EFFECT OF DIFFERENT PROCESSING METHODS ON THE CHEMICAL COMPOSITION OF AFRICAN YAM BEAN (*SPHENOSTYLIS STENOCARPA*) FLOURS AND ORGANOLEPTIC CHARACTERISTICS OF THEIR GRUELS.

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UNIVERSITY OF NIGERIA, NSUKKA

MARCH, 2012.

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A PROJECT REPORT SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIRMENTS FOR THE AWARD OF MASTER'S DEGREE IN HUMAN NUTRITION

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DEDICATION

This work is dedicated to God Almighty.

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TABLE OF CONTENTS

Title page - - - - - - - - - i
Approval page - - - - - - - - - ii
Certification - - - - - - - - - iii
Dedication - - - - - - - - - iv
Acknowledgements - - - - - - - - - v
Table of contents - - - - - - - - -
List of tables - - - - - - - - - vi
List of figures - - - - - - - - - vii
List of appendices - - - - - - - - - viii
ABSTRACT - - - - - - - - - xii

CHAPTER ONE: INTRODUCTION - - - - - - 1
1.1 Statement of the problem - - - - - - - - - 3
1.2 Objectives of the study - - - - - - - - - 4
1.3 Significance of the study - - - - - - - - - 4

CHAPTER TWO: LITERATURE REVIEW - - - - -- 6
2.1 Legumes in human nutrition - - - - - - - - 6
2.2 Functional properties of legumes - - - - - - - - 7
2.3 Legumes in diet-related non communicable disease - - - - 8
2.4 Bean as a legume - - - - - - - - - 9
2.5 African yam beans (AYB) (*Sphenostylis stenocarpa)* - - - - 9
2.5.1 Nutritional/ chemical composition and organoleptic attributes of AYB - 10
2.5.2 Antinutrient composition of 3 varieties of African yam bean - - - 13
2.5.3 Economic importance/uses of AYB - - - - - - - 13
2.5.4 Constraints in the use of AYB - - - - - - - - 14
Hydrogen cyanide (HCN) - - - - - - - - 15
Hemagglutinin (Lectins) - - - - - - - - 15
2.5.5 The antinutrients in AYB - - - - - - - - 15
Phytic acid - - - - - - - - - 16
Tannins - - - - - - - - - 16
Saponins - - - - - - - - - 16
Trysin inhibitors - - - - - - - - 17
Oxalates - - - - - - - - - 17
2.5.6 AYB processing methods used - - - - - - - - 18
Roasting - - - - - - - - - 18
Fermentation - - - - - - - - - 18
2.6 Lime - - - - - - - - - - 20

CHAPTER THREE: MATERIALS AND METHODS - - - - 21
3.1 Materials - - - - - - - - - 21
3.2.1 Preparation of African yam bean (AYB) flour for gruel and chemical analysis 21
24h fermentation without lime and roasting - - - - - 21
24h fermentation with lime and roasting - - - - - 21
48h fermentation with lime and roasting - - - - - 21
Roasted only - - - - - - - - - 22
3.2.2 Preparation of gruel from AYB flours - - - - - 22
3.3 Laboratory analysis - - - - - - - - 22
3.3.1 Protein determination - - - - - - - - 23

3.3.2 Fat determination - - - - - - - - 24
3.3.3 Ash determination - - - - - - - - 24
3.3.4 Crude fibre determination - - - - - - - 25
3.3.5 Carbohydrate - - - - - - - - - 25
3.3.6 Phytate determination - - - - - - - 25
3.3.7 Tannins - - - - - - - - - 26
3.3.8 Determination of trypsin inhibitors - - - - - - 27
3.3.9 Oxalic acid determination - - - - - - - 28
3.3.10 Determination of saponins - - - - - - - 29
3.3.11 Hydrocyanic acid determination - - - - - - 30
3.3.12 Determination of Haemagglutinin by spectrometric method - - - 31
3.3.13 Raffinose and stachyose determination - - - - - 31
3.4 Organoleptic evaluation - - - - - - - 32
3.5 Statistical analysis - - - - - - - - 33

CHAPTER FOUR: RESULTS - - - - - - - 34
4.1 Proximate composition of different AYB flours on dry matter basis - -
4.1.1 Protein - - - - - - - - - 34
4.1.2 Fat - - - - - - - - - - 34
4.1.3 Ash - - - - - - - - - - 34
4.1.4 Crude fibre - - - - - - - - - 34
4.1.5 Carbohydrate (CHO) - - - - - - - - 35
4.2 Effect of treatments on the anti nutrient contents of AYB flour samples -
4.2.1 Phytate - - - - - - - - - 35
4.2.2 Tannins - - - - - - - - - 35
4.2.3 Oxalates - - - - - - - - - 35
4.2.4 Saponins - - - - - - - - - 35
4.2.5 Trypsin inhibitors - - - - - - - - 35
4.3 Effect of treatment on the raffinose, stachyose, heamagglutinins and hydrogen cyanide composition of AYB flours - - - - - -
4.3.1 Raffinose - - - - - - - - - 37
4.3.2 Stachyose - - - - - - - - - 37
4.3.3 Heamagglutinins - - - - - - - - 37
4.3.4 Hydrogen cyanide (HCN) - - - - - - - 38
4.4 Organoleptic characteristics of AYB gruel - - - - -
4.4.1 Colour - - - - - - - - - 38
4.4.2 Flavour - - - - - - - - - 39
4.4.3 Consistency - - - - - - - - - 39
4.4.4 Degree of acceptability - - - - - - - 39

CHAPTER FIVE:DISCUSSION, CONCLUSION AND RECOMMENDATIONS 41
5.1 Discussion - - - - - - - - - -
5.1.1 Effect of processing on the proximate composition of AYB flours - -
Protein - - - - - - - - - 41
Fat - - - - - - - - - 41
Crude fibre - - - - - - - - - 41
Carbohydrate - - - - - - - - - 42
5.1.2 Effect of treatments on the anti nutrient composition - - -
Phytate - - - - - - - - - 42
Oxalates - - - - - - - - - 42
Tannin - - - - - - - - - 42

	Trypsin inhibitors - - - - - - - -	43
	Effect of processing on the raffinose and stachyose contents of AYB flours	43
5.1.3	Effect of treatment on the toxic substance composition of AYB flour samples	
	Heamagglutinin - - - - - - - -	43
	Hydrogen cyanide (HCN) - - - - - - -	44
5.1.4	Effect of treatment on the beany flavour and other organoleptic characteristics of AYB gruel - - - - - - -	
	Colour - - - - - - - - -	44
	Flavour - - - - - - - - -	44
5.2	Conclusion - - - - - - - - -	45
5.3	Recommendations - - - - - - - -	45
REFERENCES	- - - - - - - - -	46
APPENDICES	- - - - - - - - -	53

LIST OF TABLES

Table 1: Proximate (% dry matter) and mineral (mg/100g dry matter) composition of cream, brown and brown spotted (speckled) raw AYB seeds - - 12

Table 2: Amino acid distribution of AYB - - - - - - 13

Table 3: Antinutrient composition of 3 varieties of African yam bean (raw seed characterization) - - - - - - - - 13

Table 4: Effect of treatments on proximate composition of different AYB flour samples on dry matter basis (%) - - - - - - - 34

Table 5: Anti nutrient composition of AYB flours (mg/g) - - - - 36

Table 6: Raffinose, stachyose, heamagglutinins and hydrogen cyanide composition of AYB flours - - - - - - - - - 38

Table 7: Organoleptic characteristics of AYB gruels - - - - - 39

LIST OF FIGURES

Figure1. Flow chart for the processing of AYB flour - - - - 23

LIST OF APPENDICES

Appendix I:	Hedonic scoring form for the evaluation of AYB gruel - -	53
Appendix II:	General acceptability rating of the sample - - - -	54
Appendix III:	Raw cream coloured AYB seed - - - - -	55
Appendix IV:	Gruels from AYB flour samples - - - - -	56

ABSTRACT

The aim of this study was to determine the effect of different processing methods on the chemical composition of African yam bean (*Sphenostylis stenocarpa*) flours and the organoleptic properties of it's gruels. The seeds of cream coloured African yam bean (AYB) and lime were purchased from Oye Igbo-Eze and Ogige Nsukka markets, respectively in Enugu State, Nigeria. The seeds were sorted and divided into 4 equal portions of 1.5kg each. One portion was washed and fermented in tap water at a seed- water ratio of 1:3 (w/v), for 24h. The second portion was fermented in tap water (1:3 w/v) containing 30 tablespoonful of freshly squeezed lime for 24h. The third portion was fermented in tap water containing lime (30 tablespoonful of lime) (1:3 w/v) for 48h. They were separately sundried for 72h and roasted in a hot sauce pan until cracking. The fourth portion (control) was washed, drained and roasted in a hot sauce until cracking. The AYB samples were separately milled into fine flours and stored in separate airtight containers for chemical analysis and gruel preparations. The flour samples were chemically analyzed for proximate, phytate, tannins, oxalates, saponins, trypsin inhibitors, raffinose, stachyose, hemagglutinins and hydrogen cyanide composition using standard laboratory methods. A nine point hedonic scale was used to collect data on sensory and acceptability tests of the gruels. Means and standard deviations were calculated and least significance difference test was used to separate means. The sample that was fermented in tap water containing lime for 48h ranked best generally. The moisture levels for the flours ranged from 3.60-5.00%, protein 19.96-31.87%, fat 3.54-5.23%, ash 2.99-3.89%, crude fibre 4.00-6.01% and carbohydrate 52.72-62.32%. The anti-nutrient values for the flours were: phytate 2.63-2.97mg/g, tannins 0.02-0.04mg/g, trypsin inhibitors 0.45-0.53mg/g, oxalate 0.01-0.03mg/g, the samples had the same saponin level of 0.01mg/100g. Raffinose contents of the samples ranged from 8.25mg-9.22/100g and stachyose (8.48-6.76mg /100g). heamagglutinins ranged from 4.87 – 6.70 mg/100g and hydrogen cyanide ranged from 0.22-0.28mg/g. In the organoleptic studies, the sample that was fermented for 48h was most preferred over others in terms of colour (6.53), the sample that was fermented in lime water for 24h ranked best for flavor (6.57) and the sample that was only roasted ranked best for general acceptability (6.03).

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

It is of great importance to know the nutrient, toxic substance as well as the anti physiological substance composition and organoleptic properties of locally available foods in any community or country. Knowledge and use of local foods can help eliminate malnutrition. One of the problems of planning therapeutic diets with local foods is limited information on their nutrient composition (Standing Committee on Nutrition (SCN), 2006). It has been proposed that the fight against malnutrition in developing countries should be on the use of mixtures of tubers, cereals and legumes indigenous to them (Nnam, 2003).Urbanization has made people forget their traditional foods and favour convenient foods which are mostly nutritionally inadequate and expensive. The most dietary deficit is protein of high biological value and this was attributed to the high cost of animal protein (SCN, 2006). Vegetable proteins however complement each other if well chosen and will have a nutritive value as good as animal protein (Achinewhu & Akah, 2003; Nnam, 2003; Obiakor, 2008).

