EFFECT OF PROCESSING (TOASTING AND FERMENTATION) ON THE NUTRITIONAL VALUE OF SICKLE POD (*Senna obtusifolia*) SEEDS

BY

ABDULKADIR MUHAMMAD

DEPARTMENT OF BIOCHEMISTRY

FACULTY OF LIFE SCIENCES

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

APRIL, 2018

EFFECT OF PROCESSING (TOASTING AND FERMENTATION) ON THE NUTRITIONAL VALUE OF SICKLE POD (*Senna obtusifolia*) SEEDS

BY

Abdulkadir MUHAMMAD BSc. (ABU ZARIA) 2011

P13SCBC8071

A DISSERTATION SUBMITTED TO SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER DEGREE IN NUTRITION

DEPARTMENT OF BIOCHEMISTRY

FACULTY OF LIFE SCIENCES

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

APRIL, 2018

DECLARATION

I hereby declare that this dissertation entitled "Effect of Processing (Toasting and Fermentation) on the Nutritional Value of Sickle Pod (*Senna obtusifolia)* Seeds" has been performed by me in the Department of Biochemistry under the supervision of Prof. D.A. Ameh and Prof. H.C Nzelibe. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at another university.

MUHAMMAD ABDULKADIR	……	…….....
(Name of student)	Signature	Date

CERTIFICATION

This dissertation entitled "EFFECT OF PROCESSING (TOASTING AND FERMENTATION) ON THE NUTRITIONAL VALUE OF SICKLE POD (*SENNA OBTUSIFOLIA)* SEEDS" by ABDULKADIR MUHAMMAD meets the regulation governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literacy presentation.

_____ Date _____

Prof. D.A Ameh

Chairman Supervisory Committee

_____ Date _____

Prof. H.C Nzelibe

Member Supervisory Committee

_____ Date _____

Prof. M.N Shuaibu

Head of Department, Biochemistry

_____ Date _____

Prof. S. Z Abubakar

Dean, Postgraduate School

DEDICATION

This work is wholeheartedly dedicated to the Almighty Allah (Subhanahu Wa Ta'ala), my beloved family, friends and Africa at large.

ACKNOWLEGEMENTS

All thanks and gratitude is due to Allah (SWT) Who provided me with the life, health and wisdom to carry out this research. I am highly indebted to my supervisors, Prof. D.A Ameh and Prof. H.C Nzelibe for their unending support in supervising this work. I am grateful for the time given from the beginning of this work to a successful and useful end. May Allah (Subhanahu Wa Ta'ala) reward them abundantly. I sincerely acknowledge the role of the Head of Department Prof. M.N Shuaibu, and all academic and non academic staff of Biochemistry Department for their useful suggestions.

I would also like to express my sincere gratitude to my parents-Muhammad Ismail and Malama Safinat for their resilience, financial support, encouragement, patience and love. I extend my gratitude to staff of Herbarium Laboratory, Department of Botany, Ahmadu Bello University Zaria, for helping me in identifying the plant. I would also like to thank my colleagues particularly Haneefat S. Ahmed, Usman Bello, Suleiman Abdullahi and DanHassan to mention but a few, for their immense contribution throughout the period of this research.

Very special thanks to all other individuals that assisted me in one way or the other towards the achievement of this work.

ABSTRACT

The effects of fermentation and toasting on the nutrients and antinutrients composition of *Senna obtusifoliai* seeds were investigated. *Senna obtusifolia* seeds were fermented with *Aspergillus niger* at an ambient (27-30°C) temperature for five days. *Senna obtusifolia* seeds were also toasted at 80^{o}C in open pan using hot plate for 10 min. Raw *Senna obtusifolia* seeds contained 7.45mg/100g moisture, 33.33mg/100g crude protein, 17.25mg/100g lipid, 9.49mg/100g dietary fibre, 4.50mg/100g ash, 37.99mg/100g nitrogen free extract. Antinutrients composition of raw *Senna obtusifolia* was 3.95mg/100g tannins, 3.74mg/100g phytates, 2.13mg/100g oxalates and 2.62mg/100g saponins, 11.23 TUI/mg trypsin inhibitor and 5.77 IC_{50} (mg/ml) alpha amylase inhibitor. Fermentation increased the crude protein of *Senna obtusifolia* but reduced nitrogen free extract, moisture, lipid and dietary fibre. Toasting reduced moisture, dietary fibre, crude protein but increased lipid, ash and nitrogen free extract. Toasting/fermentation (toasting followed with fermentation) increased moisture, nitrogen free extract and ash but reduced crude protein, dietary fibre and lipid. Fermentation reduced the tannins by 38%, phytates by 84%, oxalates by 36%, saponins by 37%, trypsin inhibitor by 12% and α-amylase inhibitor to 6.40 IC_{50} (mg/ml) while toasting reduced tannins by 63%, phytates by 89%, oxalates by 61%, saponins by 66%, trypsin inhibitor by 40% and α-amylase inhibitor to 13.05 IC_{50} (mg/ml). Toasting/fermentation reduced tannins by 70%, phytates by 93%, oxalates by 54%, saponins by 91%, trypsin inhibitor by 43% and α-amylase inbitor to 19.54 IC_{50} (mg/ml). The *in vitro* protein digestibility of raw *Senna obtusifolia* was 7.80%. The reduction in antinutritional factors was concomitant with a significant ($P \leq 0.05$) increase in protein digestibility after processing of the samples, fermentation, toasting and toasting/fermentation increased the *in vitro* protein digestibility of *Senna obtusifolia* to 45%, 22%, and 25%, respectively. Raw *Senna obtusifolia* seeds contained

both essential and non essential amino acids with fermentation and toasting increasing the amino acids content respectively but toasting/fermentation slightly reduced the amino acid content. The total minerals contents *Senna obtusifolia* seeds (Ca, Zn, K, Na, Fe and Mg) were reduced after processing. Fermentation reduced all the total mineral contents, toasting increased Fe, Mg, K and Na but reduced Ca and Zn. Toasting/fermentation reduced Ca, Mg, Zn, K and Na but increased Fe. Results obtained indicated that fermentation had significant influence on the proximate composition, *in vitro* protein digestibility, antinutritional factors, amino acids and mineral contents of *Senna obtusifolia* seeds.

Table of Contents

LIST OF TABLES

LIST OF PLATES

LIST APPENDICES

ABBREVIATIONS

Symbol	Interpretation
^{o}C	degree centigrade
AAS	Atomic Absorption Spectroscopy
AOAC	Association of Official Analytical Chemist
DNS	Dinitrosalicylic acid
D/wt	Dry weight
FAO	Food and Agriculture Organization
IFAD	International Fund for Agricultural Development
T/F	Toasting followed with fermentation
Ppm	part per million
Spp	Species
SPSS	Statistical Package for Social Science
TUI	Trypsin Unit Inhibition
UNICEF	United Nations Children Fund

UNU	United Nations University
USDA	United State Department of Agriculture
V	Volume
V/N	Voucher Number
V/V	Volume per volume
WHO	World Health Organization
WFP	World Food Program
HCN	Hydrocyanic acid
EAA	Essential Amino Acids
TEAA	Toatl essential Amino Acids
TNEAA	Total non essential amino acids
TAA	Total Amino Acids
TCA	Tricarboxylic acid

CHAPTER ONE

1.0. INTRODUCTION

Food-based approaches such as dietary diversification, fortification and biofortification are necessary strategies to prevent the debilitating effects of malnutrition by promoting the consumption of foods that are naturally rich in micronutrients. Dietary diversification has been advocated internationally for the improvement of micronutrient intake and status (Hedwig *et al.*, 2012). An increase in meat and animal products consumption is eminent in emerging and developing economies (Delgado *et al.*, 1999), yet the consumption of animal protein is still low in developing countries like Nigeria (Okojie, 1999; FAO, 2001). One reason often advanced for this scenario is the high cost of animal proteins often beyond the purchasing power of the majority and poor people of these countries. In order to boost the animal protein consumption of developing countries and achieve Millennium Developmoent Goal 1 (MDG 1), there is need to reduce the cost of livestock products by bringing down the cost of their feed. Soybean which is the main source of plant protein in the diets of farm animals is increasingly finding new uses as biodiesel stock in response to the deepening world energy crisis (Sasson, 2012). In addition, the use of soybean for food and energy by man often takes precedence over its use for livestock feeding. This dilemma has encouraged livestock nutritionists and feed millers to seek for alternatives to soybean as plant protein source in livestock diets. The alternative protein source has to be cheap, locally available and preferably not used as food by man. Dietary diversification reflects household access to varieties of foods and can also act as a proxy for individuals' nutrient adequacy. Wild plants play an important role in the diet of most rural dwellers in Nigeria. These plants tend to be drought tolerant and are gathered both in times of plenty as well as times of need. Throughout the year, the plants play an important role in supplying nutrients

and calories especially during the dry season when cultivated vegetables are scarce (Freiberger *et al.*, 1998). Although commonly eaten in the rural areas they are also consumed by urban people who buy from traders who also collect them from the wild.

The use of toxic plants as food after fermentation or heat treatment is known in Africa and other parts of the world (Ganiyu, 2005). Antinutritional factors such as enzyme inhibitors, hemagglutinin, flatulence factors, polyphenols, tannins and phytic acid inhibit the proteolytic activity of the digestive enzymes such as pepsin and trypsin as well as the availability of minerals (Deshpande and Cheryan, 1983). Minerals from plant sources, particularly those from plant seeds are less bioaccessible than those from animal's sources due in part to phytic acid, tannins and fiber content (Moelijopawiro *et al.*, 1998). These antinutritional factors chelate dietary minerals in the gastrointestinal tract reducing bioaccessibility and bioavailability (Frolich, 1995). Polyphenols can form complexes with metal cations through carboxylic and hydroxylic groups and thus interfere with the intestinal absorption of essential minerals such as calcium (Valencia *et al.*, 1999). Phytic acid is naturally occurring as a hexaphosphate derivative of inositol found in plants and in almost all mammalian cells. It consists of six phosphate moieties and chelates metal irons such as Ca, Mg, Zn, Mn, Cu and Fe to form insoluble complexes that are not readily absorbed (Graf and Eaton, 1993). Tannins also complex with enzymes of the digestive tract adversely affecting utilization of proteins and carbohydrates and resulting in reduced growth, feeding efficiency, metabolizable energy and bioavailability of amino acids (Onyango *et al.*, 2005). Several treatments have been applied to remove or reduce the amount of antinutrient from plant. Few examples among them are: fermentation, germination, thermal treatments (cooking) and soaking procedures (Abd El-Hady and Habiba, 2003; Martin-Cabrejas *et al.*, 2004). In general, these processing methods enhance the nutritive

value, reduce the amount of undesirable compounds and improve the protein digestibility of legumes (Martin- Cabrejas *et al*., 2004). Sicklepod is rich in Ca, Mg and P as major minerals while Fe, Zn and Mn were present at lower levels (Ousman *et al.*, 2005).

The plant *Senna obtusifolia* belongs to the kingdom plantae, family fabaceae, a common annual plant that grows wild in Northern Nigeria. The plant is considered a serious weed to Agriculturists in many places. It grows on well-drained fertile soil. Similarly, it is suited for cleared coastal forest countries. *Senna obtusifolia* has been regarded as indigenous leafy vegetable of the Sahel with potential of providing vegetable to the rural population in the month of August and September (Pasternak *et al.,* 2006). The young tender of *Senna obtusifolia* occasionally used as vegetable throughout Africa and elsewhere and the plant is cultivated in homes for this purpose in several countries including Senegal, Ghana, Cameroon, and Ethiopia. If eaten frequently or in large quantities could cause diarrhea (Irwin and Barneby, 1982). In Nigeria the seeds and roots of *Senna obtusifol*ia of no doubt have some laxatives effects. It is used as decoction febrifuge and for the treatment of scorpion stings, gingivitis, dysentery and diarrhea (David, 2002; Fowler, 2006). As the seeds are reputedly poisonous (Crowhurst *et al*., 1972), therefore processing is necessary before eating. The cooked vegetable tastes bitter but has an attractive consistency. *Senna obtusifolia* will probably remain a minor vegetable.

