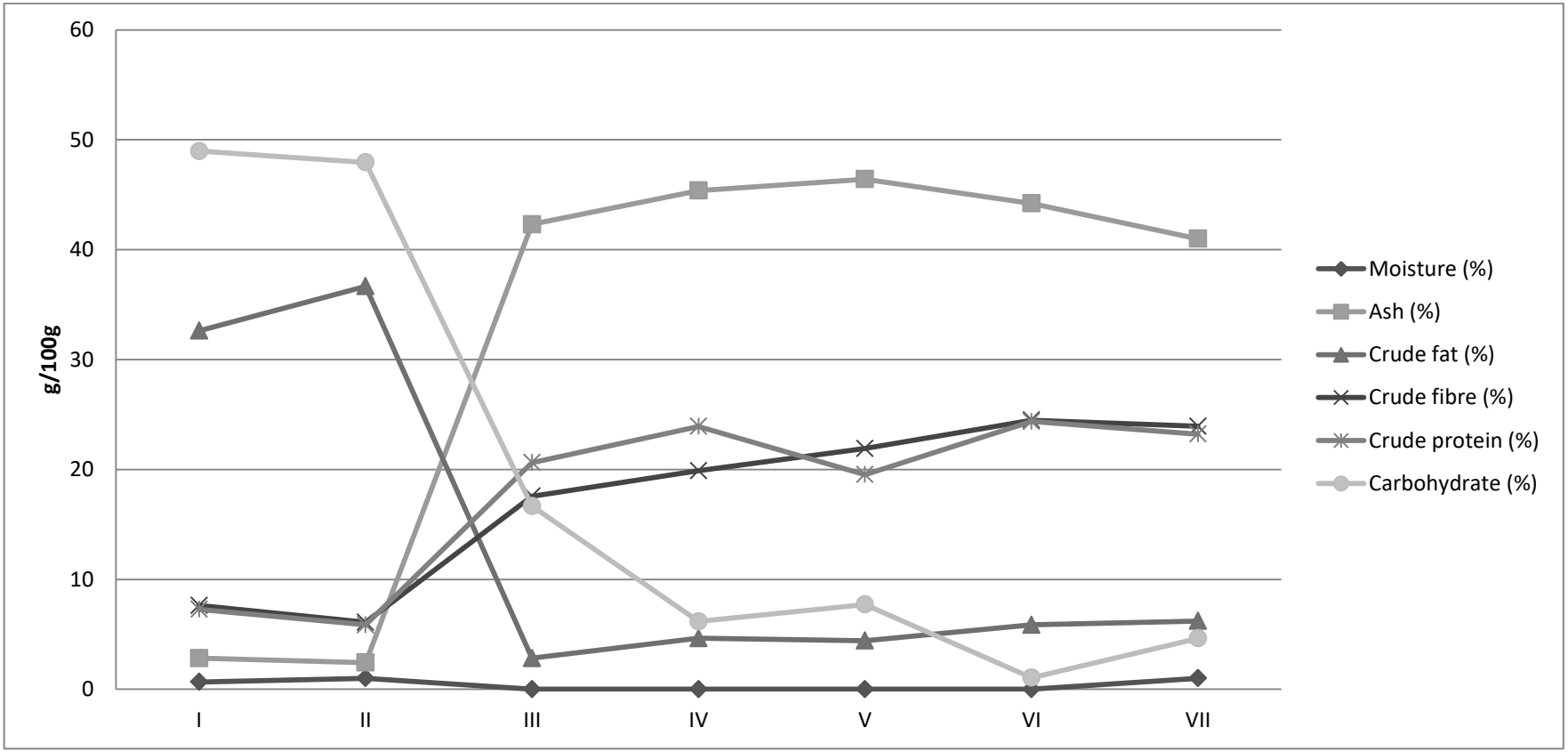
PLOT OF VOLUME OF ETHANOL RECOVERED FROM FERMENTATION OF TIGERNUT (cm^3 per Kg dry matter)



X = Un-sprouted (un-extracted) tigernut; XI = Sprouted (un-extracted) tigernut; XII = Residue of un-sprouted tigernut after oil extraction; XIII = Residue of sprouted tigernut after oil extraction.