Nutrition is coming to the fore front as a major modifiable determinant of chronic diseases, with scientific evidence increasingly supporting the view that alterations in diet have strong effects (both positive and negative) on health throughout life. Dietary adjustments may not only influence present health, but may determine whether or not an individual will develop such diseases as diabetes, obesity, hypertension, certain cancer and cardiovascular disease much later in life (WHO/FAO, 2003). Rapid change in disease pattern had occurred as a result of shifts in diet and lifestyle. The urban based Nigerian is shifting from exercise, intense agrarian life to a more sedentary urban life, with resultant obesity, diabetes and hypertension. Cheap imported foods, global markets and socio-cultural changes are placing African traditional diets at distinct disadvantages. Indigenous diets are being replaced with more refined carbohydrate fast foods (Ifeyironwa, Eyzaquirre, Matig, & Johns, 2006). In tackling the

multiple problems of food insecurity, nutrition transition and the double burden of diseases, it is essential to mobilize and employ indigenous foods like legumes as part of the solution (SCN, 2006). This is because several studies have reported immense nutritional and health protecting properties of African indigenous foods such as legumes (Obizoba & Souzey, 1989; Enwere, 1998; Ene-Obong & Carnovale, 1992; SCN, 2006; Okeke, Ene-Obong, Uzuegbunam, Simon, & Chukwuone, 2009).

For quite some time, legumes were considered not too important; but now, their food use is increasing with recent discoveries concerning their many nutritional and health properties (Pamplona-Roger, 2006).It has been documented that legumes contain 2-3 times the protein of cereal grains and no other plant food is as rich in protein as legumes in their natural state (National Academy of Science (NAS), 1997; Pamplona-Roger, 2006). Water soluble non-starch polysaccharides (NSP) that have viscous properties occur mostly in legumes and its benefit in the prevention/management of diabetes and cardiovascular diseases have been reported (Onyechi, Jude, & Ellis., 1998; Enwere, 1998). One such legume of interests is African yam bean (*Sphenostylis stenocarpa*) (AYB).

African yam bean (AYB) is an herbaceous leguminous plant occurring throughout tropical Africa (United States Department of Agriculture (USDA), 2007). It is grown as a minor crop in association with yam and cassava. AYB serves as security crop; it has the potential to meet year round protein requirements if grown on a large scale (World Health Organization (WHO), 2002). African yam bean (AYB) is highly nutritious with high protein, mineral and fibre content. Its protein content is reported to be similar to that of some major and commonly consumed legumes. Its amino acid profile is comparable if not better than those of cowpea, soy bean and pigeon pea (Obizoba & Souzey, 1989; Ene-Obong & Carnovale, 1992; Uguru & Madukaife, 2001). It has high metabolic energy, low true protein digestibility (62.9%), moderate mineral content, the amino and fatty acids contents are comparable to those

of most edible pulses (Nwokolo, 1987; Uguru & Madukaife, 2000). It has a higher water absorption capacity when compared to cowpea (Achinewhu & Akah, 2003).

The potential role of AYB in the management of many aging and chronic non-communicable diseases has been reported (Enwere, 1998; Nwachi, 2007; Alozie, Udofia, Lawal & Ani, 2009). In Ghana, the water drained after boiling may be drunk by lactating mothers to increase their milk production (Klu, Amoatey, Bansa & Kumaga, 2001). The economic potential of AYB has been recognized, especially in reducing malnutrition among Africans (Adewale, 2010).

These health benefits can be marred by the presence of anti- nutrients. Some processing methods however, such as soaking, boiling, fermentation, roasting, among others are known to achieve reduction or elimination of the anti nutritional factors which affect the nutrients (Nnam, 1994., Nnam, 1995; Ene-Obong & Obizoba, 1995; Obizoba & Atti, 1994; Messina, 1999; Nnam, 1999).

1.2 Statement of the Problem

Studies have shown that inspite of the good attributes of AYB, it is underutilized and rarely consumed in urban and rural areas in Nigeria. Its current status as a minor crop means that its potential is largely unexploited. It faces the danger of extinction (Klu *et al.,* 2001). The use of AYB may be limited by the beany flavour and long cooking time. These limitations can be overcome by processing like fermentation, soaking, roasting among others (Nnam, 1994; Ene-Obong & Obiziba, 1995; Nnam, 1999; Fasoyiro, Ajibade, Omole, Adeniyan & Farinde, 2006; Adewumi and Odunfa, 2009). Moreover, soaking or fermenting in lime medium can equally improve flavour, reduce the incidence of flatulence and acts as anti oxidant (OnlineFamilydoctors, 2000, Waladkhani, & Clemens, 2003; NewWorldencyclopedia, 2010). Citric acid treated AYB has been shown to have greater reduction in toxic substances like the cyanides (Azeke, *et* al*.,* 2007).

Some studies have been carried out on the nutrient, anti nutrient and toxic substance composition of AYB and some of its products and processing methods to improve the food use (Ene-Obong & Obizoba, 1995; Nnam, 1997; Nnam, 2003; Omeire & Ogbonna, 2006; Onyechi and Nwachi, 2008). Little has been done on the effect of 24h fermentation with and without lim e, 48h fermentation in lime water and roasting to address the beany flavour. Little has equally been done on the effects of these processing methods on nutrient, anti nutrients, toxic substances, anti physiological factors and other organoleptic characteristic of AYB flour and gruel.

There is the need therefore to determine the effect of 24h fermentation with and without lime and subsequent roasting, 48h fermentation in lime water and subsequent roasting on the chemical composition of AYB flours and organoleptic characteristics of their gruels.

1.3 Objective of the study

General objective

The general objective of the study was to determine the effect of different processing methods on the chemical composition of AYB flour and organoleptic characteristics of their gruels.

The specific objectives were to determine the processing methods on:

a. the proximate composition of the flours;
b. the anti nutrient composition of the flours;
c. the raffinose, stachyose, hydrogen cyanide and haemagglutinin contents of the flour; and
d. the organoleptic characteristics of their gruels

1.4 Significance of the study

The study would provide information on the nutrient, anti-nutrient, haemagglutinins, hydrogen cyanide, raffinose and stachyose composition of African yam bean (AYB) flour.

The gruels made from AYB flours would be a form of dietary diversification, which will enhance AYB food use and contribute to ensuring food security and sustainability in Nigeria. It may also stimulate local production and create employment for rural population. The study would serve as baseline information for researchers in this area.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Legumes in human nutrition.

According to National Academy of Science (NAS) (1997), a legume is a simple dry fruit that develops from simple carpel and usually opens along a seam on two sides. Grain legumes are plants belonging to the legume family with papilionaceous flowers and pods containing seeds. Most legumes do not need industrial fertilizers this is because of their natural symbiosis with *Rhizobium* which provides them with organic proteins made directly from atmospheric nitrogen (NAS, 1997). Grain legumes are cultivated primarily for their seeds which are rich in energy and protein. Legumes are seeds that grow in pods; they are high in fibre, low in fat and a good source of protein. Beans, lentils, peas, soybeans, and peanuts are all examples of some major common legumes. Lesser known legumes are African yam beans, baobab among others (Obiakor & Nwanekezi, 2008). A legume is a simple dry fruit that develops from a simple carpel and usually opens on two sides. The seeds are hard and dried and cannot be eaten unless prepared in the kitchen. For quite some time, legumes were considered a rather lowly food; their food is increasing with recent discoveries concerning their many nutritional and health properties (Pamplona-Roger, 2006).

Crosby (2009) stated that the plants show great diversity in both vegetative and floral form; woody, perennial species predominate, but numerous herbaceous forms and even a few aquatics also occur. The fruit is the feature by which the family is best characterized. Technically known as a legume, it is a single-chambered, flattened seedpod with two sutures. It usually splits open along the two sutures, as in the common pea. The seeds are attached along one of the sutures. The legume may be indehiscent (not splitting), as in the peanut, which matures underground; or explosively dehiscent, as in broom or lupine. It also may range from only a few millimeters long to more than 30 cm (more than 12 in) and may be single or many seeded and brightly or dully colored.

According to Crosby (2009) the family of legume is divided into three closely related subfamilies, which are often treated as three separate families. One subfamily is mostly herbaceous and is characterized by simple leaves and highly irregular flowers with ten stamens in two clusters. About 12,000 species exist, including such plants as peas, beans, peanuts, and soybeans; clover and alfalfa; and sweet pea, broom, and lupine. The second subfamily contains mostly trees and shrubs and is characterized by bipinnately compound (doubly branching) leaves and regular (radially symmetrical) flowers with ten or more stamens extending beyond the petals. This subfamily contains about 3,000 species and includes acacias and mimosas. The third subfamily is also mostly woody, but with leaves pinnately compound, and slightly too highly irregular flowers with ten stamens in one cluster. This subfamily contains about 3,000 species and includes such plants as brazil wood, carob, honey locust, Judas tree, logwood, and tamar (Crosby, 2009).

2.2 Functional properties of legumes.

Legumes contains large group of non nutritive compounds known as photochemical that are biologically active in the body, and they act as antioxidants, these photochemical were earlier thought to be harmful to the body, but, with the recent research research, they were discovered to have healing and protective properties. This phytochemicals fall in the class of phenolic acids, saponins, isoflavones, phytates among others (Gropper, Smith, & Groff, 2005). Legumes are low in fat and carbohydrate and high in protein, they can be used as substitute for animal protein, they are equally rich in magnesium, iron, copper and folic acid. Vitamins such as B_1 B_2 B_6 niacin, folates have been associated with legumes; the iron in legumes is two to three times more than that found in meat though not absorbed readily, and vitamin C from other foods in the meals of legumes increases the absorption to levels similar to heme iron in meat (Pamplona-Roger, 2006; Gropper, Smith, & Groff, 2005).

Pamplona-Roger (2006) reported that 15% to 30% of legumes' dry weight is fibre and this amount is superior to whole grains. It has been documented that they contain 2-3 times the

protein of cereal grains and no other plant food is as rich in protein as legumes in their natural state (National academy of Science, 1997, Pamplona-Roger, 2006).

2.3 Legumes in diet-related non communicable disease

Leguminous seeds have some anti-nutrients which contribute to the ability to lower blood glucose level and such anti- nutrient includes enzyme inhibitors such as phytates and possibly lectins, this has shown to produce hypoglycemic response in humans and rats (Onyechi, 2009). Studies have shown that legumes prevent arterial hypertension and lowers blood pressure because of their high levels of potassium and low sodium (Pamplona-Roger, 2006, Sacks *et al*., 2001).