1.1. Statement of Reseach Problem

Soaring food prices have triggered an increase in hunger worldwide, especially in sub-Saharan African countries like Nigeria. The increase in prices of food has been attributed to several factors that include production shortfalls due to drought and floods; impact of climate change; increased demand for biofuel; emerging consumption habits of fast growing economics of some nations as well as trade policies to stabilize the food market crisis (FAO, 2008). According to the

W. H. O. (2013), malnutrition is one of the leading causes of death globally. It is well established that majority of people in developing countries depend mainly on cereal grains as their staple food due to limited income and high prices of animal foods (Sokrab *et al*., 2012). *Senna obtusifolia* has some limitations due to the presence of antinutritional factors, such as trypsin and amylase inhibitors, phytate and tannins. These compounds are known to interfere with protein and carbohydrate digestion and mineral bioavailability. Reduction or elimination of these undesirable components is essential for improving the nutritional quality of *Senna obtusifolia* and effectively utilizing their full potential as human food. Efforts however, are directed at improving the nutritional value of *Senna obtusifolia* seeds. Various simple processing methods such as soaking, sprouting, cooking and fermentation have been found to improve the nutritional value of plant (Yagoub and Abdalla, 2007; Mohamed Nour *et al*., 2010). It is well known that fermentation decreased the values of antinutritional factors as well as increased the *in vitro* protein and starch digestibilities and thus improve the nutritional quality of plant (Elkhalifa *et al*., 2004; Idris *et al*., 2005; Abdelseed *et al*., 2011; Mohammed *et al*., 2011). Cooking on the other hand has been reported to reduce the *in vitro* protein digestibility, whereas combining fermentation with cooking had significantly improved protein digestibility over wet cooking alone (Taylor and Taylor, 2002).

Since the seeds of *Senna obtusifolia* contain antinutritional factors that can be harmful to human, this calls for the adoption of effective processing methods like boiling, soaking, roasting, fermentation and sprouting before inclusion in diets. It is also important to use the seeds in a feeding trial with the aim of evaluating their biological values to humans.

1.2. Justification

Presence of antinutritional factors in this plant is one of the factors limiting its use in food formulation (Oladele and Aina, 2007). However, there is few detailed information on the nutritional profile of the seeds in relation to human consumption within the guinea savanna environment of Nigeria, where this plant is prevalent. This is very important since the nutritional and chemical value of this plant is partly influenced by geographical location which is shaped by factors like soil and climatic conditions (FAO, 2004). Therefore to gather reliable information regarding the use of this plant as food, the chemical and nutritional characteristics of this food needs to be carefully assessed in order to prevent errors in predicting human health and environmental effects impairing the survival of mankind.

Also most developing tropical countries have depended upon soybeans and other common legume grains as protein source for both humans and animals (Vadivel *et al*., 2008) but, their production is not sufficient to meet the protein requirements of the increasing population and expanding livestock industries (Vijayakumari *et al*., 2007). The competition between human and livestock for the consumption of soybean and the increasing role of soybean in the world as biodiesel feedstock (Cotula *et al.,* 2008) have increased its cost and demand and heightened the competition between human and animal for soybeans. Consequently, the search for a novel, high quality, cheap and readily available source of plant protein to replace soybean (Adeniji *et al.,* 2005; Obun and Ayanwale, 2006). One of such legumes with great prospect as alternative and replacement for soybean is *Senna obtusifolia* (sickle pod).

1.3. Null Hypothesis

Toasting and fermentation have no effect on the nutritive value of (*Senna obtusifolia)* sickle pod seeds

1.4. Aim

The aim of this research is to propose an effective processing method for safe utilization of sickle pod seeds.

1.5. Specific Objectives

- To determine the effect of processing (toasting, fermentation and toasting + fermentation) on the proximate composition of *Senna obtusifolia* seeds.
- To determine the effect of processing (toasting, fermentation and toasting + fermentation) on the antinutritional factors content of *Senna obtusifolia* seeds.
- To determine the effect of processing (toasting, fermentation and toasting + fermentation) on the *in vitro* protein digestibility of seeds of *Senna obtusifolia.*
- To determine the effect of processing (toasting, fermentation and toasting + fermentation) on the amino acid profile of the of *Senna obtusifolia* seeds.
- To determine the effect of processing (toasting, fermentation and toasting + fermentation) on the mineral composition of *Senna obtusifolia* seeds.

CHAPTER TWO

2.0 LITERATURE REVIEW

Processing methods such as soaking, sprouting, cooking and fermentation have been found to improve the nutritional value of plant grains (Mohamed *et al*., 2010; Yagoub and Abdalla, 2007). Fermentation decreases the values of antinutritional factors and as well increases the *in vitro* protein and starch digestibilities and thus improved the nutritional quality of plant samples (Elkhalifa *et al*., 2004; Idris *et al*., 2005; Abdelseed *et al*., 2011; Mohamed *et al*., 2011). Cooking on the other hand has been reported to reduce the *in vitro* protein digestibility of plant samples, whereas combining fermentation with cooking has significantly improved protein digestibility over wet cooking alone (Taylor and Taylor, 2002).

The nutritional quality of legumes depends upon the processing methods, presence or absence of antinutritional factors and possible interaction of nutrient with other food components (Ghadge *et al*., 2008). In order to improve the nutritional quality and to provide effective utilization of legume grains for animals, it is essential that anti-nutritional factors be removed or reduced. So, it is necessary to establish processing technique(s) to ensure its optimal utilization. In order to remove or reduce antinutrients, various conventional, simple processing methods have been used in legume seeds (Barbour *et al*., 2001; Farran *et al*., 2001).

2.1 The Effects of Processing Methods on Some Anti-Nutritional Compounds

Heat treatment: Heat processing is a commonly acceptable and effective means of inactivating the thermo-labile antinutritional factors of legume grains. The nutritive quality of most tropical legume grains, particularly cowpea, soybean, pigeon pea, lima bean and winged beans is notably improved by heat treatment. Heat treatment is a usual process before legumes are used in human diet. It improves protein quality by inactivating anti-physiological factors, particularly trypsin

inhibitor and haemagglutinins and by unfolding the protein structure, thus making them more easily susceptible to attack by digestive enzymes (Sathe *et al*., 1984).

Cooking: The effectiveness of cooking in reducing tannins was reported by Kingsley (1995), who showed cooking inactivates the activities of tannins. (Bressani and Elias, 1980) also observed that about 30-40% polyphenols can be removed from *Phaseolus vulgaris* by cooking for 45 min at 100°C and discarding the cooking water solution. Cooking for 60 minutes at 100^{o}C was sufficient to inactivate over 90% of the trypsin inhibitor activity in *Phaseolus vulgaris* (Trugo *et al*., 1990). Cooking method has been used and the stability of anti-nutritional factors could be reduced by up to 15% when cooked for 45 min (Bressani, 2002). Cooking has been found to reduce the antinutritional factors and thereby enhance the nutritional value of root and tuber crops including cocoyam (Agwunobi *et al*., 2002; Akpan and Umoh, 2004). Ogundipe *et al*. (2008) reported that 71.91% tannins content was destroyed after 30 minutes of cooking. Balogun *et al*. (2001) also observed that most of the antinutritional factors in legume seeds were destroyed after cooking the seeds for a minimum of 30 min. Buba *et al*. (2010) reported that, the antinutritional properties in sickle pod seeds were reduced and safe for inclusion in livestock rations for optimum growth and development when the seeds were cooked for 45 min. Uchegbu *et al*. (2008) reported no adverse effect in terms of performance of broiler chickens fed cooked *Napoleona imperialis* diet. However, cooking in water for 60 min at 100°C may denature protein making it unavailable and may induce loss of vitamins and minerals in legumes (Austin *et al*., 1981; Bressani, 1993).

Cooking also generally inactivates heat sensitive antinutritional factors such as trypsin and chymotrypsin inhibitors and volatile compounds. (Udedibie and Nwaiwu, 1988) subjected jackbeans to four different cooking periods of 30, 60, 90 and 120 min, taking the period of

cooking as starting from boiling. This was followed by drying in the oven at 60-70^{o}C and grinding. Phytochemical analysis of raw and cooked samples showed that cooking for 60 min was enough for the elimination of most of the thermo-labile antinutritional factors in the jackbean such as saponins, cyanogenic glycosides, terpenoids and alkaloids which were detected in raw jackbeans. Carlini and Udedibie (1997) reported that it took 2 h of boiling to completely eliminate trypsin inhibitor activity in jackbean and 3 h of boiling to render the legume lectin free. Onimisi *et al.* (2017) reported that boiling at 100^{o}C at varying periods of time and oven roasting at equivalent time periods detoxified and deactivated toxins in castor oil seeds. Balogun *et al*. (2004) also reported that boiling *Delonix regia* seeds at 100^{o}C at varying time intervals (0, 20, 40, 60, 80, minutes) to remove or to reduce toxins. However moist heat has been reported to improve digestibility of proteins by opening up the protein structure (Abbey and Berezi, 1988). Smith (1986) reported that proper processing of feeds require precise control of moisture content, temperature and processing time. Adequate moisture during processing facilitates destruction of the antinutritional factors.

Legume extrusion cooking would allow reduction of antinutritional factors and therefore improve the nutritional quality at a cost lower than other heating systems (baking, autoclaving, etc.) due to a more efficient use of energy and better process control with greater production capacities (Reimerdes, 1990; Morales *et al.,* 1998; Alonso *et al*., 2001).

Two-stage cooking: Two-stage cooking, the method used in some villages in Nigeria to cook some poisonous local food stuffs, has also been applied to jackbean. This process involves cooking the beans for about an hour, then discarding the initial water used in cooking and cooking again for about 40 min with fresh water (Udedibie *et al*., 1996).

Germination (Sprouting): Germination has been documented to be an effective treatment to remove or reduce antinutritional factors in feedstuffs. According to Esonu *et al*. (1998) sprouting initiates three main types of chemical changes in the seed as follows; i) the breakdown of certain materials ii) transport of minerals from one part of the seed to another, especially from the endosperm to the embryo or from the cotyledons to the growing parts and iii) the synthesis of new materials from the breakdown product. Bvochora *et al*. (1999) reported that depending on variety, total phenolic compounds in sorghum grain decreased upon germination. Investigation carried out by Awika and Rooney (2004) showed decrease in extractable proanthocyanidins (PAs), a condensed tannins located in pericarp and testa, after germination. Similarly, Iwuoha and Aina (1997) found a decrease in (PAs) content after germination. Torki and Farahmand-Pour (2007) concluded that germinated sorghum grain could be a better alternative for maize than intact sorghum grain.

Dehulling: It has also been shown that the effect of tannins can be eliminated or neutralized by dehulling (Edwards and Duthie, 1973; Ward *et al*., 1977). Bressani (2002) reported that dehulling improved protein quality of *Phaseolus vulgaris* and suggested that this could be due to the removal of the seed coat tannins which may have caused decrease in protein digestibility. When practically feasible, the recommended method for removal of condensed tanninss is dehulling to remove the tannins rich seed coat.

Autoclaving: Autoclaving entails cooking under pressure. The time of cooking is shortened by this method. When jackbeans were autoclaved for 30 minutes at 125^{o}C and 15 Ib pressure, thermo-labile inhibitory substances such as cyanogenic glycosides, saponinss, terpenoids and alkaloids could not be detected after autoclaving (Udedibie and Nwaiwu, 1988).