APPENDIX IV

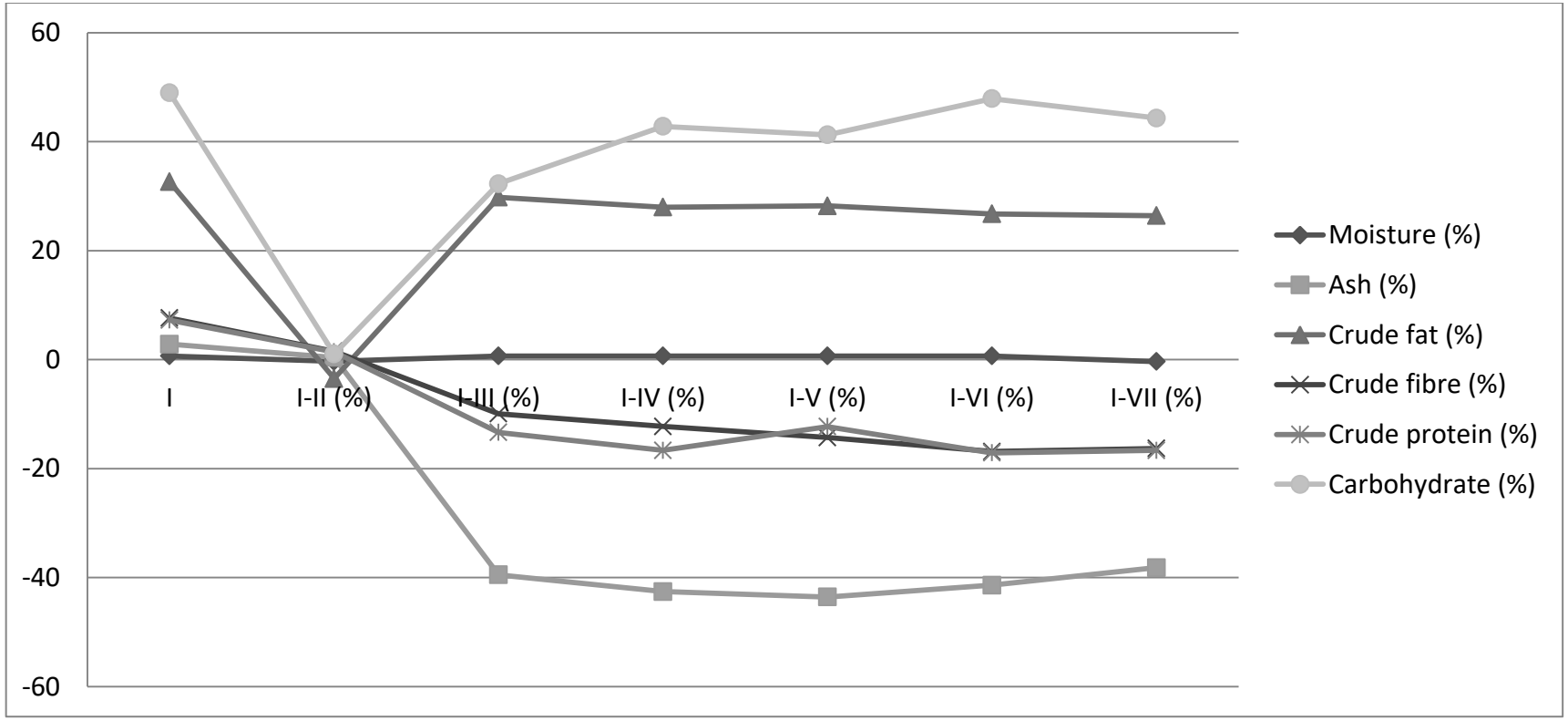
PLOT OF PROXIMATE COMPOSITION OF UN-SPROUTED AND PROCESSED TIGERNUT TUBER FLOUR (g/100g dry matter)



I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue pfollowing acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting

APPENDIX V

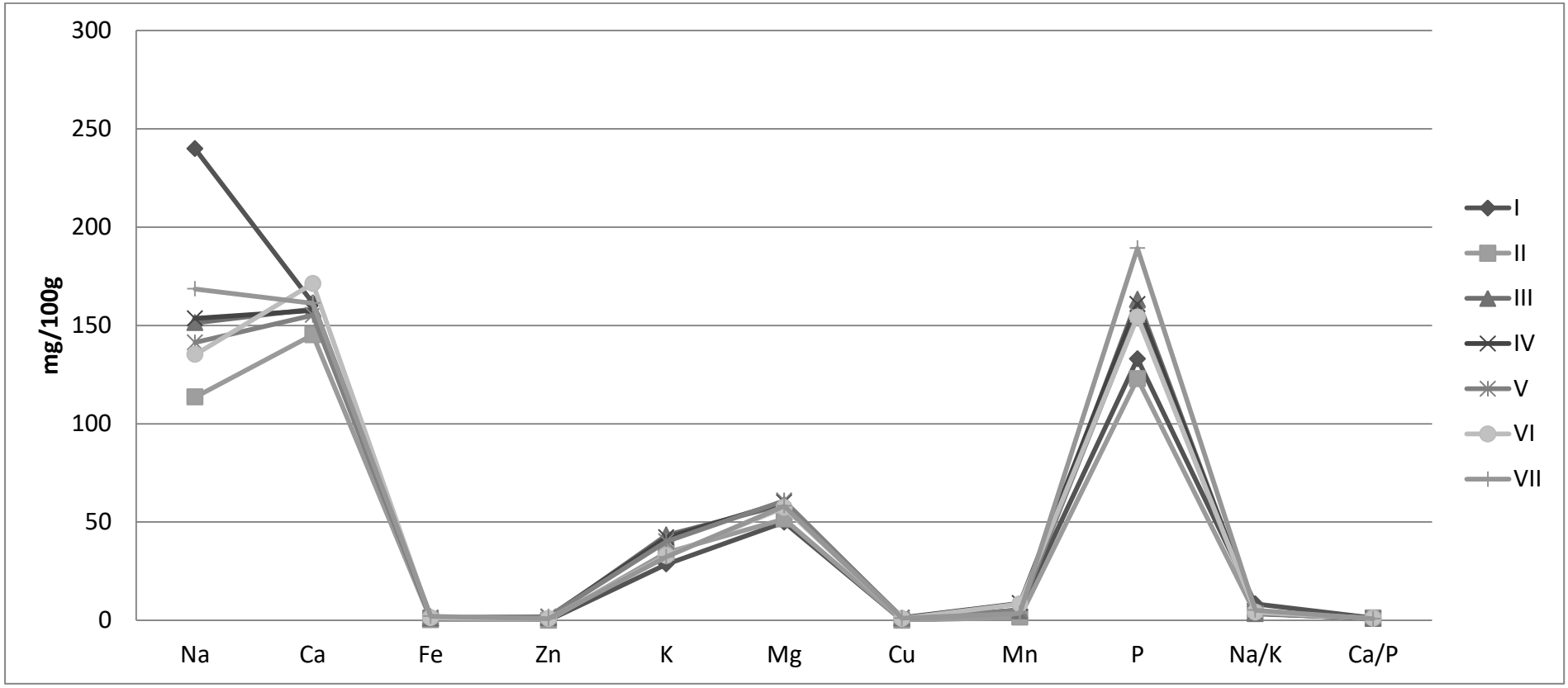
PLOT OF DIFFERENCE IN PROXIMATE COMPOSITION OF UN-SPROUTED AND PROCESSED TIGERNUT FLOUR (g/100g dry matter)



I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting.