Legumes combat damage that low density lipoprotein (LDL) cholesterol in the blood can cause. It also have an advantage of low risk of heart disease and slow down the absorption of carbohydrates. Soluble fibres in bean wards off the ups and downs in blood sugar levels therefore are good for diabetic patients. More so, soluble and insoluble fibres keep constipation at bay. Legumes have complex carbohydrates which are good in providing energy to the brain and muscles, have low glycemic index, the fibre content lowers cholesterol levels, reduce the risk of cancer and aids digestion. Water soluble non-starch polysaccharides (NSP) that have viscous properties occur mostly in legumes. They have benefit in the prevention/management of diabetes and cardiovascular diseases (Onyechi *et al.,* 1998; Enwere, 1998). Whole grain consumption was reported to be significantly and inversely associated with the development/control of diabetes (Jenkins, *et al.,* 2000)

It contains flavonoids which act as female hormone estrogen. Phytoestrogen, is the estrogen like production from plants that are good for menopausal women.

2.4 Bean as a legume.

According to Microsoft Encarta (2009) most bean belong to the subfamily Papilionoideae of the family Fabaceae and bean is common name widely applied to many plants of the legume family. The seeds and pods of these plants are used for food and forage.

The seeds themselves are also called beans and are valuable as food because of their high protein content. The term bean is also applied to plants of other families, such as the Indian bean, which is a North American species, and the sacred bean, or Indian lotus. The seeds or fruits of certain other plants, such as the coffee tree and the castor-oil plant are also called beans.

2.5 African yam bean (AYB) (*Sphenostylis stenocarpa)*

African yam bean is known and called different names by different tribes in Nigeria, some of the names are *Azama, Ijiriji, Azam,* and *Uzaaki* in Igbo; *Girigiri* in Hausa; *Akpaka* in Delta and *Nsama* in Ibibio. Other names are *Okpodudu, Ahaja, Nzamiri, Odudu* and *Sese*.in Igala. In some parts of Ghana, it is called *Kulege* or *Kutreku*. AYB belong to the family: Fabaceae (alt. *Leguminosae*) subfamily: *Faboideae* tribe: Phaseoleae subtribe: Phaseolinae., also placed in: Papilionaceae. It is also called yam pea in English and it is usually cultivated in the following African regions Northeast Tropical Africa: Chad; Ethiopia East Tropical Africa: Kenya; Tanzania; Uganda, West-Central Tropical Africa: Burundi; Central Africa Republic; Zaire West Tropical Africa: Cote D'Ivoire; Ghana; Guinea; Mali; Niger; Nigeria; Togo South Tropical Africa: Angola; Malawi; Zambia; Zimbabwe (USDA, 2007).

AYB is grown both for its edible seeds and its tubers (Klu *et al.,* 2001). The seeds are mostly used in some regions. It is a vigorous vine which twines and climbs to heights of about 3m and requires staking, with its prolific spattering of large flowers which may be pink, purple, or greenish with white, making it an attractive ornamental (NAS, 1979). The slightly woody pod which contains 20 to 30 seeds is up to 30cm long and mature within 170 days (Klu *et al.,* 2001). The seeds of AYB vary in sizes and shapes. The seed coat has a range of colours from pale white to black with spotted **or** mottled grey, cream and brown in between. In Nigeria, it is grown mostly in the northern part where it is grown mainly for its seed (Alozie, *et al.,* 2009).

2.5.1 Nutritional/ chemical composition and organoleptic attributes of AYB

Roasted AYB bread according to Onyechi and Nwachi (2008) has more acceptable colour than bread made from raw bean. Two legumes, AYB (*Sphenostylis stenocarpa*) and cowpeas (*Vigna unguiculata*), were processed into akara, moimoi and porridge then analyzed along with the raw samples for chemical, functional and sensory properties. Results showed that all the samples (raw or processed) had similar and high crude protein content with an average of 20.7%. The cowpea and AYB porridges had the highest (22.9%) and the lowest (19.9%) crude protein, respectively. This showed that the two legumes are very good sources of protein. Moimoi made from African yam beans had lower gelation capacity (19.4 w/v), higher water absorption capacity (68.0 ml/g), and lower oil absorption capacity (35.9 ml/g) than that made from cowpeas with 23.6 w/v, 54.0 ml/g and 41.0 ml/g, respectively. Similarly, akara made from African yam beans had a higher gelatin capacity of 34.5 w/v, water absorption capacity of 71.0 ml/g and a lower oil absorption capacity of 60.0 ml/g compared to that made from cowpeas (30.6 w/v, 57.0 ml/g and 62.02 ml/g, respectively). Sensory evaluation showed that moimoi and akara made from African yam beans were not significantly different ($p>0.05$) in colour, flavour, texture and overall acceptability as compared to those made from cowpeas indicating that AYB could be very useful in the preparation of moimoi and akara. In general, processing into akara and moimoi improved the oil absorption capacity of the AYB. The improved capacity to bind fat would be useful in ground meat formulations such as sausage in addition to the usefulness in making moimoi and akara (Achinewhu & Akah 2003).

According to Eneche (2006), African yam bean though deficient in methionine and cystine, has a high protein content of 21.6% and high in lysine. AYB can also be utilized as complementary protein in our carbohydrate based foods to enhance their demand and improve their quality. Incorporation of AYB flour into wheat based products greatly reduces the cost of

importation of wheat and increases the utilization of this lesser known legume as well as the protein content of our carbohydrate wheat-based foods.

A moimoi-like dish, similar to the very popular steamed cowpea dish was produced from the AYB. The AYB moimoi was compared with cowpea and soybean moimoi by a panel of eight (8) judges. The samples were compared for taste, colour, aroma texture and overall acceptability. The texture, aroma and overall acceptability of cowpea moimoi ranked highest followed by AYB moimoi and lastly soybean moimoi. Colour wise, AYB moimoi was preferred over cowpea moimoi followed by soybean moimoi. Texture wise, AYB and cowpea were scored equally followed by soybean moimoi. Proximate analysis of the product revealed total carbohydrate content was 40.8%, crude protein was 18.4%, ash was 7.1%, crude fibre was 8.3% and crude fat was 25.4% **(**Peterside, Dosumu & Njoku, 2002**).**

Proximate analysis of AYB according to Onyechi, *et al.,* (2008) showed that it contains 21.2g of protein, 1.9g fat, 3.5g ash, and 6.05g dietary fibre, 46.0g of sugars and 52.1g of total carbohydrates. Obiakor (2008), stated that AYB has a high lysine content, the crude protein vary from 21-29% of which lysine comprises of up to 8% of the protein, 50% of carbohydrates and 5-6% of fibre. Evans and Bouttler (1974) stated that amino acid of AYB analysis indicate that the lysine content is equal to or higher than that of soybean while most of the other essential amino acids corresponds to the WHO/FAO recommendation.

The raw seeds contain on the average 21.29% crude protein, with mean cystine value of 1.28 implicating it as the limiting amino acid in AYB, however, the other essential amino acids were present in higher concentrations when compared to other legumes (Uguru *et al*., 2001).

Table 1: Proximate (% dry matter) and mineral (mg/100g dry matter) composition of cream, brown and brown spotted (speckled) raw AYB seeds

	Cream	Brown	Brown spotted(speckled)
Dry matter	86.38	88.97	85.88
Protein	21.1	21.2	21.5
Ash	2.70	3.50	3.10
Fat	2.30	1.90	2.10
Dietary fibre	17.5	21.3	18.6
Sugar	5.90	46.0	48.1
Starch	50.5	46.0	48.1
Total CHO	56.4	52.1	53.8
Ca	41.0	61.0	36.3
Fe	5.08	4.37	4.64
Zn	2.44	3.02	2.44
P	267	289	308
K	1430	1490	1512
Na	3.02	3.58	1.62

Source: Ene-Obong and Carnovale (1992).

Table 2: Amino acid distribution of AYB

Amino Acids	Quantity (g/16gN)
Lysine	7.40
Histidine	4.08
Arginine	5.28
Aspartic acid	11.64
Threonine	4.14
Serine	5.80
Glutamic acid	15.20
Proline	4.71
Glycine	4.54
Alanine	4.60
Cystine	1.72
Valine	5.43
Methionine	1.17
Isoleucine	4.44
Leucine	7.59
Tyrosine	4.02
Phenylalanine	5.92

Source: Ene-Obong and Carnovale, (1992).

Table 3: Antinutrient composition of 3 varieties of African yam bean (raw seed characterization).

Antinutrient	Quantify
α-amylase inhibitor	(6-13) ug^{-1}
Saponin	(2-4) mg/kg
Trypsin inhibitor	(0.7-3.0) TIU/mg
Total oxalate	(21-35) mg/100g
Soluble oxalate	(3-6) mg/100g
Tannins	(0.9-20) mg/g
Phytic acid	(4.5-7.3) mg/g
α-galactosides	(2.3-3.4)g/100g
cyanogenic glucoside	225mg /kg

Source: Betsche, Azeke, Fretzdorff, & Buening-Pfaue (2007)

2.5.2 Economic importance/ uses of AYB

It was reproted that storage roots of AYB can be processed into 'yam bean gari.' This is similar to the current staple of West Africa, 'cassava gari,' granular flour. The bean could make a significant contribution to the improvement of food support, especially where resources are poor (The American Society of Agronomy, (ASA) (2007). In Ghana, the water drained after boiling may be drunk by lactating mothers to increase their milk production (Klu, Amoatey, Bansa and Kumaga, 2001). AYB seed can be used as ornaments for decoration (USDA, 2007).

2.5.4 Constraints in the use of AYB

AYB is one of the lesser-known legumes and has the peculiar problem associated with legumes, It has high antinutrient content and hard to cook (HTC) phenomenon. It has beany flavour which hinders its extensive utilization, coupled with the increase in the cost of domestic fuel. African yam bean belong to the family of legumes. Legumes generally contain anti nutritional factors and toxic compounds like trypsin inhibitor, tannins, phytates, hemaglutinin and oligosaccharides. These inhibit the bioavailability of nutrients. AYB is also reported to be associated with flatulence caused by flatulence inducing oligosaccharides (Nnam, 1999., Ene-Obong & Obizoba, 1995).

Flatulence is the expulsion through the rectum of a mixture of gases that are byproducts of the digestion process of mammals and other animals. The mixture of gases is known as flatus and informally as a fart, or gas, and is expelled from the rectum in a process referred to as "passing gas", "breaking wind" or "farting". Flatus is brought to the rectum by the same peristaltic process, which causes faeces to descend from the large intestine. The noises commonly associated with flatulence are caused by the vibration of the anal sphincter, and occasionally by the closed buttocks. Intestinal gas is composed of varying quantities of exogenous sources (air that is ingested through the nose and mouth) and endogenous sources (gas produced within the digestive tract). The exogenous gases are swallowed when eating or drinking or increased swallowing during times of excessive salivation. The endogenous gases

are produced either as a by-product of digesting certain types of food, or of incomplete digestion. Anything that causes food to be incompletely digested by the stomach and/or small intestine may cause flatulence when the material arrives in the large intestine. Flatulence-producing foods are typically high in certain polysaccharides, especially oligosaccharides. This flatulence inducing oligosaccharides includes namely raffinose, stachyose and verbascose which is due to the absence of α-galactosidase in humans are fermented anerobically to by micro-organisms to produce carbon dioxide, hydrogen and methane (Suarez *et al*., 1999).