The nutritive value of many legumes is enhanced by autoclaving and this effect is probably related to the destruction of haemagglutinins and other inhibitory factors. Preliminary soaking prior to autoclaving is required for complete elimination of the toxicity of kidney bean (Jaffe, 1949) and field beans (Akande and Fabiyi, 2010). Kakade and Evans (1965) found that autoclaving for 5 min was sufficient to eliminate the toxicity of finely ground navy bean meal. Kessler *et al*. (1990) stated that there was little nutritional advantage in autoclaving for more than half an hour. They reported that autoclaving of jackbeans was a satisfactory technique for ensuring survival of birds receiving jackbean diets, confirming the findings of Jayne-Williams (1973) and D'Mello *et al*. (1985). The absence of lesions on the small intestine cells of broilers fed autoclaved jackbean suggest the elimination of the lectins during the autoclaving of the jackbeans. Autoclaving is a more sophisticated, but effective method of processing seeds for eliminating antinutrients (Abeke and Otu, 2008). However autoclaving is expensive, it requires adequate supply of electricity and technical knowledge.

Pressure cooking**:** Carlini and Udedibie (1997) stated that since it is a common practice to use a pressure cooker to cook most legume grains to save time and cost, they decided to determine how long it would take the pressure cooker to completely inactivate the concanavalin A and trypsin inhibitors in *Canavalia ensiformis*. The beans were subjected to four different pressure cooking times: 15, 30, 45 and 60 min. It took 30 min of pressure cooking to completely inactivate the trypsin inhibitor in *Canavalia ensiformis* while concanavalin A required 45 min for complete inactivation, establishing the fact that concanavalin A was more resistant to heat treatment than trypsin inhibitor.

Soaking: Marfor and Oke (1965), Ayenor (1985) and Iyayi and Losel (1999) recommended soaking as a processing method to reduce tannins in non-conventional legume and cereal grains. Ahamefule and Odemelam (2008) reported that 24 hrs soaking duration produced better results and would be preferred for tannins and cyanide in feed ingredients. However, some metabolic reactions take place during soaking which will affect some of the constituent compounds (Verde *et al*., 1992).

Microwave treatment: Kadam *et al.* (1987) in their experiment with winged bean (*Psophocarpus tetragonolobus*) meal, adjusted the moisture content in winged bean meal to 15% and allowed the meal to stand overnight at room temperature. The meal was then heated for 10 min in a Philips (Model 4915) microwave oven adjusted to 50% power input. D'Mello and Walker (1991) stated that microwave cooking of jackbeans was only marginally less effective than autoclaving of $KHCO_3$ -extracted jackbeans. This contrasts with the work of Kadam *et al*. (1987) who demonstrated marked satisfactory procedures over microwave treatment of winged beans. It is recognized that further research is required to examine the effects of sample size and heating times in order to adequately evaluate efficiency of microwave treatment in the detoxification of jackbeans.

Extrusion cooking: Partial detoxification of jackbean seeds by extrusion cooking has been reported by several workers (Bressani and Sosa, 1990; Akande and Fabiyi 2010). Although (Akande and Fabiyi, 2010) claimed complete inactivation of haemagglutinins in jackbean through extrusion cooking, a feeding trial with the product indicated only partial detoxification since it still depressed growth of the experimental cockerels. It thus showed that growth retarding effects of haemagglutinins were still present in the jackbean so treated. Pinto *et al*. (1997) reported that extrusion cooking of soybean even after malting could not completely inactivate

trypsin inhibitors in the seed. Any heat treatment that cannot completely eliminate trypsin inhibitors from a seed will obviously have little effect on lectins (Carlini and Udedibie, 1997). The nutritive value of extruded jackbeans is similar to that of the field beans (Lacassague *et al*., 1988).

Chemical treatment: Various chemical treatments have been employed in an attempt to improve the nutritional value of legumes. Ologhobo *et al*. (1993) reported that the extraction of jackbean flour with different solvents yielded fractions of varying toxicities, based on the solubility or otherwise of the toxic principles in the extracting medium. They reported higher concentrations of antinutritional factors in the base-soluble fraction than in the other fractions, indicating a greater extractability of anti-nutritional factors by alkali treatment than by acid, ether or alcohol. D'Mello and Walker (1991) achieved considerable success by using the alkali, potassium bicarbonate to detoxify jackbeans. A substantial amount of trypsin inhibitors have been reported to leach out of Great Northern beans by soaking in acidic or alkaline solutions (Eicher and Satterlee, 1988). Fernandez *et al*. (1993) observed that soaking faba beans in 0.07% sodium bicarbonate solution was more effective in decreasing the trypsin inhibitor activity than soaking it in 0.1% citric acid solution, probably due to the stability of the inhibitor in acidic pH.

Toasting: This involves dry heat treatment of legume bean seeds and other seeds for incorporation in animal diets. Sometimes, some quantity of water is sprayed on the seed before they are toasted. The improvement in the nutritive quality of legumes through toasting was first reported by Borchers and Ackerson (1950). However, toasting alone could improve its nutritive value for broiler chickens only to the extent of up to 100/kg dietary inclusion (Udedibie *et al*., 1996). Studies by Esonu *et al*. (1998), have demonstrated that toasting alone as a method of processing legume seeds did not appreciably reduce the level of toxic factors.

Toasting has been reported to effectively reduce the antinutritional factors of raw soybean which are associated with growth retardation in chickens (Akpodiete *et al*. 2001). Omoikhoje *et al.* (2009) reported that inclusion of roasted fluted pumpkin pod husk waste increased broiler chicken performance and did not affect palatability of the diets (El-Boushy and Vandal Poel, 2001).

Moderate heating of soybeans reduces the soy trypsin inhibitors (TI) and lectin activities to tolerable levels by fish. Thermal denaturation and inactivation of proteinase inhibitors and agglutinating lectins requires moist heat (McNaughton and Reece, 1980). Defatted soy flakes are typically toasted (steam-cooked) at 105 °C for 30 minutes to remove solvent residues after the oil extraction procedure. This reduces the TI activity down to levels tolerable by fish. It is also paralleled by denaturation and inactivation of the lectins (Maenz *et al*., 1999).

Falayi (2003) reported that the toasting of soybeans at a controlled temperature of 100^{o}C for 45 minutes greatly reduced its toxic content. Soybeans treated by dry extrusion at temperatures ranging from 121-130^{o}C and 30 bar pressure, has been confirmed as the best for fish production (Eyo, 2003).

Fermentation: Fermentation is an age-long method of processing cereals and legumes (Siegel and Fawcet, 1978). It modifies some physical characteristics of cereals and legumes, increases the level of some nutrients, digestibility and bioavailability (Ojokoh, 2014) decreases levels of antinutrients, increases nutrient density (Nnam, 1999) and imparts some antimicrobial property (Mensah *et al*., 1990). According to Quinn *et al*. (1975), fermentation of grains and oil seeds results in increased nutritional value and wholesomeness over the starting material and it may also lead to changes in vitamin levels. Food fermentation, and especially lactic acid fermentation,

is an important technology in Africa. Fermentation actually holds promise as a food processing method that can be used to diversify the food uses of some plant foods. The traditional processing of these foods therefore needs to be changed or modified to improve their nutritional status.

Fermentation of grains has been reported to yield a significant reduction in concentration of both phytic acid (Marklinder *et al*., 1996; Mukhopadhyay and Ray, 1999; Skrede *et al*., 2002) and α-galactoside oligosaccharide (Skrede *et al*., 2002; Refstie *et al*., 2005). Sandberg, (1991) and Hurrell, (2004) reported that fermentation can induce phytate hydrolysis via the action of microbial phytase enzymes, which hydrolyze phytate to lower inositol phosphates. They further assert that such hydrolysis is important because myoinositol phosphates with five phosphate groups (i.e., IP-1 to IP-4) do not have a negative effect on zinc absorption, and those with 3 phosphate groups do not inhibit non-heme iron absorption. Microbial phytases originate either from the micro-flora on the surface of cereals and legumes or from a starter culture inoculate (Sandberg, 1991).

Low-molecular-weight organic acids (e.g., citric, malic, lactic acids) are also produced during fermentation and have the potential to enhance iron and zinc absorption via the formation of soluble ligands while simultaneously generating a low pH that optimizes the activity of endogenous phytase from cereal or legume flours (Teucher *et al.,* 2004). Pusztai, (1989) reported that fermentation helps to reduce raffinose oligosaccharides due to the alpha-galactosidase found in the bean seeds, and also phytic acid content due to the action of phytase. Microorganisms which are responsible for the fermentation also play a role (Pusztai, 1989). Similarly in germination, most of the changes occurring during the fermentation of foods are of catabolic nature, and they help in the hydrolysis of such components as proteins and carbohydrates.

Fermentation of foods can also result in significant reduction in the quantity of certain antinutrients. Depending upon the type of legume as well as fermentation, phytic acid is also hydrolysed during fermentation to a variable degree (Deshpande, 2002). Fermentation of cereals reduces phytate content via action of phytase that catalyse conversion of phytate to inorganic orthophosphate and a series of myoinositols, lower phosphoric esters of phosphate. A 3-phytase appears to be characteristic of microorganisms, while a 6-phytase is found in cereal grains and other plant food (Shahidi, 1997). According to Vin (2009) consumption of fermented food has been shown to improve the intestinal balance of beneficial lactic acid bacteria. Based on this and the phytase producing capability of the bacteria, the consumption of fermented foods may be another effective way to reduce the potential of dietary phytic acid impairing mineral absorption (Vin, 2009).

Gamma Irradiation: Gharaghani *et al*. (2008) reported the effect of gamma irradiation on anti-nutritional factors and nutritional value of canola meal for broiler chickens. Glucosinolate content was reduced to 40, 70 and 89% at irradiation dose levels of 10, 20 and 30 (Kilo gray) kGy, respectively. Their observation indicated that the irradiation treatment has a significant effect on the glucosinolate content of canola meal. Gamma irradiation treatment with its radiolytic effects can destroy glucosinolate molecules. Other investigators reported that anti nutritional factors, such as protease inhibitors (Farag, 1989; El-Morsi *et al*. 1992; Farag, 1998), a-amylas inhibitors (Al-Kahtani, 1995; Abu-Tarboush, 1998), phytohamagglutinins (Farag, 1989) and (Farag, 1998), oligosaccharides (Rao and Vakil, 1983; Ghazy, 1990) and tannins (Abu-Tarboush, 1998) were significantly inactivated by gamma irradiation.

2.2 History of Commercial Usage of *Aspergilus niger*

The primary uses of *A. niger* are for the production of enzymes and organic acids by fermentation. Fermentations to produce these enzymes may be carried out in large volumes (Finkelstein *et al*., 1989). *A. niger* is also used to produce organic acids such as citric acid and gluconic acid.

The history of safe use for *A. niger* comes primarily from its use in the food industry for the production of many enzymes such as a-amylase, amyloglucosidase, cellulases, lactase, invertase, pectinases, and acid proteases (Bennett, 1985; Ward, 1989). Citric acid fermentation using *A. niger* is carried out commercially in both surface culture and in submerged processes (Berry *et al*., 1977; Kubicek and Rohr, 1986; Ward, 1989).

A. niger has some uses as the organism itself, in addition to its products of fermentation. For example, due to its ease of visualization and resistance to several anti-fungal agents, *A. niger* is used to test the efficacy of preservative treatments (Jong and Gantt, 1987). In addition, *A. niger* has been shown to be exquisitely sensitive to micronutrient deficiencies prompting the use of *A. niger* strains for soil testing (Raper and Fennell, 1965). There is also interest in using this fungus to perform certain enzymatic reactions that are very difficult to accomplish by strictly chemical means, such as specific additions to steroids and other complex rings (Jong and Gantt, 1987).

Aspergillus niger is a member of the genus *Aspergillus* which includes a set of fungi that are generally considered asexual, although perfect forms (forms that reproduce sexually) have been found. *Aspergilli* are ubiquitous in nature. They are geographically widely distributed, and have been observed in a broad range of habitats because they can colonize a wide variety of substrates. *A. niger* is commonly found as a saprophyte growing on dead leaves, stored grain,

compost piles, and other decaying vegetation. The spores are widespread, and are often associated with organic materials and soil.