APPENDIX VI

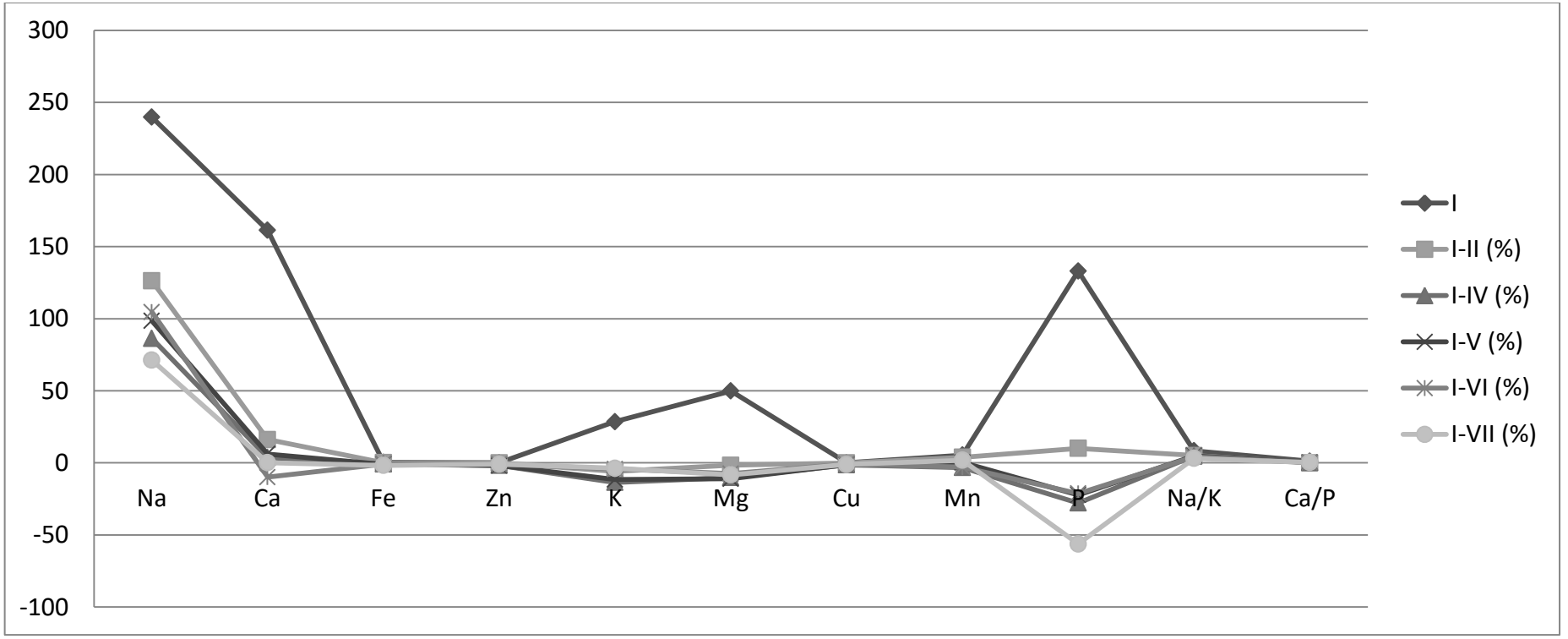
PLOT OF MINERAL COMPOSITION OF UN-SPROUTED AND PROCESSED TIGERNUT (mg/100g dry matter)



I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting.

APPENDIX VII

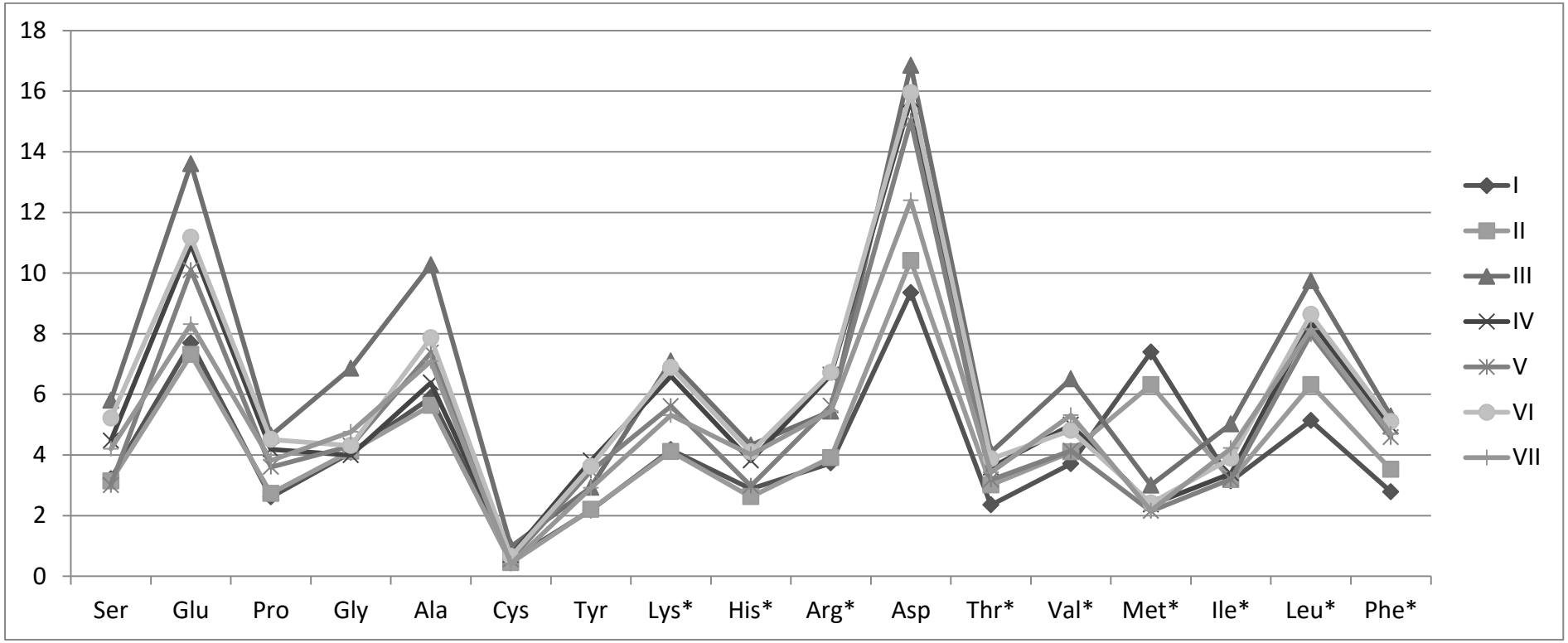
PLOT OF DIFFERENCE IN MINERAL COMPOSITION OF UN-SPROUTED AND PROCESSED TIGERNUT FLOUR (mg/100g dry matter)