In beans, endogenous gases seem to arise from complex oligosaccharide (carbohydrates) that are particularly resistant to digestion by mammals. These oligosaccharides pass through the upper intestine largely unchanged. When they reach the lower intestine, bacteria feed on them, producing copious amounts of flatus. Oligosaccharides when reduced will have a phytochemical effect of growth promotion on bifidobacteria and because of that it is said to promote the health of the colon, increase longevity and decrease colon cancer risk (Hata, Yamamoto, Nakajima, 1991).

Hydrogen cyanide (HCN): Inhibits the activity of cytochrome C oxidase of the mitochondrial electron transport chain thus preventing electron transfer from cytochrome a/a3 (electron carrier) to molecular oxygen (the final electron acceptor of the respiratory chain). It may do this by combining with iron, (the catalytic group of the enzyme), thus eliminating the active group involved in electron transfer to molecular oxygen hindering cellular oxidation and the supply of energy to the cell. Heat treatment, thus, reduces the risk of dietary exposure of consumers of AYB based diet to cyanide poisoning (Onyeike, & Omubo-dede, 2002; Oke, 1967).

Hemagglutinin (Lectins): refers to a substance that causes red blood cells to agglutinate. They are carbohydrate-binding proteins (Hudson, 1984). The occurrence of lectins is widespread in the plant kingdom, though more abundant in legumes.

2.5.4 The antinutrients in AYB

Antinutrients are natural or synthetic compounds that interfere with the absorption of nutrients (Atwood *et a*l., 2006). Nutrition studies focus on those antinutrients commonly found in food sources and beverages. The poor digestitibilty of the nutrients (especially protein) has been attributed to the presence of anti nutrients and this limits the nutritional potentials of such food for both humans and animals (Liener, 1973). Proteins can also be antinutrients, such as the trypsin inhibitors and lectins found in legumes (Tan, Gyllenhaal, Soejarto, 2006). These enzyme inhibitors interfere with digestion.

Phytic acid

Phytic acid is present in many plant systems, constituting about 1 to 5% by weight of many cereals and legumes. Concern about its presence in food arises from evidence that it decreases the bioavailability of many essential minerals by interacting with multivalent cations and/or proteins to form complexes that may be insoluble or otherwise unavailable under physiologic conditions (Cheryan and Rackis, 1980). Phytic acid content can be reduced through soaking or other forms of processing (Realfoodmedia, 2009).

Tannins

Polyphenols (tannins) are usually located in the pericarp and/or testa, especially on pigment cultivars of legumes and millets. Tannin concentration is reported to be higher in coloured seed coats with a range of 38-43mg/g and low in white coated beans (1.3mg/g). However, values ranged from 3.8-5.9mg/g in the cotyledons (Elias, Fernandez & Bressani, (1979).

Saponins

Saponins are glycosides composed of a lipid-soluble aglycone that consists of either a sterol or more commonly, a triterpenoid structure attached to water-soluble sugar residues that differ in their type and amount. The major sources of dietary saponins are legumes, and many types of saponins can be present in the same bean (Messina, 1999). Saponins are very poorly

absorbed. They can kill or inhibit cancer cells without killing normal cells (Rao, 1996). Most saponins form insoluble complexes with 3-ß-hydroxysteroids and are known to interact with and form large, mixed micelles with bile acids and cholesterol (Malinow, Marbin & delaCastra, 1985).

Trysin inhibitors

Trypsins inhibitors when ingested by man in large quantity disrupt the digestive process and may lead to undesirable physiological reactions (Booth, Robbins & Kibellin, 1960). They co-exist with anti α-amylases and are located mostly in the outer layer of the cotyledon of legumes. Trypsin inhibitors from beans interfere with protein digestion and in some species of animals do cause pancreatic enlargement and enhance chemically induced pancreatic tumors (Grant, 1989). However, heat-treating dry beans generally reduces the trypsin inhibitor content by 80–90% (Duarte-Rayas, 1992). Other processing methods like soaking in water through leaching, fermentation and germination has been shown to also reduce trypsin inhibitors (Nnam, 1997; Obiakor, 2008; Obizoba & Atti, 1991; Ene-Obong and Obizoba, 1995).

Oxalates

Oxalate occurs widely in the plant kingdom, examples of foods containing oxalates are black pepper, parsley, poppy seed, amaranth, spinach, chard, beets, cocoa, chocolate, most nuts, most berries, fishtail palms, New Zealand spinach (Tetragonia tetragonioides) and beans. Excess consumption of oxalates may result in kidney disease or even death due to oxalate poisoning (Streitweiser & Heathcock, 1976). Oxalic acid can induce toxic as well as anti nutritive effects. To humans, it can be acutely toxic. However, it would require massive doses of 4 to 5 g to induce any toxic effect (Oke, 1969). The oxalic acid levels usually found in food, however, are no cause for concern. Like phytic acid, oxalic acid reduces the availability of essential bivalent cations. Oxalic acid is a strong acid and, with alkaline earth metal ions and

other divalent metal ions, it forms salts that are hardly soluble in water. Calcium oxalate is insoluble in water at neutral or alkaline pH, and dissolves easily in an acid medium. Oxalates produce irritation in the mouth and thereby preventing the absorption of calcium and iron in foods (Osisiogu, Uzor, Ugochukwu, 1974; Oke, 1969). A study by Dresbach (1980) showed that oxalate toxicity on calcium metabolism acts by combing with serum calcium to form an insoluble calcium oxalate complex bringing about reduction in serum calcium level and violent muscular stimulation with convulsion and eventual collapse. The reduction of oxalate to a physiologically tolerable level by processing may enhance cellular utilization of some nutrients for metabolic activities of some enzymes. Oxalates functions as chelating agents and may chelate many toxic metals such as mercury and lead. Unlike other chelating agents, oxalates trap heavy metals in the tissues (Shaw, 2010).

2.5.5 AYB processing methods used

Roasting

Roasting is a traditional processing technique, it has the capacity to develop attractive flavours in foods so treated. It also induces important functional properties, attributes that should be compatible with nutritional value (Bressani, 1983).

Fermentation

Fermentation is one of the oldest and cheapest traditional processing methods used in the home and industries to improve the nutritional quality of food and reduces anti nutrient and toxic substances like phytic acid, polyphenols and oxalic acids, Hydrogen cyanide, raffinose and stachyose among others to improve food use (Gibson, 2007; Obizoba & Egbuna, 1992; Nnam, 1994; Mahungu, Yawaguchi, Almazar, Kahan, 1987; Obizoba & Atti, 1991, Ogunsua, 1980). It is the metabolic process in which carbohydrates are oxidized with the release of energy. During fermentation, the microbial enzymes converts storage nutrient in foods to readily utilizable form (Rajalakshimi & Ramakrishanan, 1977). Fermentation begins when a

food rich in simple sugars, yeast, and water are combined and left at room temperature. During the first stage, the yeast cells multiply, using the sugars for energy, and produce small amounts of alcohol (Byrd-Bredbenner, Moe, Bestgetoor, Berning, 2007). Many foods which are inedible are made edible in their unfermented form and this is brought about by the extensive hydrolysis of the indigestible components and the removal of antinutritional factors by the micro-organisms (Odunfa, 1983).

Reasons for fermentation

Some reasons why fermentation of legumes is used in the preparation of foods are:

(1) Legumes often contain substances that are undesirable, such as the trypsin, phytates among others. The treatments of the beans (soaking or heating) in preparation for fermentation or the enzymes produced by the microorganisms remove or destroy these factors.

(2) Moist products spoil readily, however, after fermentation, some products will keep without refrigeration for extended periods of time. This is especially true of the fermented products that are high in salt.

(3) All the legume fermentations involve the action of proteolytic and lipolytic enzymes with the result that the final products are more digestible.

(4) Almost invariably the final product has a changed flavor more acceptable to the consumer.

(5) In many instances the microorganisms increase nutrients such as vitamins, including riboflavin and vitamin B12 in some of the bacterial fermentations.

(6) Finally, fermentation may reduce energy requirements. Thus, a short cooking may be the major energy input required while the microorganisms do the rest of the work by using energy from the substrate.

Source: Hesseltine & Wang (1980).

In addition, fermentation equally extends shelf life and level of safety (Hesseltine & Wang, 1980). It increases essential amino acids like methionine, improved palatability,

increase non protein nitrogen (Reddy & Salunkhe, 1980). Proteolytic acid and amylolytic enzymes from micro-organism in fermentation process enhance digestibility and nutritional quality (Murato, Ikehata & Myamoto, 1967). It equally enhances flavour, aroma, texture, keeping quality and improves nutritive values (Eka, 1980). Fermented corn, cowpea and AYB are known to have higher nitrogen balance than its unfermented counterparts (Nnam, 1999).

2.6 Lime

Lime is a citrus fruit. The lime tree (Citrus aurantifolia) belongs to the Rue family (Rutaceae). Limes are small, oval citrus fruits with porous and smooth skin. Lime colour ranges from light to medium green, sometimes with a slight yellow cast. Limes and lemons look similar, but limes are smaller and green (when ripe) lemons are yellow (NewWorldencyclopedia, 2010). Its fruits like other citrus fruits are very rich in vitamins A, B and C. They have a tangy flavour and are used in cooking, baking, pickling, soaking among others. It also contains various other minerals and acids. The fruit juice is an efficacious remedy in scurvy, anaemia, intestinal disorders, cough and cold, gastric troubles, constipation, fevers, typhoid and high blood pressure. It is a tonic, rejuvenative and refresher (onlinefamilydoctors, 2000).Citric acid treated AYB has been shown to have greater reduction in anti nutritional factors like the cyanides (Azeke *et al.,* 2007). Citric acid treated AYB was also found to be bacteria and cyanogenic glucoside free.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Cream coloured African yam bean (AYB) seed was purchased from Oye Igbo-Eze and lime was purchased from Ogige Nsukka both in Enugu State, Nigeria.

3.2 Methods

3.2.1 Preparation of AYB flour for gruel and chemical analysis

Fifty-two cups (6kg) of cream coloured AYB seeds were sorted, weighed and divided into 4 equal portions of 1.5kg each. These portions were labeled 24h fermentation without lime and roasting (SO1), 24h fermentation with lime and roasting (SL2), 48h fermentation with lime and roasting (FL3) and Roasted only (RO4).