2.3 Senna obtusifolia species

Senna obtusifolia belongs to the *Fabaceae* (Leguminoseae) or bean (pea) family, and sub-family *Ceasalpinoideae.* It is an annual weed taxonomically known as *Senna obtusifolia*. This weed is found in Myanmar, China, and Central America and in Nigeria. It is locally called Tapasa in Hausa language. This is a small fetid weed growing up to an average height from 30 to 90cm (Parsons and Cuthbertson, 1992 and Holm *et al*., 1997).The stem is light green cylindrical or grooved and profusely branched. The leaves are paripinnate; leaflets are in 3- pairs and obovate-oblong. Leaves are arranged on a rachis which is grooved and up to 10 cm in length. Conical glands are found between each two lowest pairs of leaflets. The leaflets are usually in three pairs, opposite, obovate and oblong with oblique base. Flowers are found in pairs in axils of leaves. The flowers bloom in August to September. Corolla consists of five petals in yellow or pale colour. The pod is long and slender, 4 sided sharp and pointed. Each pod has 20-30 seeds that are rhombhedral in shape. The authors (Parsons and Cuthbertson, 1992 and Holm *et al*., 1997) further described *Senna obtusifolia* as an erect branched bushy annual or perennial that grows up to 90cm high and reproduces from seeds. The pod is 10-15cm long, 3-5mm wide slender and sickle shaped. The seeds are about 5mm long and 2mm wide somewhat rhomboid brown and smooth. Muhammad *et al*. (2002) reported that *Senna obtusifolia* is very abundant on rangeland. Sicklepod is a weed of pastures and it has the potential to become a major weed of several other crops. In pasture, it is an aggressive invader and can completely dominate grass species, eradicating pasture growth and excluding stock. Although it is generally unpalatable to stock, if eaten, sicklepod is toxic to cattle. Sicklepod is dispersed by water (stream flow and floods), in

mud on machinery and stock, in mulch and by stock which ingest the pods. Seed production is profuse (8000 seeds per plant) and as the seed is long lived, an abundant seed bank can become established in the soil. Scarification of the seed is required for germination to occur, and sometimes fires can cause mass emergences of seedlings because of this. The plant grows on a variety of soil types and is usually an annual, although it can perenniate.

Plate I: ***Senna obtusifolia*** **Plant with Fresh Fruits**

Source: www.harbalshop.com

Plate II. *Senna obtusifolia* Seeds

Source: www.herbalshop.com

2.4 Application and Utilization of *Senna obtusifolia*

2.4.1 Food Formulation

Different parts of *Senna obtusifolia* plant are used for food in various parts of the world; depending on the culture and season, the seeds and leaves are used. In northern Nigeria the sickle pod leaves are used as (kwado) where the leaves are boiled for 5 minutes and the water is drained and it is mixed with grind peanut bars (garin kulikuli) or it can be eaten in a form of vegetable soup. In Sudan Sicklepod (kawal) leaves are prepared by a solid state process of fermentation and used as an ingredient of sauces destined for consumption with porridge. It imparts a savoury and a meaty flavour on the sauce (Dirar, 1993). Kawal is used in relatively large quantities in the preparation of sauces as a meat substitute or meat extender by poor people and in small quantities as a spice by some urban rich people. It is used wildly by Fur who invented it and other neighbouring tribes (Dirar, 1993). During famine years, kawal, a protein source, and its juice, probably protected many children against kwashiorkor (Dirar, 1993). The seeds are poisonous but when roasted they are used as a substitute for coffee (Crowhurst, 1972) and they contain a gum of commercial importance in addition to protein and fat (Wu and Abbott, 2004).

2.4.2 Feed Formulation

The leaves and the seeds have also been assessed as dietary ingredients for poultry (Gupta *et al.*, 1970; Charles and Müller, 1975; Page *et al.*, 1977; Herbert and Flory, 1983) and found to cause poor growth rate and reduced egg production. However, the leaves and seeds, are rich in protein (Sigh, 1964; Gohl, 1975; Taparia *et al.*, 1978) and might, therefore, be of considerable value.

2.4.3 Uses of underutilized crops

Nigeria has been catalogued as an epicentre of diversities of wild varieties of plant species which are grown for various purposes such as food, oil, fiber, fodder, and as sources of traditional medicine, and play a major role in the subsistence of local communities and frequently are of special social, cultural and medicinal value (Elbert, 2014). With good adaptation to often marginal lands, they constitute an important part of the local diet of communities providing valuable nutritional components, which are often lacking in staple crops (Jain and Gupta, 2013).

Underutilized crops especially traditional vegetables such as jute mallow (*Corchorus olitorius*), African nightshade (*Solanum scabrum*), eggplant, drumstick tree (*Moringa oleifera*), bitter gourd (*Momordica charantia*), water spinach (*Ipomoea aquatica*), Chinese kale (*Brassica oleracea* var. *alboglabra*), edible rape (*Brassica napus*), roselle (*Hibiscus sabdariffa*), Malabar spinach (*Basella alba*), slippery cabbage (*Abelmoschus manihot*), winged bean (*Psophocarpus tetragonolobus*) and many gourd species are of considerable commercial value and thus can make a significant contribution to household income. (Hughes and Ebert 2013) cite a number of examples of profitable cultivation of traditional vegetables in East and West Africa such as worowo (*Solanacio biafrae*), cockscomb (*Celosia argentea*), African eggplant (*Solanum macrocarpon*), and amaranth (Elbert, 2014).

In Eastern Africa and Southeast Asia selected traditional vegetables are becoming an increasingly attractive food group for the wealthier segments of the population and are slowly moving out of the underutilized category into the commercial mainstream (Weinberger, 2007). Attracted by the strong market demand, seed companies are beginning to explore and develop

these popular crops, thus strengthening the formal seed sector (AVRDC, 2008). Apart from their commercial, medicinal and cultural value, traditional vegetables are also considered important for sustainable food production as they reduce the impact of production systems on the environment (Elbert, 2014).

2.5 Proximate, Antinutrients and Amino Acids Composition of Raw *Senna obtusifolia* Seeds

The proximate composition of the raw seeds (Table 2.1) show a high dry matter (92.50%), crude protein (29.54%), ether extract (2.31) nitrogen free extract (NFE) (46.77) and crude fibre (10.18%) as reported by Ingweye *et al*. (2010). It also contains alkaloids, saponins, tannins, oxalates and phytates contents in various concentrations. The hydrogen cyanide concentration is reported by Ingweye *et al*. (2010) to be 6.98mg/100g. The essential amino acids (EAA) of *S obtusifolia* include phenylalanine, lysine, valine, leucine, isoleucine, threonine, methionine, cystine and tyrosine while the non-essential amino acids are aspartic acid serine, glutamic acid, proline, glycine and alanine (Table 2.2).

Table 2.1: Proximate Composition and Antinutrients of Raw *Senna obtusifolia* Seeds

Parameters	Concentration (%DM)
Dry matter	92.50
Crude protein	29.54
Ether extract	2.31
Crude fibre	10.18
Nitrogen free extract	46.77
Ash	3.70
Calorific value (Kcal/100g)	325.28
Phytochemicals	Concentration (mg/100g)
Alkaloids/antinutrients	360.00
Saponins	185.00
Tannins	388.50
Oxalates	83.25
Hydrogen cyanide	6.98
Phytates	240.50

Source: Ingweye *et al*. (2010)

Table 2.2: Amino Acids Composition of Raw *Senna obtusifolia* Seeds

Amino acids	Concentration (g/100g protein)
Phenylalanine	4.50
Lysine	4.02
Valine	4.30
Leucine	7.60
Isoleucine	3.01
Threonine	2.20
Methionine	1.20
Cystine	0.92
Tyrosine	3.54
Histidine	7.11
Arginine	4.67
Aspartic acid	8.30
Serine	3.00
Glutamic acid	9.16
Proline	2.33
Glycine	4.11
Alanine	3.71

Source: Ingweye *et al*. (2010)

2.6 Biochemical Effects of Major Antinutrients in Legumes

The antinutritional factors (ANFS) can be defined as those substances generated in natural food stuffs by the normal metabolism of species and by different mechanisms (such as inactivation of some nutrients or metabolic utilization of feed) which exert effects contrary to optimum nutrition (Kumar, 1992). The biochemical and adverse effects of plant's secondary metabolites (antinutritional factors) have been reviewed by several authors (Cheeke and Shull, 1985; Aletor, 1993; Osagie, 1998; Fu *et al*., 2002). However, their adverse effects will be briefly highlighted. Antinutritional factors diminish animal productivity but may also cause toxicity during periods of scarcity or confinement when the feed rich in these substances is consumed by animals in large quantities (Kumar, 1992).

Oxalate, phytate and tannins are antinutrients, which could be toxic when consumed in an unprocessed food (Ojiako and Igwe, 2008). The bioavailability of the essential nutrients in plant foods could be reduced by the presence of some anti-nutritional factors such as oxalates and cyanogenic glycosides (Akindahunsi and Salawu, 2005).

2.6.1 Cyanogenic glycoside

Cyanogenic glycoside on hydrolysis yields toxic hydrocyanic acid (HCN). The cyanide ions inhibit several enzyme systems, it depresses growth through interference with certain essential amino acids and utilization of associated nutrients. They also cause acute toxicity, neuropathy and death (Osuntokun, 1970; Fernando, 1987).

2.6.2 Alkaloids

Alkaloids cause gastrointestinal and neurological disorders (Aletor, 1993). The glycoalkaloids, solanine and chaconine present in potato and *Solanum spp*. as reported by Saito *et al*. (1990) and

Aletor (1991) alkaloaids are haemolytically active and toxic to fungi and humans. Some of the toxicological manifestations of potato glycoalkaloids involve gastrointestinal upsets and neurological disorders, especially in doses in excess of 20 mg/100 g sample.

2.6.3 Tannins

Tannins are complex polyphenolic compounds found widely in the plant kingdom (Hagerman and Butler, 1978). They are found in leaves, twigs, flowers, fruits and tree barks. Tannins are usually subdivided into two major groups, the hydrolysable and condensed tannins.

The hydrolysable tannins: these are split into sugars and phenolic carboxylic acids in acid and alkaline conditions (White, 1957) and are further classified according to the products of hydrolysis into gallo tannins (gallic acid and glucose) and ellagic tannins (ellagic acid and glucose) (Mcleod, 1974). Two other categories, tara-gallo tannins (gallic and quinic acid plus glucose) and caffe-tannins (caffeic acid and quinic acid plus glucose) have also been suggested (Haslam, 1966). Hydrolysable tannins can be hydrolyzed upon heating by weak acid, weak base or enzymatically (Gemede and Ratta, 2014).

Condensed tannins: Condensed tannins are referred to as proanthocyanidins because they produce red anthocyanidins when heated in acid (Haslam, 1982). Proanthocyanidins are phenylpropanoid polyphenols and are categorized by type of monomer they contain: either flavan-3-ols or flavan-3,4-diols into catechin or lecoanthocyanidin (Horvath, 1981). Generally, the high molecular weight condensed tannins (i.e >10 flavonol units) have limited solubility and extractability and hence may have little nutritional significance (Mehansho *et al.*, 1987). Condensed tannins polymerize on heating in acids to amorphous phlobaphenes (Foo *et al.*, 1989).

The beta tannins: Beside hydrolysable and condensed tannins, beta tannins can be added (Swain, 1979; Horvath 1981). Beta-tannins are protein precipitating compounds which are insoluble in water. They form very stable bond with protein and appeared to have lower molecular weight than other tannins (Woodward and Reed, 1989).

Tannins cause decreased consumption of feed in animals by binding dietary protein and digestive enzymes to form complexes that are not readily digestible (Aletor, 1993). The precipitation can be as a result of hydrogen bonding (Loomies and Battaile, 1966), covalent bonding (Swain, 1979), ionic bonding (Gustavson, 1956) or hydrophilic interaction (Oh *et al.*, 1980). The interaction between tannins and protein is very specific (Hagerman and Butter, 1981) and dependent on their molecular weight (Kumar and Horigome, 1986), water solubility (Addisu, 2016), conformation, compatibility of binding site and the solvent pH (Wooward and Reed 1989; Reed *et al*. 1985) showed that condensed tannins seem to be more important in forming complex in feed than hydrolysable tannins. The effect of tannins on quality of forage includes reduction in palatability and feed intake of feeds in animals (Bate-Smith, 1973), growth rate (Woodward and Reed, 1989) digestion of fiber fractions (Barry and Manley, 1984; Reed, 1986). They also cause decrease in palatability and reduced growth rate (Roeder, 1995).