I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting.

APPENDIX VIII

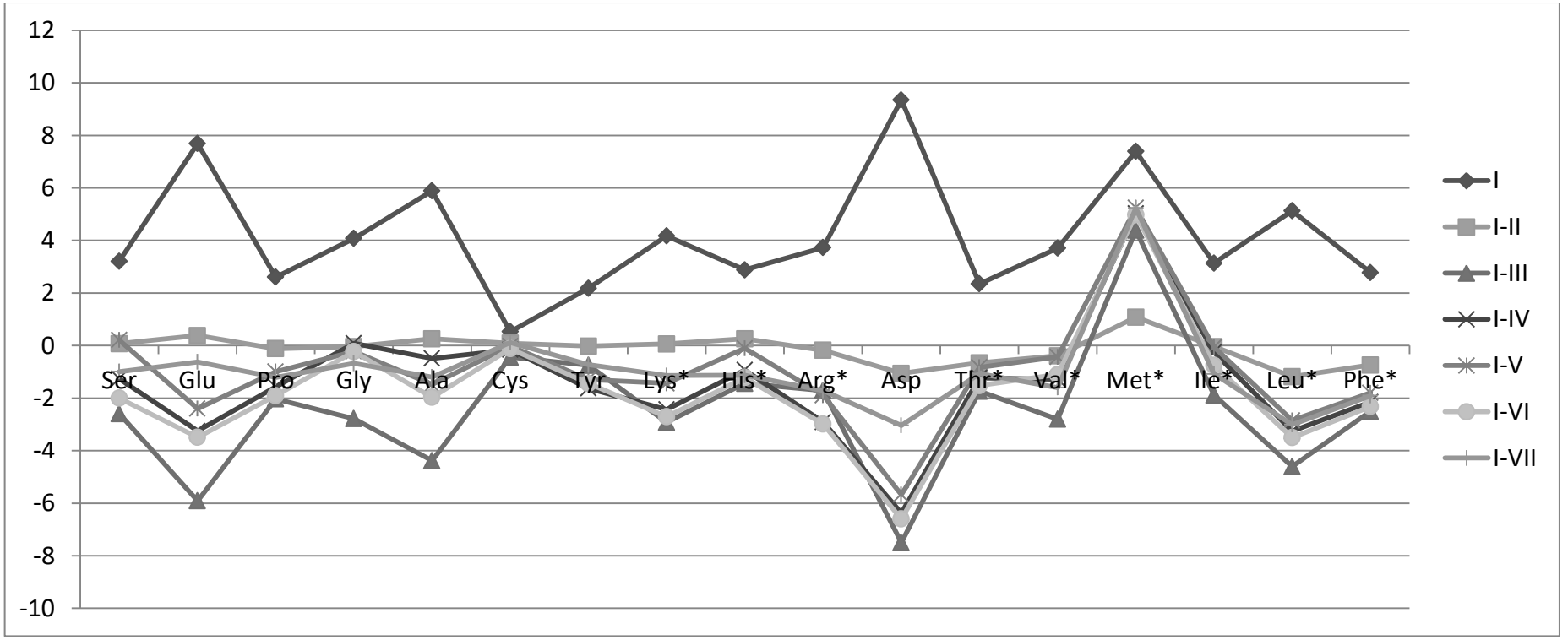
PLOT OF AMINO ACID COMPOSITION OF UN-SPROUTED AND PROCESSED TIGERNUT FLOUR (mg/100g crude protein)



* = Essential amino acids; I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting.