- **24h fermentation without lime and roasting (F24)**: - The sorted AYB seeds were washed and fermented in tap water in a ratio of 1:3(w/v) for 24h at room temperature (25 ± 28^0C). The water was changed every six hours. At the end of 24h fermentation, the seeds were sundried for 72h and roasted till cracking sets in. The roasting was carried out by placing the sauce pan on fire and allowing it to heat. The AYB seeds were poured into the saucepan and wooden spoon was used to stir the AYB seeds to prevent burning. After roasting, the seeds were allowed to cool and milled whole into fine flour using laboratory mill.
- **24h fermentation with lime and roasting (FL24): -** The sorted AYB seeds were washed and soaked in tap water containing 30 tablespoonful of freshly squeezed lime in a ratio of 1:3(w/v) at room temperature (25 ± 28^0C) for 24hours. The water was changed every six hours with addition of the same quantity of lime. At the end of 24 hours fermentation, the seeds were sundried for 72 hours and roasted till cracking sets in: The AYB seeds were poured into the saucepan and wooden spoon was used to stir the AYB

seeds to prevent burning. After roasting, the seeds were allowed to cool and milled whole into fine flour using the same method above.

- **48h fermentation with lime and roasting (FL48): -** The sorted AYB seeds were washed and fermented in lime water (30 table spoonful of lime) for 48h at room temperature (25±28^{0}C) in a ratio of 1:3(w/v). At the end of 48 hours of fermentation, the seeds were sundried for 72 hours and roasted in a hot sauce pan till cracking set in. The AYB seeds were poured into the saucepan and wooden spoon was used to stir the AYB seeds to prevent burning. After roasting, the seeds were allowed to cool and milled whole into fine flour using laboratory mill.
- **Roasted only (OR): -** The AYB seeds were washed, drained and roasted in a hot saucepan until it starts cracking. The roasting was as thus: the pan was put on fire to heat and the AYB seeds were poured into the pan using wooden spoon to turn the seed to prevent burning. After roasting, the seeds were allowed to cool and milled whole into fine flour using laboratory mill.
- The samples were separately stored in airtight containers for chemical analysis and preparation of gruel.

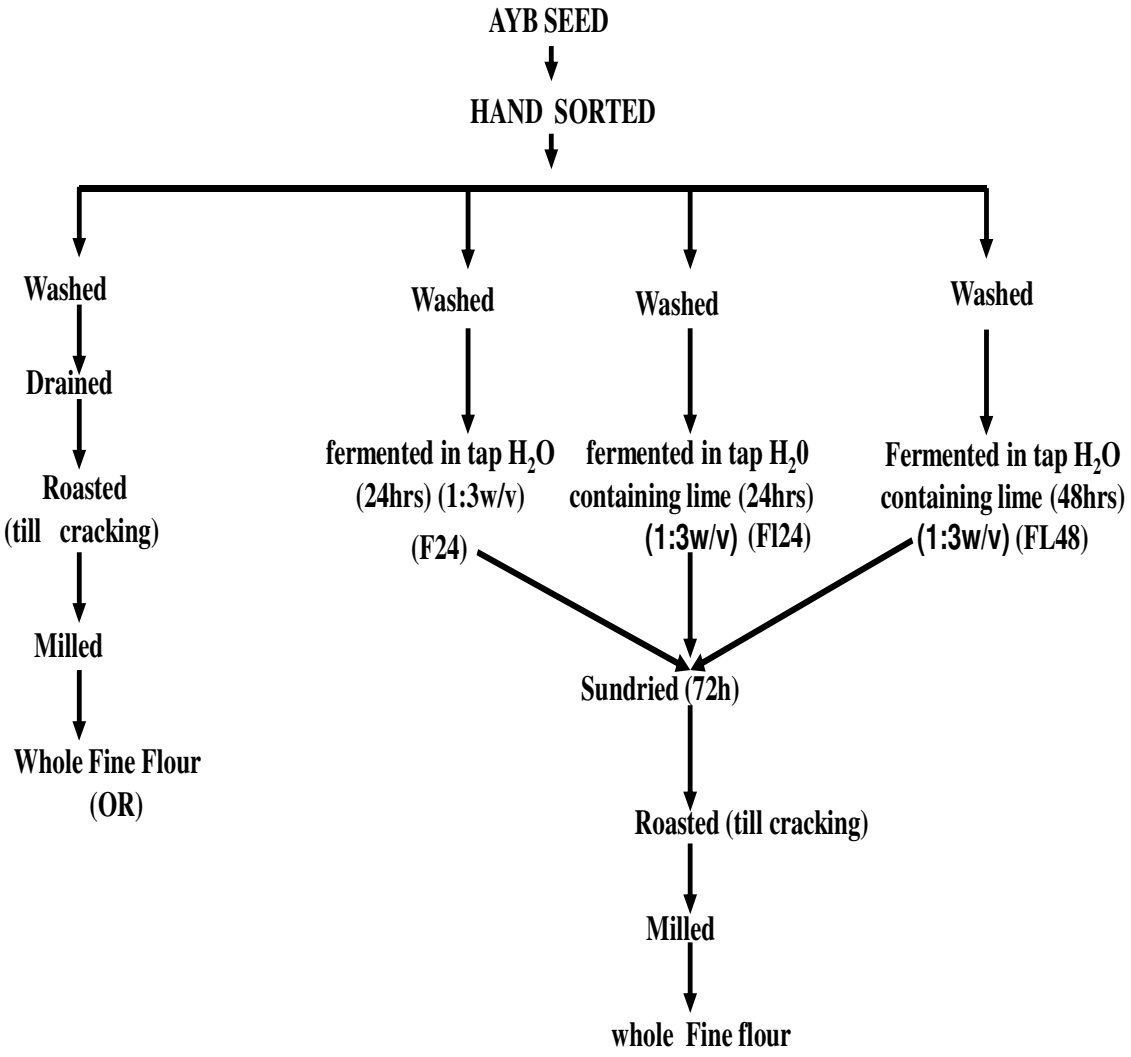

Fig. 1: Flow chart for the processing of AYB flour

3.2.2 Preparation of gruel from AYB flours

Gruel was prepared from each of the AYB flour samples.

Ingredient for the gruel preparation:

Flour ------- 400g

Water------- 2.5 litres (10 cups)

Sugar------- 4 cubes

Method of preparation:

500ml water was used to reconstitute the flour

1. 2 litres of water was brought to boil.
2. The boiled water was gradually added to the reconstituted flour while stirring continuously to avoid the formation of lumps.
3. The mixture was allowed to simmer for some minutes and stirred continuously till cooked (approximately 5 minutes).
4. Sugar was added and stirred
5. It was served hot

3.3 Laboratory analysis

Four samples of milled AYB flour were subjected to chemical analysis.

3.3.1 Protein determination

Protein was determined by automated micro-Kjedahl method described by AOAC (1995). One gramme of each sample was weighed into the micro- Kjeldahl flask and 20ml concentrated H_2SO_4, 2g Na2SO4, 0.5 CUSO4 (as catalyst) and 0.1g, selenium was added in the flask. The mixture was boiled on a digester until the black solution became clear after which it was made up to 100ml with distilled water. About 5 ml samples were drawn from the solution and subjected to steam, boric acid, blue methyl and red methyl. The end product was titrated against 0.01N HCL. The percentage nitrogen was gotten from the formula:

$$\% \text{ Nitrogen} = \frac{\text{Titre} \times N \times DF \times Nmw \times 100}{\text{Weight of samples in mg}}$$

Titre = Final burette reading -initial burette reading
N = Normality of acid
DF = Dilution factor
NMN =Molecular weight of nitrogen.
Percentage protein = % Nitrogen x 6.25 (conversion factor).

3.3.2 Fat determination

The fat content of the sample was determined by Soxhlet extraction method (AOAC, 1995). Extraction flask was weighed; two grammes of each sample were weighed into a filter paper and introduced into the extraction thimble. The thimble was placed into the soxhlet extractor, some quantity of petroleum ether was placed into the flask and connected to the soxhlet apparatus. The extraction lasted for about 6 hours at 40-60^{0}C after which the solvent (petroleum ether) was recovered leaving only the extract in the flask. The extract was dried at 100^{0}C to expel the remaining solvent, then cooled in the dessicator and weighed.

$$\% \text{ fat} = \frac{(\text{weight of flask + oil}) - (\text{weight of flask}) \times 100}{\text{Weight of sample}}$$

3.3.3 Ash determination

The ash content was determined using the official method of the AOAC (1995). Two grammes of each sample were weighed into a weighed crucible and incinerated in a muffle furnance at 600^{0}C for about 6 hours. The crucible was removed and cooled in a desiccator and reweighed.

$$\% \text{ Ash} = \frac{(\text{Weight of crucible + Ash}) - (\text{Weight of crucible}) \times 100\%}{\text{Weight of sample}}$$

3.3.4 Crude fibre determination

The method of Joslyn (1970) was used. Two grammes of defatted sample were hydrolyzed in a beaker with 200ml of 1.25% H2S04 for 30 minutes, and then filtered under suction, washed with hot distilled water and boiled again for another 30 minutes with 200ml of

1.25%NaOH. The digested samples were washed with 1%HCLto neutralize the NaOH for several times with hot distilled water. The residue collected was put into a weighed crucible and dried at 100^{o}C for 2 hours in an air oven. It was cooled and weighed. The ash was cooled and weighed. The % crude fibre was calculated using the expression:

$$\% \text{ crude fibre} = \frac{\text{loss in weight after ignition}}{\text{Weight of sample}} \times 100$$

$$\text{ie Crude fibre} = \frac{\text{weight after drying} - \text{weight after ignition}}{\text{weight of sample}} \times 100$$

3.3.5 Carbohydrate determination

This was determined by difference. The determined percentages of protein, fat, crude fibre and moisture were summed up and subtracted from 100%.

3.3.6 Phytate determination

Phytate was determined by a simple and rapid colourimetric method by Eskin and Latta, (1980). The equipment used were Anion exchange column, spectrophotometre, centrifuge,volumetric flask and Vortex mixer. The reagents used were HCL, 2.4% HCL (0.65N), Wade reagent: 0.03% $FeCl_3.6H_2O$ and 0.3% sulfosalicylic acid) in distilled water

NaCl, 0.1M, 0.7M NaCl

Procedure

Five grammes (5g) of milled sample were weighed into a 250 ml conical flask, 100ml of 2.45HCL was added and extracted for 1 hour at room temperature 25^{0}C±28^{0}c and centrifuged. Supernatant was decanted. 1ml of 2.4% extract supernatant was diluted to 25 ml with distilled water. Ten milliliters (10mls) of diluted sample was passed through the AG1-X8 chloride anion exchange column (0.5 g). Phytate was eluted with 0.7M NaCl. 3ml of 0.7M eluent fraction was pipetted into 15 ml conical test tubes, and mixed on a vortex mixer for 5 seconds, and centrifuged for 10 minutes. Absorbance of supernatant was read at 500 nm using water to zero the spectrophotometer

Preparation of standard curve

Series of sodium phytate dilutions were made from 5-40 µg phytate in distilled water. Three millimetres (3ml) of solution was pipetted into 115ml. One millimetre (1ml) of Wade reagent was added within 30 minutes of elution. It was mixed on a vortex mixer for 5 seconds and centrifuged for 10 minutes. Absorbance of supernatant was read at 500 nm using water to zero the spectrophotometer

Phytate content was estimated from the standard curve.