2.6.4 Saponins

The saponins are naturally occurring surface-active glycosides which are composed of carbohydrate and non carbohydrate or aglycone portion. The aglycones are often refered to as sapogenins. The sapogenin nucleus may be either of steroid or triterpenoid structure. They are mainly produced by plants, but also by lower marine animals and some bacteria (Riguera, 1997; Yoshiki *et al.* 1998). They derive their name from their ability to form stable, soap-like foams in

aqueous solutions. This easily observable character has attracted human interest from ancient times. Saponinss consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin) which may be triterpenoid or steroid in nature (Das *et al.*, 2012). The aglycone may contain one or more unsaturated C–C bonds.

Saponins cause hypocholesterolaemia by binding cholesterol, making it unavailable for absorption. They also cause haemolysis of red blood cells and are toxic to rats (Johnson *et al*., 1986). Saponinss from *Bulbostermma paniculatum* and *Pentapamax leschenaultii* have also been reported to have anti-spermal effects on human spermatozoa (Su and Guo, 1986; Pant *et al*., 1989). They significantly inhibite acrosine activity of human sperms and the spermicidal effect might be attributed to strong damage of the spermal plasma membrane (Su and Guo, 1986).

2.6.5 Trypsin inbitor

Trypsin (a protese inhibitor) causes pancreatic enlargement and growth depression (Aletor and Fetuga, 1987). Proteins like haemagluttinins are known for agglutinating red blood cells. They depress animal growth by interfering with the digestion and absorption of nutrients in the gastrointestinal tract (Aletor and Fetuga, 1987).

2.6.6 Phytates

Among all the antinutritional components, phytic acid is of a major concern for human nutrition and health management. The unique structure of phytic acid (myoinositol (1,2,3,4,5,6) hexakisphosphoric acid) offers it the ability to strongly chelate cations such as calcium, magnesium, zinc, copper, iron and potassium to form insoluble salts. It therefore adversely affects the absorption and digestion of these minerals by animals (Raboy, 2001). Salts of phytic

acid contains mono and divalent cations such as K^+, Mg^{2+} and Ca^{2+} which accumulates in the seeds during ripening and are the main storage form of both phosphate and inositol in plant seeds and grains (Loewus, 2002). Besides, phytate has also been reported to form complexes with proteins at both low and high pH values. These complex formations alter the protein structure, which may result in decreased protein solubility, enzymatic activity and proteolytic digestibility. In spite of many negative aspects on human health, the consumption of phytate, however, has been reported to have some favourable effects, including its anticarcinogenic effect (Shamsuddin, 2002). The metal binding characteristics of phytate endow it with an anti-oxidant function, inhibiting the production of hydroxyl radicals that normalise cell homeostasis (Minihane and Rimbach, 2002) and it also acts as a natural food antioxidant (Raboy, 2003). Dietary phytate may have health benefits for diabetic patients because it lowers the blood glucose response by reducing the rate of starch digestion and slowing gastric emptying (Thompson, 1993). Likewise, phytate has also been shown to regulate insulin secretion (Barker and Berggren, 1999). It is believed that phytate reduces blood clots, cholesterol and triacylglycerols and thus prevents heart diseases (Jariwalla *et al*. 1990). It is also suggested that it prevents renal stone development (Grasses *et al.*, 2000; Selvam, 2002).

Phytates is, therefore a common constituent of plant derived foods like cereals or legumes, which are the main staple food of people in developing countries. The daily intake of phytate for humans on vegetarian diets, on an average, is 2000–2600 mg whilst, for inhabitants of rural areas in developing countries, on mixed diets, it is 150–1400 mg (Reddy, 2002).

2.6.7 Oxalates

Oxalates like phytates also bind minerals like calcium and magnesium and interfere with their metabolism. They also cause muscular weakness and paralysis. Oxalates also cause

gastrointestinal tract irritation, blockage of the renal tubules by calcium oxalate crystals, development of urinary calculi and hypocalcaemia (Oke, 1969; Blood and Radostits, 1989). Too much of soluble oxalate in the body prevents the absorption of soluble calcium ions as the oxalate binds the calcium ions to form insoluble calciumoxalate complexes. As a result of this, people with the tendency to form kidney stones are advised to avoid oxalate-rich foods (Adeniyi *et al*., 2009).

2.6.8 Alpha amylase

Alpha amylase is responsible for the breakdown of oligo and/or disaccharide to monosaccharides. Inhibitors of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time causing a marked decrease in the rate of glucose absorption thereby blunting the post prandial plasma glucose rise (Rhabasa and Chiasson, 2004).

2.7 Nutritional Effect of Major Minerals in Plants

Calcium, according to Vasudevan and Sreekumari (2007) is highly recommended for animals for activation of enzymes, mediation of muscle excitation and contraction, bone and teeth formation, blood coagulation, transmission of impulses, regulation of heartbeat, maintenance of acid-base equilibrium in the body of animal and reduction of allergic exudates. Phosphorus plays more known functions in the animal body than other mineral elements (McDonald *et al.,* 1995). It associates with Ca in bone and teeth formation, involved in energy metabolism and low dietary intakes are associated with infertility, poor growth and live weight gain in livestock (McDonald *et al.,* 1995). Phosporus in the form of phosphate ion is needed for, production of high energy compounds e.g ATP, DNA and RNA formation and activation of enzymes.

Sodium is present in the soft tissues and body fluids. It is associated with acid base equilibrium. A chief cation of blood plasma, it functions in nerve impulse transmission and absorption of sugars and amino acids from the gut. A deficiency of sodium leads to body dehydration, poor growth and reduced utilization of digested proteins (McDonald *et al*., 1995). Sodium also helps in maintaining body fluid pH, support nerve transmission and muscular contraction in livestock (McDonald *et al.*, 1995). Magnesium is necessary for efficient metabolism of carbohydrates and lipids, involved in cellular respiration and general cellular biochemistry and function (McDonald *et al.*, 1995). As reported by Vasudevan and Sreekumari (2007), that beans and other vegetable foods like cereals are rich in Mg. Mg is also important in lowering the irritability of neuromuscular system and activates enzymes like phosphatase which require ATP (Banerjee, 2004). The requirement for Fe is most critical in young suckling animals because milk is a poor source of Fe, the deficiency which causes iron deficiency anemia (Vasudevan and Sreekumari, 2007), could be prevented by feeding nursing animals with diets rich in Fe. Fe content was high compared to 11.5 and 8.4mg/100g for soy bean and mung beans (Vasudevan and Sreekumari, 2007). This indicates the possibility of *Senna obtusifolia* if fed livestock, to improve transport of oxygen to the tissues as well as enzymes of electron transport chain (McDonald *et al.*, 1995). Chatterjea and Shinde (2007) reported that legumes, pulses, oil seeds and un-milled cereals are good sources of Zn. Zn is an antioxidant and aids in Cu absorption in some disease conditions. Many enzymes are dependent on Zn for proper functioning.

This implies that feeding animals with high level of Zn could improve electrolyte balance; hence production, storage and secretion of hormones; improve immune system and prevent parakeratosis, a skin ailment (McDonald *et al.*, 1995). Potassium is necessary for nerve transmission, maintenance of osmotic pressure and acid-base equilibrium, activation of certain

enzymes uptake of certain amino acids as well as carbohydrates and protein metabolism (Banerjee, 2004). The inclusion of Cu in livestock diets could protect the heart by increasing high density lipoprotein (HDL) tyrosinase activity and Fe absorption and incorporation into haemoglobin

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. Collection of Plant Sample and Preparation

The mature pods of *Senna obtusifolia* were harvested from the wild within Sabon Gari local government area of Kaduna state at the onset of dry season (first week of October 2016). The pods were properly dried, threshed and the seeds obtained were properly dried in the sun. The identity of the plant was confirmed at the herbarium of Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna state, Nigeria, and a voucher number was deposited (V/N:- 573). The dried seeds were pounded in a laboratory mortar and sieved using a 1mm mesh sieve. All chemicals and reagents used were of analytical grade

3.2. Processing of Sickle Pod Seeds

Toasting was carried out by the method described by Oladele *et al*. (2009). The seeds were toasted for 10 minutes at 80^{o}C in an open pan using hot plate. The fermentation of the seeds was carried out according to the method described by Gabriel *et al.* (2015) by mixing the *Senna obtusifolia* seed with water in the ratio 2:1 (1 part of *Senna obtusifolia* seed to 2 parts of water); 0.25ml of cultured *Aspergillus niger* was prepared. The *Aspergillus niger* was collected from the Department of Microbiology laboratory of Ahmadu Bello University Zaria, it was dissolved using Tween 80 detergent and then pipetted using foster pipette and mixed with 100ml of water. The mixture was packed in a plastic container, firmly sealed with cotton wool before being kept in a room at ambient temperature of 25°C. The fermentation period lasted for five days. The fermented samples were then washed and spread on a polythene sheet in a room and dried for six (6) days up to about 90% dry matter. The seeds were ground into powder using hammer mill.

Toasting followed by fermentation for 5 days were used to detoxify the seeds again and it was ground into powder using hammer mill.

3.3. Determination of Proximate Composition

3.3.1. Determination of moisture

The method employed for the determination of moisture content of the sample was based on the measurement of the loss in weight due to drying at a temperature of about 105^{o}C as described by AOAC (2000). Six watch glasses were washed and dried in an oven at 105^{o}C after which they were cooled and weighed empty. Two grams of each sample was weighed into their respective watch glasses. The watch glasses and their contents were dried in an air circulated oven at 105^{o}C to a constant weight. The watch glasses and their contents were cooled in desiccators and reweighed.

The percentage moisture content of each sample was calculated using the expression

$$\%\, moisture = (Loss\ of\ weight\ on\ drying(g) \times 100)/(Initial\ sample\ weight)$$

3.3.2. Determination of ash content

The term ash refers to the residue left after the combustion of the oven dried sample and is a measure of the total mineral content. Determination of ash content was carried out according to the method described by AOAC (2000). Six crucibles were preheated in a muffle furnace at 550^{o}C. Each crucible was cooled in a desiccator and weighed. Approximately 1g of each sample was weighed into the different crucibles. The crucibles and their contents were then transferred into the muffle furnace at 550^{o}C and allowed to stay for 5 hours. The weights of the crucible contents were taken and recorded. Percentage ash was calculated using the expression below:

$$Ash = \frac{Weight\ of\ ash \times 100}{Weight\ of\ dry\ sample}$$

3.3.3. Determination of crude lipid content

The lipid content of each sample was determined by the procedure described by AOAC (2000). A clean dry round bottom flask containing anti bumping granules was used. Exactly 210cm^3 of petroleum ether (60 – 80^{o}C) was put into a flask fitted with soxhlet extraction unit. The weighed sample was transferred into a thimble already fixed into the Soxhlet extraction unit. Cold water was put into circulation. The heating mantle was then switched on and the heating rate adjusted until the solvent is refluxed at a steady rate. Extraction was carried out for 8hours.

The sample was removed and dried to a constant weight in an oven, cooled in a desiccator and reweighed and the percentage crude lipid content was determined thus;

$$\%\, lipid \;=\; (Weight\ of\ lipid\ extracted \times 100)/(Weight\ of\ dry\ sample)$$

Where the weight of lipid extracted was the loss in weight of the sample after extraction, drying in an oven and cooling in a desiccator.