APPENDIX IX

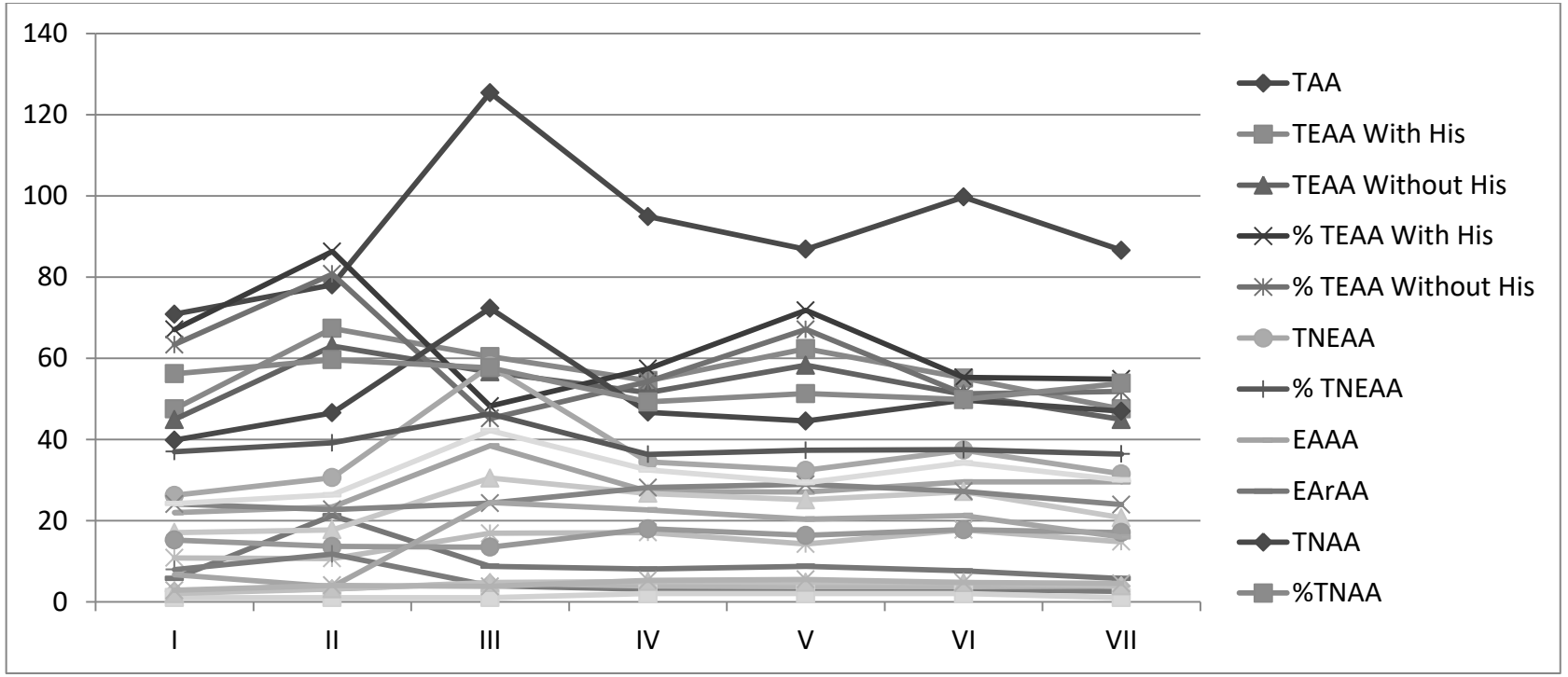
PLOT OF DIFFERENCE IN AMINO ACID COMPOSITION OF UN-SPROUTED AND PROCESSED TIGERNUT FLOUR (mg/100g dry matter)



* = Essential amino acids; I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting.

APPENDIX X

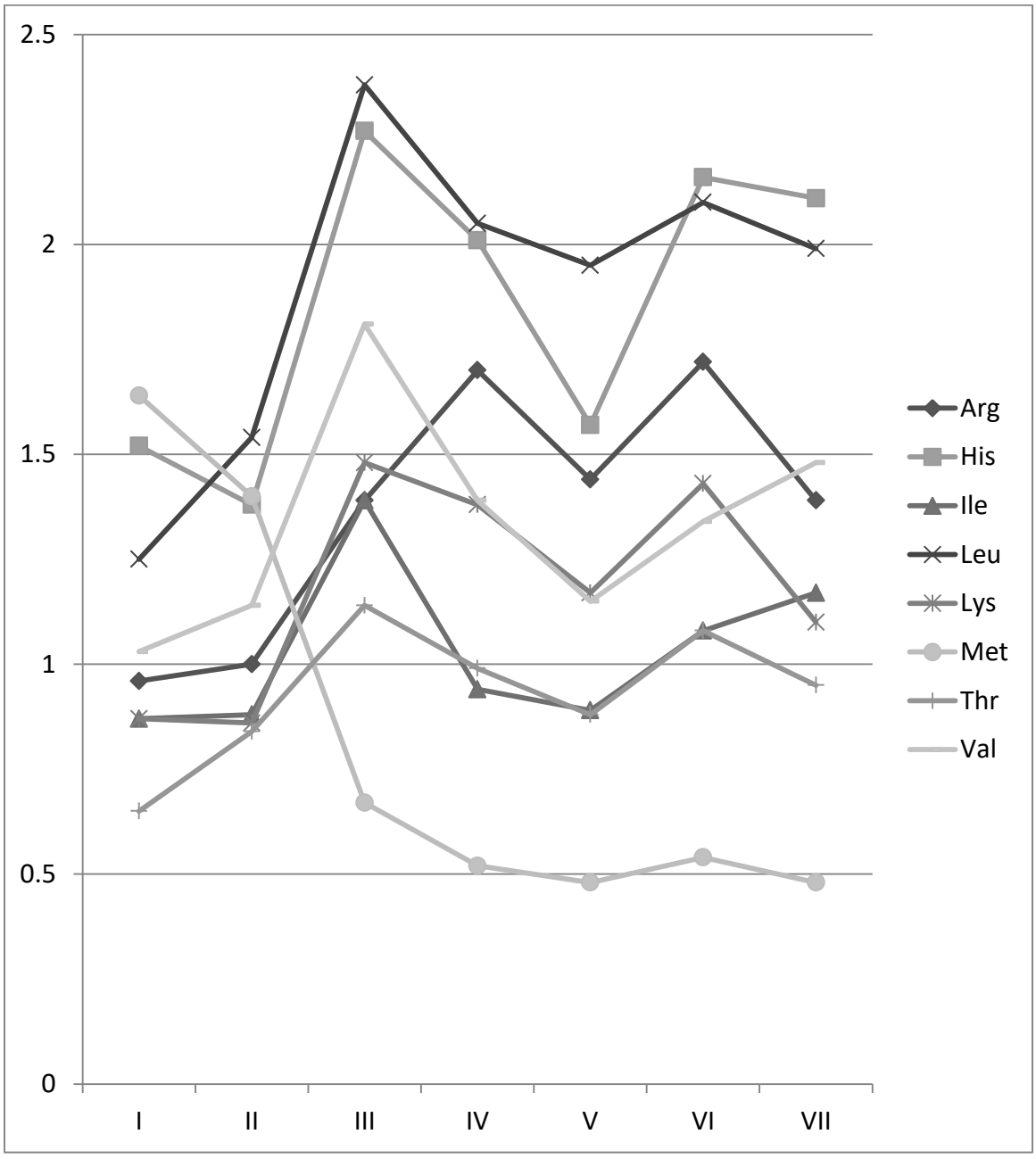
PLOT OF COMPOSITIONS OF ESSENTIAL, NON-ESSENTIAL, ACIDIC, NEUTRAL, SULPHUR, AROMATIC OF UN-SPROUTED AND PROCESSED TIGERNUT FLOUR