3.3.7 Tannins

Tannins was determined using Joslyn, (1970) method. The reagents used were methanol, sodium carbonate; 350g $NaCO_3$ dissolved in 1 Litre of distilled water at 70-80 ^{0}C cooled and filtered through glass wool. Tannic acid (100ppm) (0.01g tannic acid was weighed and dissolved in 100ml of distilled water). Sodium tungstate, orthophosphoric acid, phosphomolybdic acid, folin-Denis reagent: (50g of Sodium tungstate, 10g of phosphomolybdic acid and 25ml of phosphoric acid was added to 375ml distilled water and reflux for 2h, and allowed to cool and made up to 500ml mark.

Preparation of standard curve

1. 0, 0.05, 0.1,0.2, 0.3, 0.4 and 0.5ml of tannic acid standard solution was pipetted into test tubes and made up to 5ml by adding 5, 4.95, 4.9, 4.8, 4.7, 4.6 and 4.5ml of distilled water (These corresponds to concentrations of 0, 1, 2, 4, 6, 8 and10 ppm.)
2. 0.3ml Folin-Denis reagent was added
3. 0.6ml of Na_2CO_3 solution was added
4. The solution was allowed to stand for 25-30 mins. The absorbance of blue color was read at 760nm.

Analysis of sample

One hundred miligrammes (0.01g) of sample were weighed; 20ml cold (4^{o}C) methanol was added. It was vortexes and centrifuged at 3,000rpm for 20minutes.

1. An aliquot of 0.01 to 5ml of supernatant was taken for assay.
2. % Tannins was calculated from standard curve as follows:

$$\%\text{tannins} = \frac{(A-I)\ xVx100xD.\ F}{BxWx10^6}$$

A= Absorbance of sample.

I= Intercept

V= Total volume of extract

B= Slope of standard curve

W= Weight of sample

3.3.8 Determination of trypsin inhibitors

Trypsin inhibitor was determined using Kakade, Racis, Mcchee, & Puski (1974) method. The reagents were prepared by dissolving 1.21g of hydroxymethyl amino methane and 0.59g of $CaCl_2$ H_20 in 180 ml of distilled water in Tris-buffer and BAPA solution. The pH was adjusted to 8.2 with 1N HCl and put in water bath for 2 hours at 37^oC 0.08g of BAPA was weighed and dissolved in 2ml of dimethisulfuroxide and added to the already prewarned trisbuffer in a measuring cylinder and adjusted to 200 ml with distilled water. It was returned to water bath for another 1 hour. Trypsin solution was prepared by weighing 0.0040g of trypsin into a 250ml volumetric flask and added 200 ml of .001N HCl. and kept in the refrigerator before and after use. Thirty grammes (30g) of glacial acetic acid were weighed and dissolved in 70 ml of distilled water.(30% glacial acetic acid), 1 ml of 1N NaOH was used in 100 ml of distilled water . One normal hydrochloric acid (IN HCl) was used in 100 ml of distilled water.

Procedure

One grammes (1g) of sample was weighed and 50 ml of 0.01N NaOH was added to extract the sample. P^H was adjusted between 8.4-10 0. 1N HCL was used and pH and IN NaOH was reduced to increase the pH to required level. The sample was allowed to stay for 3 hours stirring at intervals to maintain the sample in suspension. 1ml of the extract was withdrawn in 33ml of distilled water for dilution from the diluted extract; 2ml was taken and

poured in 3 test-tube each. 2ml of each samples of trypsin solution was added to 2 test-tubes. 2ml of distilled water was withdrawn in 3 test-tubes and 2mls of trypsin solution was added to 2 test tubes. The samples in the test-tubes were returned to the water bath and allowed to warm for 10 minutes. 5mls of BAPA was put to the entire test-tubes, vortex and warmed again for 10 minutes. 1ml of glacial acetic acid solution was added to all the test tubes and vortexed. 2ml of trypsin solution was added to all the 3rd test tubes that do not contain trypsin solution initially. The samples were filtered and the absorbance was read at 410nm using a spectrophometer.

Calculations:

$$\text{T.I mg/g of sample} = \frac{\text{Abs of Standard - Abs of sample}}{0.019 \times \text{sample weight}} \times \frac{\text{dilution factor}}{1.000 \times \text{sample size (ml)}}$$

$$\text{In 1g sample: T.I mg/g of sample} = \frac{(\text{Abs of standard} - \text{Abs sample}) \times \text{dilution factor}}{19}$$

$$\text{Therefore} = \frac{(\text{Abs of standard} - \text{Abs of sample}) \times 50 \times 33}{19}$$

3.3. 9 Oxalic acid determination

Oxalate was determined by the method of Nwinuka *et a*l. (2005). One hundred milligrammes (0.1g) of the flour were extracted thrice by warming it at 40-50 degree centigrade with constant stirring with magnetic stirrer for 1 hour with 20ml of 0.3 N HCL The extract was diluted to 100ml with distilled water. 5ml of the extract was made alkaline with 1ml of 5 N NH4OH. This was made acidic with glacial acetic acid and phenolphthalein served as an indicator (2 drops). 1 ml of 5% calcium chloride was added and the mixture was allowed to stand for 3 hours, centrifuged using (IEC Centra GP8) at 1400 rpm for 15 minutes. The supernatant was discarded and the precipitate was washed thrice with hot water, thorough mixing and centrifuging each time. 0.2ml of 3 N H2SO4 was pipette to each test tube.

The precipitate was dissolved by warming in water at 70^{o}C. for 30 mins. The content of each test tube was titrated with freshly prepared 0.01 N Potassium permanganate solutions. Titration was done at room temperature 29^{o}C until the colour of the solution become pink. The

solution was allowed to stand until it became colourless. It was warmed at 70 degree C and titrated until a pink color persisted for 30 seconds.

Calculations =oxalate content=W* 100/5

W= Mass of oxalate in 100ml

3.3.10 Determination of saponins

Saponins determination was carried out by Fenwick and Oakenfull, (1983) procedure. The reagents used were reagent grade acetone, methanol, solution of concentrated *n*-buthanol-methanol-ammonia (3.5:1:2.5), Standard solution of saponins purified in methanol, Solution of sulphuric acid in methanol (100 ml per litre). The materials and equipment used were Soxhlet extractor, Rotary evaporator, Densitometer with plotter,Planimeter, Dryer,Silica gel chromatography plate (Kieselgel 60 F-254 Merck).

Method

The sample was finely ground and dried at constant weight. 40g were weighed and placed in the Soxhlet reflux extractor with acetone for 24 hours. The solvent was changed for methanol and extraction was continued for another 24 hours. The methanolic extract was cooled and made to 250 ml with methanol.

At this point there was a modification to the method, proposed by Miriam Monforte (CICY, Merida, Yucatan, Mexico). Instead of bringing the sample up to 250 ml as suggested in the original method it was concentrated. In this, methanolic extract was transferred into a rotary evaporator and concentrate until dry. The residue was concentrated again in a minimum of methanol and transferred to a reweighed vial. The vial was weighed with the dry sample and the weight of the residue was calculated.

Fine drops of a standard solution of saponins were placed on the chromatography plates. The points of extract were placed so that each one is at the side of standard saponins drops. The plates were revealed and the drops with aspersion and a solution of sulphuric acid

in methanol, it heated at 110°C for 30 min. The intensity of the saponins stains was measured with a densitometer and the peak areas were calculated on the plotter with a planimater. The results were expressed as the relation (R) of the peak areas of the unknown sample in respect to those of the standard. R^2 was plotted against the volume of the drop of methanolized extract on the plate. The downslope of the line (was calculated by the least squares method), divided by the gradient of a line derived from a master curve, to give the concentration of saponins in the extract and thus the saponins content of the sample.

3.3.11 Hydrocyanic acid determination

Hydrocyanic acid was determined according to AOAC (1995) method. Using this method, 10 g of the dried sample were allowed to soak in 30 ml of water for 4 hours before steam distillation into 2.5% (w/v) NaOH. Eight milliliters of 6 N NH4OH and 2 ml of 5% (w/v) potassium iodide were added to the distillate before titrating with 0.02 N AgNO3.

3.3.12 Determination of haemagglutinin by spectrometric method

Haemagglutinin was determined according to the method described by Onwuka (2005). Two grammes (2g) of the sample were weighed into 40ml normal saline solution buffered at P^H 6.4 with 0.01m phosphate buffer solution. It was allowed to stand at room temperature for 30mins and centrifuged to obtain the extract. Half of a mililitre (0.5ml) of the extract was diluted in a test tube, 1 ml of heparinized rabbit blood was poured. The blank was prepared by adding 1ml of blood into a test tube and allowed to stand for 4h at room temperature. 1ml of normal saline was added to all the test tubes and it was allowed to stand for 10min. after which the absorbance was read at 620nm.

Haemagglutin unit/g= (b-a) x F

Where b = absorbance of the blank

F= experimental factor given by

F= (1/w x f/va) D

Where

W= weight of sample

VF= total volume of extract

VA= volume of extract used in the assay

D= dilution factor

3.3.13 Raffinose and stachyose determination

Raffinose and stachyose (oligosaccharides) were determined by the method described by Tanaka, Thanakul, Lee, and Chichester (1975). Five grams each of both raw and processed flour were extracted with 50ml of 70% (v/v) aqueous ethanol and kept on an orbital shaker at 130 rpm for 13h. Extracts were further washed with 25ml of 70% (v/v) ethanol. The filtrates obtained were then concentrated on a water bath. The concentrated sugar syrup was dissolved in 5ml of distilled water. Separation of oligosaccharides was done by thin layer chromatography (TLC). A 100g silica gel was dissolved in distilled water and stirred well until the slurry was homogeneous. The TLC plates were washed, dried and cleaned with chloroform to remove any grease from the plates. TLC plates were then coated with the slurry and air-dried. Spotting of the sugar samples was done by using capillary tubes. Each sample was spotted twice separately and dried using electronic hand drier. The plate were developed by using a solvent system of n-propanol, ethyl acetate and distilled water (6:1:3), and dried. The separated sugars' colours were developed with iodine crystals. The separated spots were compared with the standard sugar spots. The separated sugars that appeared were stachyose, raffinose and sucrose. The stachyose and raffinose spots were scrapped, eluted in 2ml of distilled water, kept over night and filtered throughWhatman No.1 filter paper. The filtrates were then subjected to quantitative estimation. The eluted individual oligosaccharide was estimated. One ml of the eluted and filtered sugar solution was treated with one ml of concentrated HCl. The tubes were boiled in water bath for exactly 6 min. After cooling, absorbances of the oligosaccharide contents were read using spectrophotometer 259 at 432nm.

The absorbance values were used to calculate the concentration and mass of the oligosaccharides. Average values of duplicate estimations were calculated and the oligosaccharide contents expressed on dry weight basis.