3.3.4. Determination of crude fiber

Crude fibre was determined by the method of AOAC (2000). Two grams of the grounded sample was placed in a round bottom flask. 100ml of 0.25M H_2SO_4 was added and the mixture was boiled under reflux for 30minutes. The insoluble matter was washed several times with hot water until it was acid free (C1). It was then transferred into a flask containing 100ml of 0.25M NaOH solution. The mixture was boiled again under reflux for 30 minutes and filtered under suction. The insoluble residue was washed with hot water until it was base free, then the residue and crucibles were oven dried at 105^{o}C overnight and weighed (C2). It was then ashed in a furnace at 550^{o}C for 2 hours. The furnace was then put off and allowed to cool down. The sample was then removed and cooled in a dessicator and weighed (C3). The crude fibre content was then

calculated as loss of weight in ashing. Weight of original sample was used as W.

$$\%\, crude\ fiber\ =\ (C2 - C3\ \times 100)/W$$

3.3.5. Determination of dietary fibre

Dietary fiber was determined by AOAC (2000) method. Samples were defatted and gelatinized in the presence of heat stable alpha amylase, and then enzymatically digested with protease and amyloglucosidase to remove digestible protein and starch. Four volumes of ethanol were added to precipitate soluble dietary fibre. Total residue was filtered off and washed with ethanol and acetone. The residue was weighed after drying. The remaining material was analyzed for protein and ash content respectively. Subtracting the amounts measured for protein, ash and a blank control from the dry weight of the filtered residue yields a value for total dietary fiber content.

3.3.6. Determination of nitrogen content and crude protein

Principle

Proteins are major compounds containing nitrogen primarily in the form of amino acids which are their building blocks. Nitrogen is used as an index termed crude protein as distinct from true protein. The Kjedahl method of AOAC (2000) was used for crude protein determination.

Steps for determination

A mineralization steps of organic substance in boiling sulphuric acid.

$$2H_2SO_4 \longrightarrow 2SO_2 + 2H_2O + O_2$$

$$R{-}CH(COOH)(NH_2) + O_2 \longrightarrow CO_2 + 2H_2O + 2NH_3$$

B. Distillation Steps of Ammonium sulphate after alkalisation of the Boric acid Solution

$$(NH_4)_2SO_4 + NaOH \longrightarrow 2Na_2SO_4 + 2H_2O + 2NH_3$$

$NH_3 + H_2O \longrightarrow NH_4OH$

$H_3BO_3 + 3NH_4OH \longrightarrow (NH_4)_3BO_3 + 3H_2O$

C. Titration of ammonium with hydrochloric acid of standardized concentration

$(NH_4)_3BO_3 + 3HCl \longrightarrow 3NH_4Cl + H_3BO_3$

Method.

Two grams of each sample was weighed into 100ml Kjedahl flask and a few anti bumping granules were also added. One gram of the mixed catalyst ($CuSO_4$ and K_2SO_4 in the ratio 8:1 respectively) and 15ml of concentrated sulphuric acid were added. The flask was placed on a Kjedahl digestion rack and heated until a clear solution was obtained. At the end of the digestion, the flask was cooled and the sample was quantitatively transferred to a 100ml volumetric flask and made up to the mark with distilled water. Ten milliliters of the digest was pipetted into Markham semi micro nitrogen steel tube, 10ml of 40% NaOH solution was then added cautiously. The sample was then steam distilled liberating ammonia into a 100ml conical flask containing 10ml of 4% boric acid and a drop of methyl blue indicator until the colour changed from pink to green. 30ml of sample volume was then collected. The content of the conical flask was then titrated with 0.1M HCl. The end point was indicated by a colour change from green to pink and the volume (v) of the acid for each distillate was noted. Percentage nitrogen per sample was calculated using the expression below:

$$\%Nitrogen = (M \times v \times 14 \times 100 \times 100)/(Weight\ of\ sample \times 1000 \times 10)$$

Where, M = Molarity of HCl

14 = Atomic weight of nitrogen.

100 = Total volume of digest.

100 = % conversion.

10 = Volume of the digest taken.

1000 = Conversion to litre.

The crude protein was calculated as:

% Protein = 6.25 x % nitrogen.

3.3.7. Determination of nitrogen free extract.

Nitrogen free extract (carbohydrate) was calculated by subtracting the total percentages of moisture, crude protein, crude lipid, ash and crude fibre from 100%.

NFE (%) = 100 – (moisture* + protein + lipid + ash + fibre**)

* In case of dry matter basis, moisture was excluded

**** Fibre is included here so that NFE represents potentially available carbohydrate**

3.4. Determination of Antinutritional factors

3.4.1. α-Amylase inhibition assay

This assay was carried out using a modified procedure of McCue and Shetty, (2004). A total of 250µL of extract was placed in a tube and 250µL of 0.02m sodium phosphate buffer pH 6.9 containing α-amylase solution was added. This solution was pre-incubated at 25^{o}C for 10minutes, after which 250µL of 1% starch solution in 0.02M sodium phosphate buffer pH 6.9 was added at timed interval and then further incubated at 25^{o}C for 10mins. The reaction was terminated after incubation by adding 500µL of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5minutes and cooled to room temperature. The reaction mixture was diluted with 5ml distilled water and the absorbance was measured at 540nm using spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water. Individual blanks were prepared for correcting the background absorbance. In this case, the colour reagent solution was added prior to the addition of starch solution and the

mixture then placed in the water bath immediately. The α-amylase inhibitory activity was calculated as percentage inhibition.

%Inhibition $=\{(A_{control} - A_{samples})/A_{control}\} * 100$

Where $A_{control}$ is the absorbance of each control and $A_{samples}$ is the net absorbance of each sample. The net absorbance of each sample was calculated using the following equation:

$A_{samples} = A_{test} - A_{blank}$

Where A_{test} is the absorbance of each test and A_{blank} is the absorbance of each blank. Concentrations of extract resulting in 50% inhibition of enzyme activity (IC_{50}) were determined.

3.4.2. Determination of trypsin inhibition

Trypsin inhibition was determined by the method described by Onwuka (2005). Extraction of sample: A known weight (1g) of ground sample was weighed and dispersed into $50cm^3$ of 0.5M NaCl solution. The mixture was stirred for 30mins at room temperature and centrifuged. The supernatant was filtered through whatman filter paper. The filtrate (extract volume) was used for the assay.

Preparation of standard trypsin and substrate: 1mg/ml of trypsin in 0.1M HCl was prepared, 1% casein substrate in 0.1M phosphate buffer pH7.7 was prepared. To $2cm^3$ of trypsin standard solution, $1cm^3$ of trypsin inhibitor was added and incubated for 10mins at 37^oC for 10minutes. A blank of $5cm^3$ substrate was prepared in a test-tube (with no trypsin inhibitor extract added). The contents in the test-tube was left for 10minutes and the reaction was stopped by adding $3cm^3$ 5% TCA. It was then filtered and absorbance was measured spectrophotometrically at 410nm. The trypsin inhibitor activity was expressed as the number of trypsin unit inhibited (TUI) per unit weight of the sample analyzed.

Calculation: TUI/mg = (b-a)/0.1 X F

Where b = absorbance of test sample solution

A = absorbance of the blank

F = (1 x Vf/Va x D)/W

Where W = weight of sample

Vf = total volume of extract used in the assay

D = dilution factor

Va = volume of standard

3.4.3. Determination of phytic acid

Phytic acid was determined using the procedure described by Lucas and Markaka (1975). Exactly 2.0g of each powdered sample was weighed into 250ml conical flask, 100ml of 2%HCl acid was used to soak each sample in the conical flask for 3 hours and then filtered through a double layer of harden filter paper, 50ml of each filtrate in 250ml beaker and 100ml of distilled water was added in each case to give proper acidity.

Ten milliliters of 0.3% ammonium thiocyanide solution was added into each solution as indicator and titrated with standard iron (III) chloride solution which contains 1.95mg of iron/ml. The end point was brownish colour persisting for 5minutes. The percentage of phytic acid was calculated using the formula:

$$Phytic\ acid\ (mg/100g) = (Y \times 1.19 \times 100)/(Sample\ weight)$$

Where ;

Y = titre value x 1.95mg.

3.4.4. Determination of oxalates

Oxalate was determined using method of Oke, (1965). The total oxalic acid content of the powdered sample was determined by weighing 2g of sample into 250ml of conical flask.190ml of distilled water and 10ml of 6MHCl acid were then added. The mixture was warmed for 1hr on a boiling water bath, cooled, transferred into a 250ml volumetric flask, diluted to volume and filtered. Four drops of methyl red indicator were added, followed by concentrated ammonia till the solution turned faint yellow. It was then heated to 100^{o}C, allowed to cool and filtered to remove precipitate containing ferrous irons. The filtrate was then boiled and 10ml of 5% $CaCl_2$ added with constant stirring. It was then allowed to stand overnight.

The mixture was filtered through Whatman No. 4 filter paper. The precipitate was then washed several times with distilled water and transferred to a beaker and 5ml of 25% sulphuric acid was added to dissolve the precipitate. The resultant solution was maintained at 80^{o}C and titrated against 0.5% potassium permanganate until the pink colour persisted for approximately one minute. A blank was ran for the test sample. From the amount of potassium permanganate used, the oxalate content of the unknown sample was calculated using the equation below:

1ml potassium permanganate = 2.24mg oxalate.

3.4.5. Determination of tannins

Tannins content was estimated according to the method described by Joslyn (1970). Two grams (2g) of the sample was poured into a beaker containing 50 ml distilled water and heated to 60^{o}C. it was thereafter filtered and the residue discarded. Ten (10ml) of 4% copper acetate solution was added to the hot filtrate and boiled again for 10 min. The precipateg was filtered and the filtrate was discarded. The residue was dried using filter paper and the dried sample scraped from filter paper into a pre-weighed crucible. The weight was recorded as W. The crucible (which contained

the sample) was incinerated in a muffle furnace at 550^{o}C, cooled in a dessicator and then reweighed as W1. The difference between the weight of the sample before ashing and the ash residue after incineration represent the tannins content.

3.4.6 Determination of saponins

The saponins contents of the sample were determined following the AOAC (1990) method. Saponins extraction was done using acetone and methanol. Crude lipid content of the sample was extracted with acetone while methanol was used to extract saponins. Samples (2.0g) were folded in a filter paper and put in a thimble and extracted by refluxing in a soxhlet extractor. Extraction was done with 200cm^3 of acetone in a 250cm^3 capacity round bottomed flask for 3hr after which the apparatus was dismantled and another 150cm^3 capacity flask containing 100cm^3 methanol was fitted to the extractor and extraction sustained for another 3hr.

Weight of flask before and after the second extraction was taken to note the change in weight. Methanol was recovered by distillation after the second extraction and the flasks oven-dried, allowed to cool at room temperature and weighed. Saponins content was calculated from the formula:

$$\text{Saponins: (mg/100g)} = \frac{\text{A- B}}{\text{Sm}} \times 100$$

Where A= mass of flask and extract

B= mass of empty flask, Sm= sample mass

3.5. *In Vitro* Assay for Protein Digestibility

The *in vitro* protein digestibility was carried out according to the method of Mertz *et al.* (1984). Two hundred (200) milligrams of the powdered bean sample was dispersed in 20cm^3 of pepsin

reagent (1.5mg/ml in 0.1M phosphate buffer of pH 2.0) and shaken vigorously. All the tubes were kept in a water bath at 37^{o}C for three hours with constant shaking at 15 minutes interval. After three hours, the digestion was stopped by removing the tubes from the water bath and placing them in ice bath for 30 minutes. The samples were then filtered through whatman No.1 filter paper and the residue washed with buffer and dried at 80^{o}C for 2 hours. The dried residue was placed in a 50cm^{3} micro-kjedahl flask and analyzed for nitrogen by mico-kjedahl digestion. The indigestible nitrogen was subtracted from total nitrogen of the sample to obtain digestible nitrogen using the following:

Digestible N (mg) = Total N in sample (mg) – N in residue (mg).

Digestible protein = Digestible N (mg) × Conversion factor.

$$\% \text{ in vitro digestibility} = \frac{\text{Digestible protein}}{\text{Total protein in sample}} \times 100$$

3.6. Determination of Amino Acids Profile

The amino acids profile of the sample was determined using the method 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 Analyzer (TSM).