TAA= Total Amino Acid; TEAA= Total Essential Amino Acid; TNEAA= Total Non-Essential Amino Acid; EAAA= Essential Aliphatic Amino Acid; EArAA= Essential Aromatic Amino Acid; TNAA= Total Neutral Amino Acid; TAAA= Total Acidic Amino Acid; TBAA= Total Basic Amino Acid; Total Sulphur Amino Acid; I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting.

APPENDIX XI

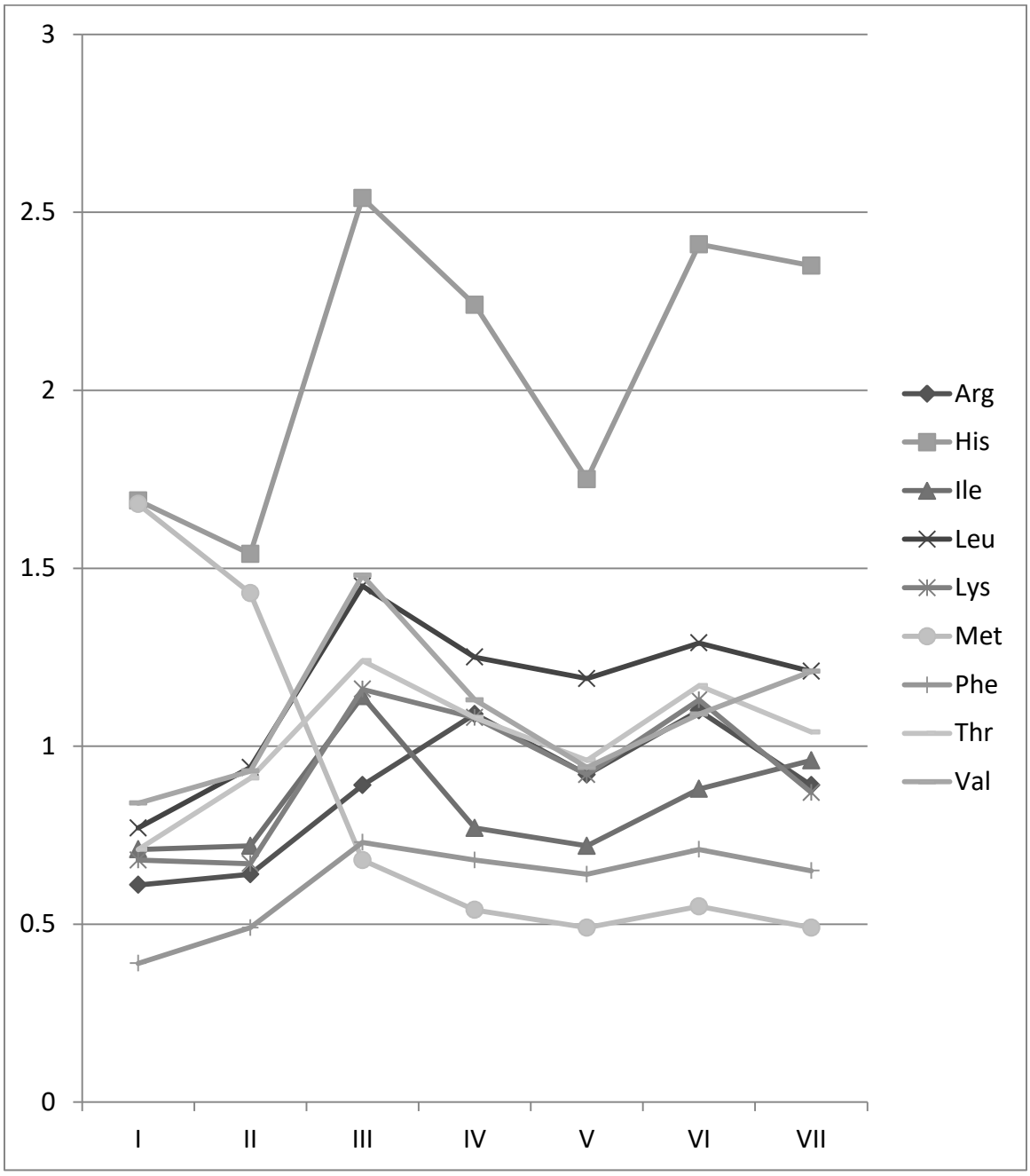
PLOT OF ESSENTIAL AMINO ACID SCORES FOR FINGERLINGS



I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting.