3.4 Organoleptic evaluation

Gruel made from flour fermented without lime for 24h and roasted, soaked with lime for 24h and roasted, fermented with lime for 48h and roasted and roasted only were displayed for sensory evaluation using the preference test 9- point hedonic scale as described by Ihekoronye and Ngoddy (1985). With this method, a 30 member panelist was selected to rate the sample on a 9- point hedonic scale where 1 represents lowest and 9 represents the highest for colour, consistency, flavour and general acceptability. The panelists were made up of lecturers and post graduate students of the department of Home Science, Nutrition and Dietetics, University of Nigeria, Nsukka. The gruels were presented in a food warmer and coded. On arrival, the judges were served a hot coded AYB gruel using a soup plate. An evaluation form was also given immediately to each of the judges. A glass of water was given to rinse the mouth after each tasting; this was to avoid a carry over taste from preceding samples.

3.4 Statistical analysis

Statistical Package for Social Sciences (SPSS) 2.0 computer software was used to analyze the data. Means, standard deviation were calculated where appropriate. Analysis of variance (ANOVA) was used to determine the treatment that was different from others in the various parameters tested.

CHAPTER FOUR

4.0 RESULTS

4.1 Proximate composition of the different AYB flour on dry weight basis

Table 4 presents the proximate composition of the different AYB flours on dry matter basis. The results are determined at $p<0.05$.

4.1.1 Protein

The protein for each sample varied. The sample fermented in lime water for 24h and roasted had the least protein content (20.96%). The sample that was fermented in tap water for 24h and roasted had the highest protein (33.08%). The sample that was only roasted had 24.19% protein and the sample fermented in lime water for 48h and roasted had 27.86% protein.

4.1.2 Fat

The sample fermented in lime water for 48h and roasted had the least fat value (3.68%). The sample that was fermented in lime water had comparable fat with the sample that was only roasted (5.46% vs. 5.49%). The sample that was fermented in tap water for 24h had 4.90% fat.

TABLE 4: Effect of treatments on proximate composition of different AYB flour samples on dry matter basis (%).

Samples	Protein	Fat	Ash	Crude fibre	CHO
F24	33.08 [a]	4.90[b]	3.11 [a]	4.28[b]	54.63 [c]
FL24	20.96[d]	5.46[a]	4.08 [a]	4.24[b]	65.26 [a]
FL48	27.86[b]	3.68[c]	3.84[a]	4.16[b]	60.46[b]
OR	24.19[c]	5.49[a]	3.13[a]	6.31[a]	60.88[b]
LSD	3.23	0.56	1.02	2.03	4.38

*Means of three replications.
[abcde] values with different superscripts on the same column are significantly different ($P< 0.05$).
F24- AYB fermented in tap water for 24hrs & roasted
Fl24- AYB fermented in lime water for 24hrs & roasted
FL48- AYB fermented in lime water for 48hrs &roasted
OR- AYB Only roasted (control)

4.1.3 Ash

The ash values varied but not significant. The sample fermented in tap water for 24h and roasted had the least ash value (3.11%). The sample that was fermented in lime water for 24h and roasted had the highest value for ash (4.08%). The samples that were only roasted and that fermented in lime water for 48h had ash values of 3.13% and 3.84%, respectively.

4.1.4 Crude fibre

The sample that was only roasted had the highest fibre content (6.31%) which was significantly higher than the other samples. The fibre for other samples was not significantly different from one another. The sample that was fermented in lime water for 48h and roasted had the lowest fibre content (4.16%). The samples that were fermented in lime water for 24h and roasted and that fermented in tap water for 24h and roasted had fibre values of 4.24% and 4.28% , respectively.

4.1.5 Carbohydrate (CHO)

The sample that was fermented in lime water for 24h and roasted had the highest CHO value (65.26%) which was significantly different from the other samples. The sample that was fermented in tap water for 24h and roasted had the least CHO level (54.63%) which was significantly different from the other samples. The samples that were fermented in lime water for 48h and roasted and that which was only roasted had CHO values of 60.46% and 60.90% CHO respectively. There was no significant difference between the values.

4.2 Effect of treatment on the anti-nutrient content of AYB flour samples

Table 5 presents the effect of treatments on the anti nutrient contents of AYB flour samples. Results are reported at $p<0.05$

4.2.1 Phytate

The sample that was only roasted had the least phytate (2.68mg/g) level, which was similar to the phytate value of the sample fermented with lime for 48h and roasted (2.73mg/g). The sample that was fermented in lime water for 24h and roasted had the highest level of

phytate (2.97mg/g), which was significantly different from the levels in the other samples. The sample that was fermented in lime water for 24h and roasted had a phytate level of 2.81mg/g which was significantly different from the other samples ($p<0.05$).

Table 5: Anti nutrient composition of AYB flours (mg/g)

Samples	Phytate	Tannins	Saponins	Oxalate	Trypsin inhibitors
F24	2.81^{b}	0.02^{c}	0.01^{a}	0.01^{b}	0.48^{ab}
FL24	2.97^{a}	0.03^{ac}	0.01^{a}	0.01^{b}	0.51^{ab}
FL48	2.73^{c}	0.04^{ab}	0.01^{a}	0.03a	0.53^{a}
OR	2.68^{d}	0.03^{ac}	0.01^{a}	0.01^{b}	0.45^{b}
LSD	0.05	0.01	0.02	0.16	0.08

*Means of three replications.
^{abcde} values with different superscripts on the same column are significantly different ($P< 0.05$).
F24- AYB fermented in tap water for 24hrs & roasted
FL24- AYB fermented in lime water for 24hrs & roasted
FL48- AYB fermented in lime water for 48hrs &roasted
OR- AYB roasted only (control)

4.2.2 Tannins

The sample that was fermented in tap water for 24h and roasted had the least tannin level (0.02mg/g), which differed from the sample that was fermented in lime water for 48h and roasted. The sample fermented in lime water for 48h and roasted had the highest level of tannins (0.04mg/g). The samples that were only roasted and the one fermented in lime water for 24h and roasted had the comparables tannins (0.03mg/g).

4.2.3 Oxalates

The differences in the oxalate levels were not significant. The sample fermented in tap water and roasted, the sample fermented in lime water and roasted and the sample that was only roasted had the lowest oxalate level of 0.01mg/g. The sample that was fermented in lime water for 48h and roasted had the highest oxalate level of 0.03mg/g.

4.2.4 Saponins

The saponins content of all the samples were comparable (0.01mg/g) and did not differ ($p>0.05$).

4.2.5 Trypsin inhibitor

The roasted sample had the lowest trypsin inhibitor (0.45mg/g), which was significantly different from the sample fermented in lime water for 48h and roasted (0.53mg/g). The sample fermented in lime water for 48h and roasted had the highest trypsin inhibitor. The samples fermented in tap water for 24h and roasted and that fermented in lime water for 24h and roasted had trypsin inhibitor levels of 0.48mg/g and 0.51mg/g, respectively.

4.3 Effect of treatment on the raffinose, stachyose, hydrogen cyanide and haemagglutinin composition of AYB flours

Table 6 presents the effect of treatment on the raffinose, stachyose, haemagglutinins and hydrogen cyanide composition of AYB flours. Results are reported at $P<0.05$

4.3.1 Raffinose

The raffinose values for all the samples varied. Raffinose level was highest in the sample that was only roasted (9.22mg/100g). The sample that was fermented in tap water for 24h and roasted had the lowest raffinose (8.25mg/100g). The samples that were fermented in lime water for 48h and roasted and the one fermented in lime water for 24h had a raffinose level of 8.45mg/100g and 8.78mg/100g, respectively.

4.3.2 Stachyose

The sample fermented in lime water for 24h and roasted had the least stachyose (6.76mg/100g), while the sample that was only roasted had the highest stachyose (8.48mg/100g). The samples that were fermented in tap water for 24h and roasted and the sample fermented in lime water for 48h and roasted had a stachyose level of 7.19mg/100g and 7.51mg/100g respectively.

4.3.3 Haemagglutinin

The values for haemagglutinin differed. The sample that was fermented in lime water for 24h and roasted had the least haemagglutinin (4.87hu/100g). The sample that was

fermented in tap water for 24h and roasted had the highest haemagglutinin (6.70hu/100g). The samples that were only roasted and the one fermented in lime water for 48h and roasted had 5.30hu/100g and 5.80hu/100g, respectively.

4.3.4 Hydrogen cyanide (HCN)

The sample fermented in lime water for 24h and roasted had significantly lower hydrogen cyanide (0.22mg/g) than other samples. The sample fermented in lime water for 48h and roasted had a HCN level of 0.23mg/g, the sample that was fermented in tap water for 24h and roasted had 0.24mn/g HCN level. HCN was highest in the sample that was only roasted (0.28mg/g).

Table 6: Raffinose, stachyose, heamagglutinins and hydrogen cyanide composition of AYB flours

Samples	Raffinose (mg/100g)	Stachyose (mg/100g)	Haemagglutinins mg/100g	Hydrogen cyanide mg/g
F24	8.25[d]	7.19[b]	6.70[b]	0.24[b]
FL24	8.78[b]	6.76[b]	4.87[e]	0.22[c]
FL48	8.45[c]	7.51[b]	5.80[c]	0.23[b c]
OR	9.22[a]	8.48[a]	5.30[d]	0.28[b]
LSD	0.20	0.32	0.25	0.04

*Means of three replications.
[abcde] values with different superscripts on the same column are significantly different (P< 0.05).
F24- AYB fermented in tap water for 24hrs & roasted
FL24- AYB fermented in lime water for 24hrs & roasted
FL48- AYB fermented in lime water for 48hrs &roasted
OR- AYB roasted only (control).

4.4 Effect of processing on the organoleptic properties of the gruels

Table 7 presents the organoleptic characteristics of AYB gruels.

4.4.1 Colour

The sample that was fermented in lime water for 48h and roasted had the highest ($p<0.05$) for colour (6.53). The colour of the gruel made from the sample that was fermented in lime water for 24h and roasted had the least score for colour. It was neither liked nor disliked (4.83), the colour of the gruels made from the samples that were fermented in tap water for 24h and roasted (5.87) and the sample that was roasted only (6.10) were liked slightly.

4.4.2 Flavour

The sample fermented in lime water for 24h and roasted had the highest score ($p<0.05$) for flavour, it was liked moderately (6.57).The sample that was only roasted and the sample fermented in lime water for 48h and roasted were liked slightly and had scores of 5.67 and 6.00 respectively. The gruel made from the sample that was fermented in tap water for 24h and had the least ($p<0.05$) rating for flavour (5.50), though it was liked slightly.

4.4.3 Consistency

The consistency of all the gruels was similar. The consistency of the gruel fermented in lime water for 24h and roasted (6.17) and fermented in lime water for 48h (6.13) and roasted were liked slightly, while the sample that was fermented in tap water for 24h (6.87) and roasted only (6.97) were liked moderately with scores of 6.87 and 6.07, respectively.