Defatting Sample**:**

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

ii. Nitrogen Determination:

A small amount (200mg) of ground sample was weighed, wrapped in Whatman filter paper (No.1) and put in the Kjedhal digestion flask. Concentrated sulphuric acid (10ml) 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. Four pieces of anti-bumping granules were added.

The flask was then digested for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (10m1) of the diluted solution with 10ml of 40% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70ml of distillate was collected.

The distillate was then titrated with standardized 0.01 N hydrochloric acid to grey colour.

$$Percentage\ Nitrogen\ =\ ((a-b)\times 0.01\times 14\times V\times 100)/(W\times C)$$

Where:

a. = Titre value of the digested sample

b. = Titre value of blank sample

v. = Volume after dilution (100ml)

W. = Weight of dried sample (mg)

C. = Aliquot of the sample used (10ml)

14. = Nitrogen constant in mg.

Hydrolysis of the sample

Approximately 1.4g of the defatted sample was weighed into glass ampoule. 7ml of 6NHCL was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis (e.g methionine and cystein). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105^0C±

5^0C for 22 hours. The ampoule was allowed to cool, then broken open at the tip and the content was filtered. It should be noted that tryptophan is destroyed by 6N HCL during hydrolysis.

The filtrate was then evaporated to dryness at 40^oC under vacuum in a rotary evaporator. The residue was dissolved with 5ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles, which was kept in the freezer.

Loading of the Hydrolysate into TSM analyzer

The amount loaded was between 5 to 10 microlitre. This was then dispensed into the cartridge of the analyzer. The TSM analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of an Analysis lasted for 76 minutes.

Method of Calculating Amino Acid Values from the Chromatogram Peaks.

An integrator attached to the Analyzer calculates the peak area proportional to the concentration of each of the amino acids.

3.6.1. Determination of tryptophan

Tryptophan is a difficult amino acid to determine in proteins and peptides because it chemically decomposes during acid hydrolysis. It should be noted that tryptophan is destroyed by 6N HCl during hydrolysis. Antioxidants such as thioglycolic acid or dodecanethiol have been used with 6N hydrochloric acid (HCl) to preserve tryptophan. Alkaline hydrolysis has also been studied and was shown to produce higher tryptophan recovery than acid hydrolysis. The addition of phenol has also been reported. Alkaline hydrolysis was improved by using sodium hydroxide (Na0H) instead of barium hydroxide to prevent problems with both precipitation and adsorption of tryptophan.

Determination:

The tryptophan in the sample was hydrolyzed with 4.2 M Sodium hydroxide (Robel, 1967). The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

Defatting Sample

A known weight of the dried sample was weighed into extraction thimble and the fat was extracted with chloroform/methanol (2:1 mixture) using soxhlet extraction apparatus as described by AOAC (2006) the extraction lasted for 15 hours.

Hydrolysis of the Sample

Approximately 1.3g of the defatted sample was weighed into glass ampoule. 10ml of 4.2M NaOH was added and oxygen was expelled by passing nitrogen into the ampoule. The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at $105^{o}C \pm 5^{o}C$ for 22 hours. The ampoule was allowed to cool then broken open at the tip and the content was filtered to remove the humins. The filtrate was neutralized with 6N HCl and evaporated to dryness at $40^{o}C$ under vacuum in a rotary evaporator. The residue was dissolved with 5ml of acetate buffer (pH 7.0) and stored in plastic specimen bottles, which were kept in the freezer.

Loading of the Hydrolysate into Analyzer

The amount loaded was 60 microlitre. This was injected into the analyzer.

Method of Calculating Amino Acids Values

An integrator attached to the analyzer calculates the peak area proportional to the concentration of each of the amino acids.

3.7. Mineral Analysis

Zinc (Zn), iron (Fe), calcium (Ca), and magnesium (Mg) were determined by atomic absorption spectrometry while sodium (Na) and potassium (K) was determined by flame photometry according to the method of AOAC (2003).

WET DIGESTION OF SAMPLE: approximately (1.0g) of the powdered sample was taken in digesting glass tube. Twelve milliliters (12ml) of HNO_3 was added to the samples and mixtures were kept overnight at room temperature. Then 4.0ml perchloric acid ($HClO_4$) was added to the mixture and kept in the fumes block for digestion. The temperature was increased gradually starting from 50^oC and increasing up to $250 – 300^oC$. The digestion was completed in about 70-85 minutes as indicated by the appearance of white fumes. The mixture was left to cool and the contents of the tubes were transferred to 100ml volumetric flasks and the volumes of the contents were made to 100ml with distilled water. The wet digested solution was transferred to plastic bottles labeled accurately and stored for mineral determination.

Proceedure: the absorption measurement of the elements for the samples was read out. Different electrode lamps were used for each mineral. The equipment was run for standard solutions of each of the mineral before and during determination to check that it is working properly. The dilution factor for all minerals except Mg was 100, for determination of Mg, further dilution of the original solution was done using 0.5ml original solution and enough distilled water was added to it to make the volume up to 100ml. Also the determination of calcium (Ca), 1.0ml lithium oxide solution was added to the original solution to unmask Ca from Mg. The concentration of minerals was determined using the formular below:

Mw = absorbance (ppm) × dry wt. × D/wt. of sample × 100

Where Mw = Conc. of minerals

D = Dilution factor

The same wet digested sample solutions as used in AAS was used for the determination of Na and K. Standard solutions of 20, 40, 60, 80 and 100milli equivalent/L were used for both Na and K. The calculations for the total minerals involve the same procedure as given in AAS.

3.8. Statistical Analysis

The data obtained were expressed as mean ± standard deviation (SD). The data generated were analyzed using (ANOVA). Duncan multiple range test was used for multiple mean comparison tests to determine differences between the means. Significance at 0.05 level of probability ($p<0.05$) was accepted.

CHAPTER FOUR

4.0 RESULTS

4.1 Effects of Processing Methods on Nutrient Composition of Senna obtusifolia Seeds

The proximate compositions of raw, fermented, toasted and toasted/fermented *Senna obtusifolia* seeds are presented in Table (4.1). The crude protein content of raw *Senna obtusifolia* sample was 33.33±0.02g/100g and for fermented *Senna obtusifolia* 36.86±0.06g/100g. The crude protein content for the fermented sample differed significantly ($p<0.05$) from the toasted 31.17±0.37/100g and toasted/fermented sample 25.39±0.62/100g. The toasted/fermented significantly reduced the crude protein contents compared to the toasted seeds. In all the processing methods there was a significant difference ($P<0.05$) in crude protein amongst treatment.

The crude lipid content of the raw *Senna obtusifolia* seed was 17.25±0.05/100g but the toasted 10.86±0.06g/100g, fermented 16.37±0.30g/100g and toasted/fermented 15.21±0.15 *Senna obtusifolia* seeds had lower lipid content than the raw. All the processing methods decreased the lipid contents of the seeds. In all the processing methods there were significant differences ($P<0.05$) in crude lipids contents.

The ash content of the raw *Senna obtusifolia* 4.50±0.35g/100g was significantly from that of the fermented 4.10±0.07g/100g and toasted 4.82±0.03g/100g but not significantly ($p>0.05$) than that of the toasted/fermented seeds which recorded a higher value of 5.33±0.13g/100g which is significant ($p<0.05$) when compared with the other processing methods (fermentation and toasting).

The crude fiber contents of raw *Senna obtusifolia* seeds sample was 15.38±0.35g/100g and significantly ($p<0.05$) from fermented sample 13.39±0.0g/100g *Senna obtusifolia* seeds. Toasting significantly ($p<0.05$) increased fibre contents of *Senna obtusifolia* seeds. As well as toasting/fermentation increased crude fiber of *Senna obtusifolia* significantly ($p<0.05$) from the raw sample. The crude fibre content of the fermented sample of *Senna obtusifolia* was lower than the raw sample and significantly ($P<0.05$) among treatments. Also the dietary fibre of all the processed seeds were significantly ($p<0.05$) from raw seeds.

The moisture content of raw *Senna obtusifolia* seeds was 7.45±0.13g/100g the moisture content of the fermented sample 7.44±0.14g/100g was not significantly ($p>0.05$) from the raw. There was a significant ($P<0.05$) in the moisture contents among toasted 3.23±0.35g/100g and toasted/fermented treatment 7.79±0.08g/100g with the toasted/fermented *Senna obtusifolia* seeds recording the highest moisture content 7.79g/100g The nitrogen free extract content of the raw *Senna obtusifolia* seeds was 22.09±0.16g/100g while fermentation decreased the nitrogen free extract content 21.84±0.21g/100g but there was a significant increase ($P<0.05$) in nitrogen free extract content in the toasted and toasted/fermented seeds 26.92±0.38g/100g and 26.33±0.08g/100g, respectively.

4.2. Antinutritional Compounds of Raw and Processed *Senna obtusifolia* Seed

The antinutritional compounds of raw and processed *Senna obtusifolia* seeds is presented in Table 4.2. There were significant ($p<0.05$) in antinutritional compounds between raw and the differently processed seeds, the levels of these compounds reduced significantly. The phytates concentration in the raw seed 3.74±0.03g/100g was reduced significantly ($p<0.05$) to 0.58±0.09g/100g for the fermented seeds representing 84.50% reduction. Similarly, the concentration of phytates reduced to 0.42±0.06g/100g for the seed toasted representing a

reduction of 89% of phytates and also the concentration of phytates reduced to 0.26±0.07g/100g in the toasted/fermented seeds representing 93% reduction in phytates respectively. The tannins in the raw seed was 3.95±0.21g/100g which was significantly ($p<0.05$) reduced by 38% to 2.45±0.49g/100g in the fermented seed. The tannins was also reduced by 63% to 1.48±0.71g/100g in toasted seed, while the tannins in the seed toasted/fermented also reduced by 70% to 1.18±0.11g/100g. The oxalate concentration was 2.13±0.01g/100g in the raw seed it was reduced to 1.36±0.28g/100g in the fermented seed representing a 36% reduction, the concentration was reduced to 0.83±0.07g/100g in the toasted seed representing 61% reduction and in the toasted/fermented seed concentration was also reduced to 0.99±0.15g/100g representing 54% reduction.

Saponins concentration was 2.62±0.68g/100g in the raw seeds and it was significantly ($p<0.05$) reduced to 1.65±0.35g/100g in the fermented seeds, representing a reduction of 37%, the concentration was also reduced to 0.90±0.07g/100g in the toasted seed representing a reduction of 66% and in the toasted/fermented seeds, the saponins was reduced to 0.23±0.04g/100g representing a reduction of 91%. Trypsin inhibitor concentration was 11.23±0.68TUI/mg in the raw seeds and it was significantly ($p<0.05$) reduced to 9.92±0.01TUI/mg in the fermented seeds representing a reduction of 12%, the concentration was also reduced to 6.72±0.01TUI/mg in the toasted seeds representing a reduction of 40% and in the toasted/fermented seeds the trypsin inhibitor was reduced to 6.39±0.01TUI/mg representing a reduction of 43%.

Table 4.1: Effect of Processing on the Proximate Composition of *Senna obtusifolia* Seeds.