APPENDIX XII

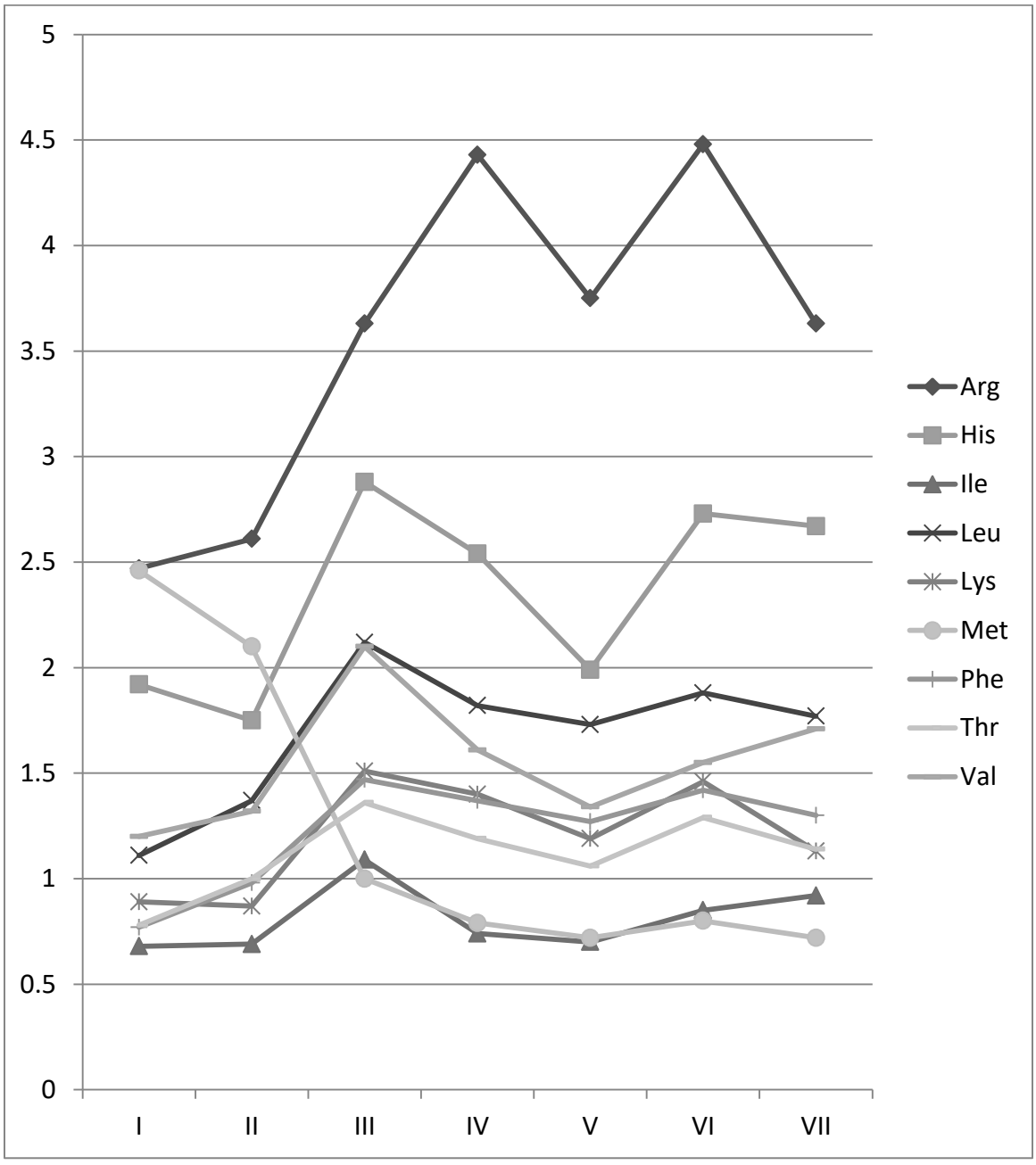
PLOT OF ESSENTIAL AMINO ACID SCORES FOR CHICKS



I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting.

APPENDIX XIII

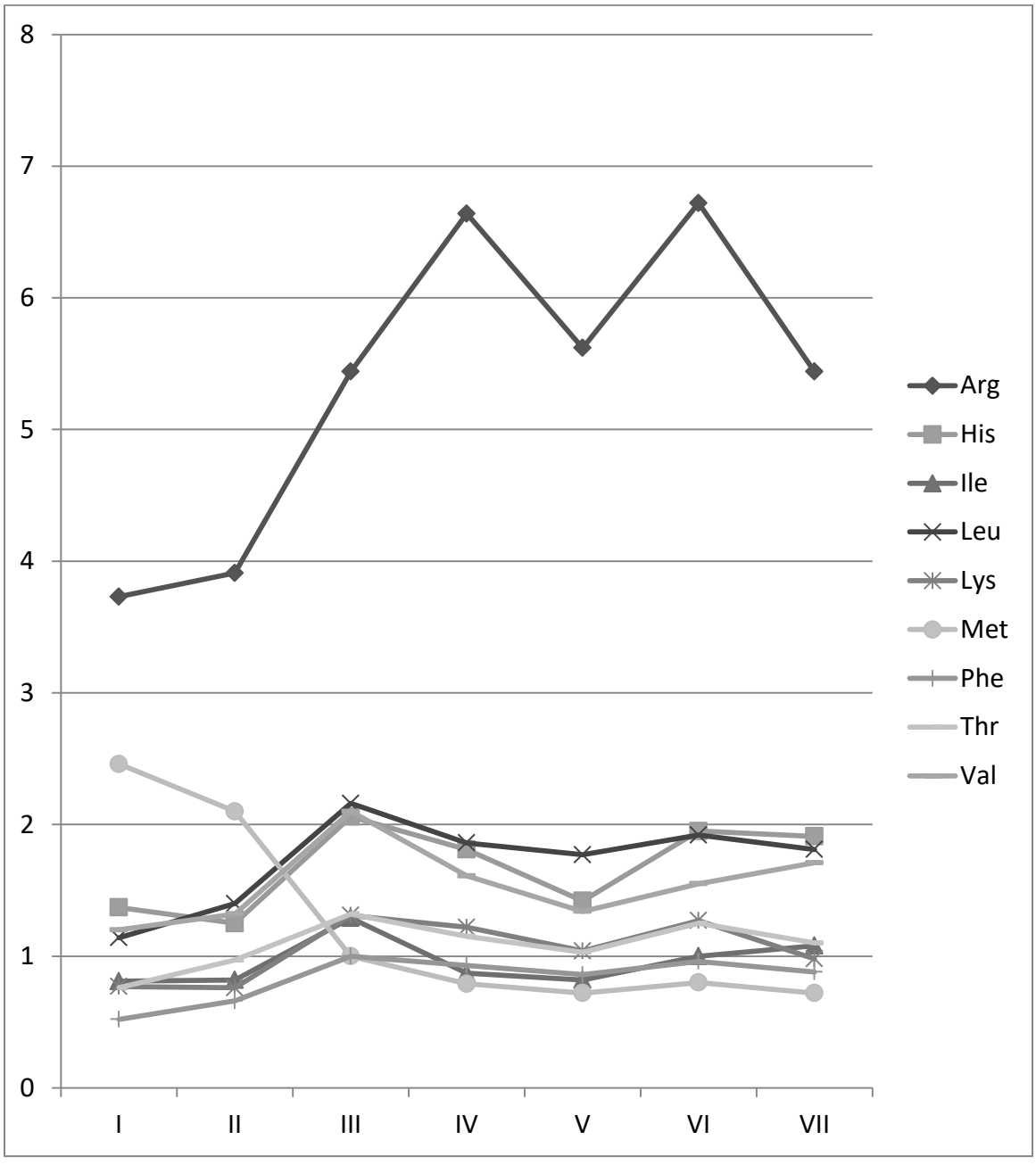
PLOT OF ESSENTIAL AMINO ACID SCORES FOR PIGLETS