4.4.4 Degree of acceptability

There were variations in the general acceptability of the gruels. The gruels made from the sample that was fermented in lime water for 48h and roasted, fermented in tap water for 24h and roasted and fermented in lime water for 24h and roasted were neither liked nor disliked. The roasted was liked slightly (6.03) which was the highest score for general acceptability statue.

Table 7: Organoleptic characteristics of AYB gruels

	Colour	Flavour	Consistency	Degree of acceptability
F24	5.87^{a}	5.50^{b}	6.87^{a}	5.17^{b}
FL24	4.83^{b}	6.57^{a}	6.17^{a}	5.30^{a}
FL48	6.53^{a}	6.00^{a}	6.13^{a}	4.93^{b}
OR	6.10^{a}	5.67^{b}	6.97^{a}	6.03^{a}
LSD	1.04	0.33	0.70	0.13

*mean± standard deviations of 30 panelist response on a 9-point hedonic scale with 9 = like extremely to 1 = dislike extremely.

^{abcde} values with different superscripts on the same column are significantly different ($P< 0.05$).

F24- AYB soaked in tap water for 24hrs & roasted
FL24- AYB soaked in lime water for 24hrs & roasted
FL48- AYB fermented in lime water for 48hrs &roasted
OR- AYB roasted only

Key: organoleptic scores

1. Dislike extremely
2. Dislike very much
3. Dislike moderately
4. Dislike slightly
5. Neither like or dislike
6. Like slightly
7. Like moderately
8. Like very much
9. Like extremely

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

5.1.1 Effect of processing on proximate composition of AYB flours

Protein

The increases and decreases in protein when compared with the control (roasted only) were solely attributed to treatments. The lower protein content for the sample fermented in lime water for 24h could be due to leaching of protein in the treatment medium. This observation is in conformity with the work of Wanjekece, Wakasa, & Mureithi (2003). The high protein content of the sample fermented in tap water for 24h could be attributed to the processing method used (Mugendi, Ngaji, Kuria, Mwasaru, Mureithi & Apostolides, 2010; Bressani, 1983; Borhade, Kadam & Salunkhe, 1984). This observation was because, during fermentation there was hydrolysis of protein enzyme tannin-complexes to release free amino acids for new protein synthesis (Tongul, Nanson, & fields, 1981). Fermentation is equally known to cause gradual increase in crude protein of AYB and cowpea flours (Nnam, 1997).

Fat

The lower fat content of the 48h lime fermented sample might be due to metabolic activities of micro organism and length of fermentation (48h). Vander Riet, Wight, Ciller & Datel (1987) observed that fat decreases as fermentation time increases. The reducd fat content of the fermented sample implies better keeping quality due to less rancidity of the flour.

Crude fibre

The decrease in the fibre content of the fermented samples is in line with other studies that reported decrease in crude fibre during fermentation (Eka, 1980; Achinewhu & Iscichei, 1990).

Carbohydrate

The decrease in the carbohydrate content for the sample fermented for 24h in tap water might be due to high utilization of energy by micro flora during fermentation as observed in earlier studies (Obiakor & Nwanekezi; 2009, Nnam, 1995), the decrease might also be due to the significant increase in the protein content.

5.1.2 Effect of treatments on the anti nutrient composition of AYB flours

Phytate

The high phytate level for the sample fermented for 48h in lime could be as a result of the lime treatment. All the samples, including the control appeared to be safe. The low anti-nutrients levels suggest that minerals otherwise chelated by phytate can be much more available. Reports from an earlier study showed that low phytate levels of 3.01mg/g had significant increase in calcium absorption (Heaney, Weaver & Fitzsimmons, 1991).

Tannins

The higher tannins in 48h lime fermented sample could be as a result of the addition of lime. Recent research shows that the consumption of tannins within 0.05-0.2% (150-200mg/100g) of a diet can be regarded as safe (Schiavone, *et al.,* 2007). Therefore, the tannin level observed in this study would not exert negative effect associated with tannins such as the lowering of available protein by antagonistic competition, this can elicite protein deficiency syndrome.

Oxalate

The higher oxalate for the 48h lime fermented sample could be as a result of the addition of lime. The levels of oxalates in all the samples were within safe level (4-5mg/g). Pearson (1973) reported that lethal dose of oxalate is between 200mg/100g and 500mg/100g. Oke (1969) reported that low levels of oxalates (4-5mg/g) is known to cause no irritation in the mouth or interfere with iron or calcium absorption. Dresbach (1980) reported that the reduction

of oxalate to a physiologically tolerable level by processing enhanced cellular utilization of some nutrients for metabolic activities of some enzymes.

Trypsin inhibitors

The higher trypsin inhibitors observed in the 48h lime fermented sample could be as a result of the addition of lime. However, the level of trypsin inhibitor observed from all the treated samples could be regarded as low and might be safe for consumption as the nutrient which trypsin inhibitors interfers with can be made more available. Trypsin inhibitors in the samples may not cause pancreatic enlargement and enhance chemically induced pancreatic tumors. Low levels of trypsin inhibitors (0.54mg/g) have been shown to have no damage to the pancrease (Harwood, 1986).

5.1.3 The effect of processing on the raffinose and stachyose contents of AYB flours.

The greater reduction of raffinose in all the samples treated is of interest. This is because raffinose is implicated in flatulence. The observation is similar to the reports of many workers (Nnam, 1997; Nwibani, Nwinuka, Bene, Abbey & Ayalogu, 2009). Fermented milk has been shown to have greater reduction in raffinose over stachyose (Nnam, 1997).The much more reduction in raffinose due to lime treatment agrees with the results that acidic medium fastens the reduction of oligosaccharide, especially raffinose sugar (Jean *et al*, 2004*)*.

5.1.4 Effect of treatment on the haemagglutinin and hydrogen cyanide composition of AYB flour samples.

Heamagglutinin

The much higher reduction of haemagglutinin in the 24h lime treated sample could be due to the intermittent changing of water and lime treatment provided acidic medium which lowered haemagglutinin on the sample (Puri, Booy, Doms, White & Blumenthal, 1990). The combination of fermentation in lime water for 24h and roasting appears to be most effective in reducing haemagglutinin in AYB.

Hydrogen cyanide (HCN)

The reductions of HCN in all the samples were far beyond the 35mg/100g lethal value (Oke, 1969). The much more reduction in lime treated samples agreed with previous result. This result indicated that treated AYB in citric acid medium led to the reduction of cyanide in AYB (Azeke, 2007). From this study, fermentation in lime water for 24h in combination with roasting is the most effective way to reduce HCN in AYB.

5.1.5 Effect of treatment on the beany flavour and other organoleptic characteristics of AYB gruel

Colour

The higher rating for colour in AYB gruel made from the sample that was fermented in lime water for 48h and roasted might be associated with the brown colour due to these treatments. This assumption is in line with previous study of Hesseltine & Wang (2009). They reported that fermentation and roasting improves colour by imparting brown colour to the product, this brown colouration could be enzymatic browning, caramelization of sugar or even Millard reaction. Nnam (1997) reported that the colour of the fermented AYB milk was slightly preferred to the unfermented milk. Another study by Onyechi and Nwachi (2008) reported that roasting improves colour. The brown colour observed in this sample can also be attributed to the higher tannin content of the sample. Tannins are known to be responsible for the pigmentation and browning of foods (Friday, Uhegbu, Onwuchekwa, Iweala, & Kanu, 2009).

Flavour

The preference of flavour to the control was a confirmation of previous studies of the importance of processing for improved usage of traditional foods (Nnam, 1997; Betsche *et al.,* 2005; Obizoba, & Egbuna, 1992; Nnam, 1994; Nnam, 2003). The higher preference for lime treated gruels might be attributed to masking of beany flavour of AYB due to synergistic effect of roasting and fermentation in lime water. Lime is known to improve flavour of grains such as

legumes and cereals (OnlineFamilydoctors, 2000; NewWorldencyclopedia, 2010; Fisher and Bender, 1975). Anecdotal reports and daily practice showed that lime was used successfully to mask strong flavour associated with foods such as fresh sea fish; *Kunun Gyda* (Drink made from groundnut) usually used by the Hausas; in the bakery industries as flavouring agents and in beverage industries among others. From the study, fermentation in lime water for 24h in combination with roasting has shown to be a better way of improving the flavour of AYB.

5.2 Conclusion

This work has shown that adequate knowledge of processes to increase nutrients and reduce anti nutrients and anti physiological factors would increase food usage of AYB. It also shows that the increased nutrients and reduced anti-nutrients, anti physiological factors and toxic substances in AYB were attributed to synergistic effects of food processing methods adopted. The 24h fermentation in tap water in combination with roasting increased protein much more than other methods. Fermentation in lime water for 24h decreased protein and increased carbohydrates. Fermentation in lime water for 48h reduced fat for better shelf life. The samples that were only roasted had better fibre content. The 48h fermentation in lime water increased the antinutrients, though the values are still within the safe levels. Fermentation in lime water for 24h had greater reduction in the haemagglutinins level of the flour samples, generally, lime treated samples had greater reduction in the raffinose, stachyose and HCN contents of the flours. The 24h fermentation in lime water in combination with roasting had positive effect on the beany flavour better than in the other products.

5.3 Recommendation

It is recommended that the combinations of different techniques of processing are important to produce (wholesome) flours free of beany flavour. Therapeutic studies of AYB products are imperative. Detailed studies are required in the area of lime treatment of AYB flours. Food industries are recommended to process AYB flours using these processing methods, package it well and make it available for buyers.

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APPENDIX 1

HEDONIC SCORING FORM FOR EVALUATION OF AFRICAN YAM BEAN GRUEL

Taste these samples and check how much you like or dislike each one on the hedonic scale by giving a score corresponding to each description below. Please give a reason for this attitude. An honest expression of your personal feeling will help us.

	FLAVOUR	COLOUR	CONSISTENCY	TEXTURE
F24				
FL24				
FL48				
OR				

KEY: Organoleptic Scores

10. Dislike extremely
11. Dislike very much
12. Dislike moderately
13. Dislike slightly
14. Neither like or dislike
15. Like slightly
16. Like moderately
17. Like very much
18. Like extremely

Reasons for your attitude for:

F24:----------

FL24----------

FL48:----------

OR----------

APPENDIX II

GENERAL ACCEPTABILTY RATING OF THE SAMPLES

	DEGREE OF ACCEPTABILITY	SAMPLES			
		F24	FL24	FL48	OR
9	I WOULD EAT THIS AT EVERY OPPORTUNITY				
8	I Would eat this often				
7	I would eat this occasionally				
6	I would eat this when available				
5	I would when there is no option				
4	I don't like this but would manage				
3	I would hardly ever eat this again				
2	I would eat this only if I were forced				
1	No account would I eat this				

APPENDAGE III

Raw cream coloured AYB seeds

Gruel from AYB flour samples

OR (control)