I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting.

APPENDIX XIV

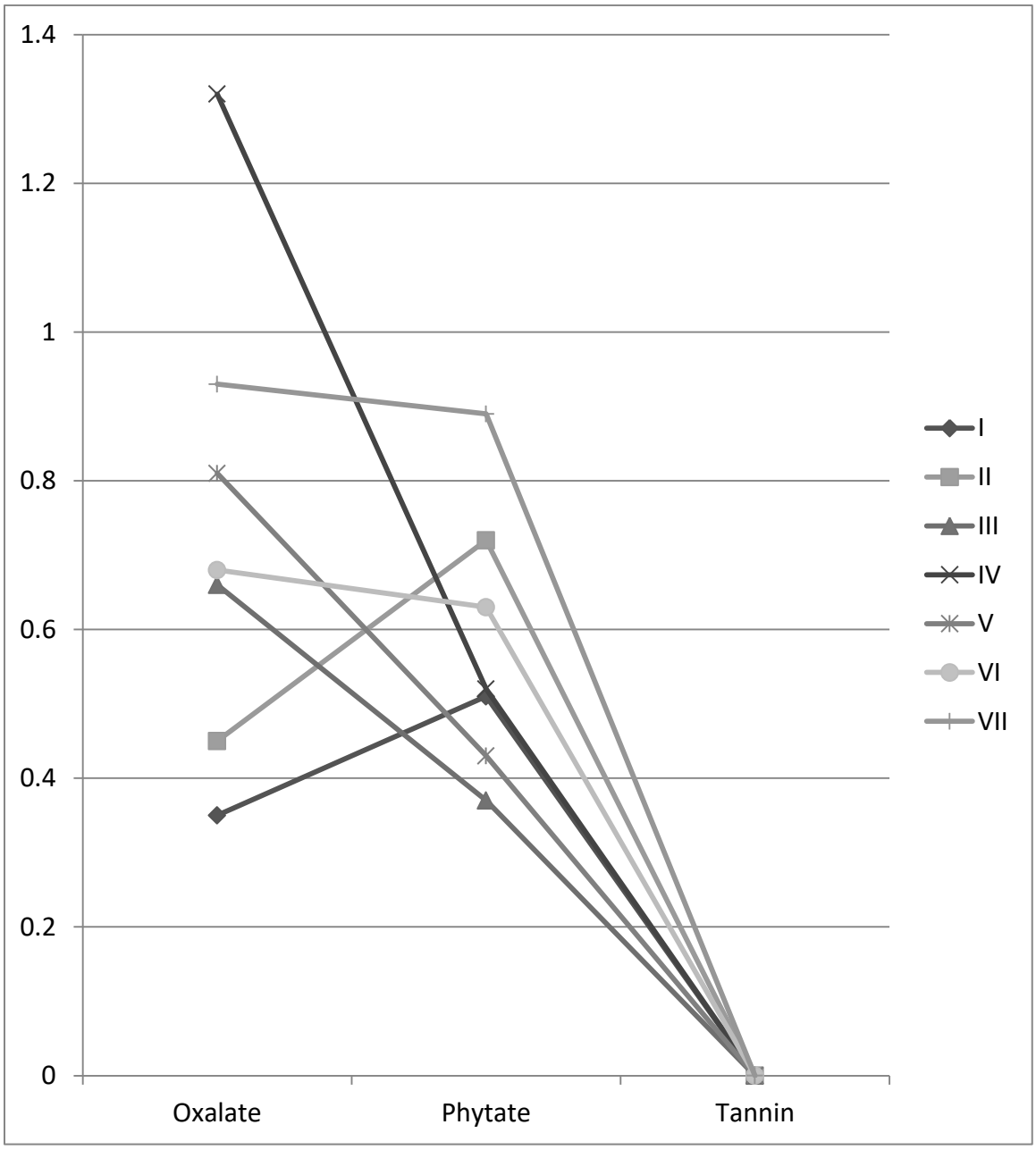
PLOT OF ESSENTIAL AMINO ACID SCORES FOR RATS



I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting.

APPENDIX XV

PLOT OF ANTI-NUTRIENT CONTENTS OF UN-SPROUTED AND PROCESSED TIGERNUT TUBER FLOUR (mg/100g dry matter)



I = Un-sprouted sample; II = Sprouted sample; III = Fermentation residue following crude fermentation; IV = Fermentation residue following enzyme hydrolysis; V = Fermentation residue following acid hydrolysis; VI = Fermentation residue following enzyme/acid hydrolysis; VII = Fermentation residue following sprouting.