	MOISTURE (g/100g)	LIPID (g/100g)	ASH (g/100g)	PROTEIN (g/100g)	Crude FIBRE (g/100g)	D FIBRE (g/100g)	CARBOHYDRATE (g/100g)	CALORIFIC VALUE (kcal/100g)
Raw	7.45±0.13^{b}	17.25±0.05^{d}	4.50±0.35^{ab}	33.33±0.02^{c}	15.38±0.35^{b}	9.49±0.02^{d}	37.99±0.16^{b}	440.53
Fermented	7.44±0.14^{b}	16.37±0.30^{c}	4.10±0.07^{a}	36.86±0.06^{d}	13.39±0.01^{a}	6.72±0.06^{a}	35.06±0.21^{a}	435.01
Toasted	3.23±0.35^{a}	10.86±0.06^{a}	4.82±0.03^{bc}	31.17±0.37^{b}	23.00±0.14^{d}	8.27±0.08^{c}	50.03±0.38^{d}	422.54
Toasted/ Fermented	7.79±0.08^{c}	15.21±0.15^{b}	5.33±0.13^{c}	25.39±0.62^{a}	19.95±0.50^{c}	7.68±0.22^{b}	46.04±0.08^{c}	422.61

Values are expressed as Mean ± SD of duplicate determination and values with different superscripts on the same column are significantly different ($p<0.05$).
R-Raw, F-Fermented, T-Toasted and T/F-Toasted followed with fermentation

Table 4.2: Effect of Processing on the Anti-nutritional Contents of *Senna obtusifolia* Seeds

	PHYTATE (g/100g)	TANNINS (g/100g)	OXALATE (g/100g)	SAPONINS (g/100g)	TRYPSIN INHIBITOR (TUI/mg)	α-AMYLASE INHIBITOR IC_{50} (mg/ml)
Raw	3.74 ± 0.03^{a}	3.95 ± 0.21^{a}	2.13 ± 0.01^{a}	2.62 ± 0.68^{a}	11.23 ± 0.03^{a}	5.77 ± 0.50^{a}
Fermented	0.58 ± 0.09^{a}	2.45 ± 0.49^{bc}	1.36 ± 0.28^{b}	1.65 ± 0.35^{ab}	9.92 ± 0.01^{b}	6.40 ± 1.57^{b}
Toasted	0.42 ± 0.06^{ab}	1.48 ± 0.71^{bc}	0.83 ± 0.07^{c}	0.90 ± 0.07^{b}	6.72 ± 0.01^{c}	13.05 ± 0.88^{c}
Toasted/ Fermented	0.26 ± 0.07^{ac}	1.18 ± 0.11^{c}	0.99 ± 0.15^{c}	0.23 ± 0.04^{bc}	6.39 ± 0.01^{d}	19.54 ± 1.46^{d}

Values are expressed as Mean ± SD of duplicate measurement and values with different superscripts in the same column are significantly ($p<0.05$).

4.3 Effects of Different Processing Methods on the *In Vitro* Protein Digestibility of *Senna obtusifolia* Seeds.

The *in vitro* protein digestibility of the differently processed samples showed significant (P< 0.05). The fermented seeds had the highest digestibility followed by toasted/fermented and toasted seeds. Fermentation significantly (P<0.05) improved the *in vitro* protein digestibility of *Senna obtusifolia* seeds (Table 4.3), to 45.88g/100g. Toasting of the raw *Senna obtusifolia* seeds significantly (P<0.05) increased the *in vitro* protein digestibility to 22.27g/100g and toasting/fermentation also significantly (P<0.05) increased the *in vitro* protein digestibility to 25.02g/100g from 7.80g/100g of the raw seeds.

4.4. Amino Acids Composition of Processed and Unprocessed *Senna obtusifolia* Seeds

The amino acid (g/100g protein) composition of the raw and differently processed *Senna obtusifolia* seeds are presented in Table.4.4. Leucine 5.75g/100g protein had the highest value of essential amino acid while glutamic and aspartic acids 10.86 and 8.20g/100g, protein respectively were the most abundant non-essential amino acids in the raw seeds. Methionine (Met.) had the least value both in the raw and processed seeds. Toasted and toasted/fermented significantly (p<0.05) increased the percentage amino acid, while fermentation significantly (p<0.05) improved the amino acids.

4.5 Concentration of Amino Acid (g/100g) of Raw and Processed Seeds of *Senna obtusifolia* Seeds

The evaluation of the amino acids based on the classification of the differently processed *Senna obtusifolia* seed is presented in Table 4.5. The result revealed that the total amino acid (TAA) and Total Essential Amino Acid (TEAA) of the raw seeds were 67.73 and 31.66, respectively.

Toasting and toasting/fermentation increased the TAA while fermentation equally enhanced the total TAA. Similarly, the TEAA were enhanced by fermentation, toasting and toasting/fermentation while fermentation progressively enhanced it from 31.66 to 38.12, 35.71 and 32.24, respectively.

4.5.1 Amino Acid Score of Raw and Processed Senna obtusifolia seeds

Using Amino acid score according to FAO/WHO/UNU (2007) guidelines, lysine was found to be the limiting amino acid whose values were found to be less than 100mg/g in both raw (69.78mg/g) and the processed (fermented (94.88mg/g), toasted (75.33mg/g) and toasted/fermented (68mg/g)) *Senna obtusifolia* seeds

Table 4.3: Effect of Processing on the *In Vitro* Digestibility of Protein of *Senna obtusifolia* Seeds

	***IN VITRO* PROTEIN DIGESTIBILITY (g/100g)**
RAW	7.80 ± 0.71^{a}
FERMENTED	45.88 ± 0.91^{b}
TOASTED	22.27 ± 1.41^{c}
TOASTED/FERMENTED	25.02 ± 0.71^{d}

Values are expressed as Mean±SD of duplicate measurement and values with different superscripts in the same column are significantly different ($p<0.05$).

Table 4.4: Effect of Processing on the Amino Acids contents of *Senna obtusifolia* Seeds (g/100g)

	Raw	Fermented	Toasted	Toasted/Fermented
EAA				
Lys	3.14±0.01[a]	4.27±0.06[c]	3.39±0.06[b]	3.06±0.06[a]
Hist	2.31±0.15[a]	2.38±0.02[a]	2.29±0.03[a]	2.22±0.16[a]
Arg	5.56±0.07[a]	6.72±0.13[c]	6.07±0.07[b]	5.38±0.07[a]
Thr	3.06±0.08[a]	3.70±0.01[d]	3.40±0.01[c]	3.22±0.01[b]
Val	3.39±0.04[a]	3.39±0.04[a]	3.97±0.06[b]	4.03±0.02[b]
Met	1.24±0.01[a]	1.62±0.13[b]	1.38±0.04[a]	1.25±0.07[a]
Iso	2.67±0.06[a]	3.11±0.04[c]	2.64±0.02[a]	2.43±0.01[b]
Leu	5.75±0.08[a]	7.26±0.07[c]	7.26±0.07[c]	6.36±0.08[b]
Phe	3.58±0.04[b]	4.28±0.03[d]	4.05±0.08[c]	3.39±0.03[a]
Trp	0.96±0.06[a]	1.39±0.03[b]	1.26±0.07[b]	0.90±0.01[a]
TEAA	31.66	38.12	35.71	32.24
Ala	3.50±0.01[a]	4.27±0.08[c]	3.66±0.13[a]	3.81±0.29[b]
Glu	10.86±0.36[b]	12.27±0.04[c]	11.07±0.03[b]	10.30±0.01[a]
Gly	4.02±0.01[a]	4.56±0.06[c]	4.28±0.06[b]	4.20±0.01[b]
Ser	3.17±0.06[a]	3.77±0.06[c]	3.41±0.01[b]	3.09±0.01[a]
Asp	8.20±0.01[a]	9.27±0.05[c]	8.86±0.07[b]	8.01±0.13[a]
Cys	1.38±0.06[ab]	1.69±0.24[b]	1.39±0.02[ab]	1.17±0.06[a]
Tyr	1.92±0.28[a]	2.66±0.11[b]	2.29±0.02[ab]	2.10±0.05[a]
Pro	3.02±0.11[b]	3.28±0.04[c]	2.87±0.042[b]	2.34±0.01[a]
TNEAA	36.07	41.77	37.83	35.02
TAA	67.73	79.89	73.54	67.26

Values are expressed as Mean±SD of single determination and values with different superscripts on the same row are significantly different ($p<0.05$).

Table 4.5. Amino Acid Score of Raw and Processed *S. obtusifolia* Seeds Based on FAO/WHO/UNU(mg/g protein)

Amino Acids	FAO/WHO/UNU (2007) Maintenance amino acid pattern (mg/g protein)	Chemical Score of raw *Senna obtusifolia* seeds	Chemical Score of fermented *Senna obtusifolia* seeds	Chemical Score of toasted *Senna obtusifolia* seeds	Chemical Score of toasted/fermented *Senna obtusifolia* seeds
Lys	45	69.78	94.89	75.33	68
His	15	154	158.67	152.67	148
Thr	23	133.04	160.87	147.83	140
Meth	16	77.50	101.25	86.25	78.12
Val	39	86.92	86.92	101.79	103.33
Tryp	6	160	231.67	210	150
Meth	16	77.50	101.25	86.25	78.13
Phynl	38	94.21	112.63	106.58	89.21
Leu	59	97.46	123.05	123.05	107.80

$$AAS = mg\ of\ IAA\ in\ 1g\ protein \div mgof\ same\ IAA\ in\ 1g\ of\ reference\ protein$$

IAA: Indispensable Amino Acid

4.6 Effects of Different Processing Methods on the Mineral Contents of *S. obtusifolia* Seeds

Calcium (Ca), potassium (K), magnesium (Mg), iron (Fe), sodium (Na) and zinc (Zn) contents of raw *S. obtusifolia* were 81.60, 0.94, 29.98, 0.88, 0.32 and 0.49 ppm respectively. The calcium, iron, magnesium and potassium contents were observed to be significantly ($p<0.05$) when compared to the raw seeds. Zinc and sodium were observed not to be significantly different ($p>0.05$) when compared with the raw seeds (Table 4.6). The calcium, and iron contents of toasted/fermented seeds were observed to be significantly ($p<0.05$) higher than the other two processing methods (fermentation and toasting) (Table 4.6). The magnesium and potassium contents of the toasted seeds ware observed to be higher than the raw and other processed seeds.

4.7 Antinutrient/Mineral Molar Ratios of Raw and Processed *Senna obtusifolia* seeds

The calculated [phytate/zinc], [phytate]/[calcium], [phytate]/[iron], [phytate]x[Calcium]/[Zinc] and [oxalate]/[calcium] molar ratios are presented in table (4.7). The raw seeds recorded high values of molar ratios when compared with the processed seeds which recorded lower molar ratio.

Table 4.6: Effects of Different Processing Methods on the Mineral Contents of *S. obtusifolia* Seeds (ppm)

	Fe	Ca	Mg	Zn	K	Na
Raw	0.88±0.046^{a}	81.60±0.01^{a}	29.98±0.21^{a}	0.49±0.03^{b}	0.94±0.01^{a}	0.32±0.39^{a}
Fermented	0.62±0.02^{b}	64.05±0.51^{b}	23.06±0.40^{b}	0.30±0.01^{a}	0.72±0.02^{b}	0.04±0.01^{a}
Toasted	1.48±0.04^{c}	39.65±0.66^{c}	31.06±0.37^{c}	0.43±0.13^{b}	1.16±0.07^{c}	0.34±0.39^{a}
Toasted/ Fermented	2.87±0.05^{d}	79.98±0.20^{d}	24.67±0.35^{d}	0.46±0.01^{b}	0.53±0.01^{d}	0.07±0.00^{a}

Values are expressed as Mean±SD of single determination and values with different superscripts in the column are significantly ($p<0.05$).

Table 4.7: Antinutrient/mineral Molar Ratios of Raw and Processed *Senna obtusifolia* Seeds

	[Phy]/[Zn][1]	[Ca]/[Phy][2]	[Phy]/[Fe]	[PhyXCa]/[Zn]	[OX]/[Ca]
RAW	756.06	0.34	359.62	622.82	11.87
FERMENTED	191.51	1.82	79.16	123.83	9.67
TOASTED	96.75	1.55	25.37	38.73	9.53
TOASTED/FERMENTED	55.99	5.07	7.67	45.21	5.64
CRITICAL VALUES	15	6.0	1.0	0.5	2.5

[1](mg phytate/MWof phytate :mg Zn/MWof Zn)

[2](mgCa/MWofCa :mgphytate/MWof phytate)

(mg phytate/MWof phytate :mg Fe/MWof Fe)

(mol/Kg of phytate × mol/Kg of Ca : mol/Kg of Zn)

(mg oxalate/MW of oxalate :mg Ca/MW of Ca).

CHAPTER FIVE

5.0 DISCUSSION

Among the processed *Senna obtusifolia* seeds, the fermented seeds gave the highest mean value of crude protein this trend may be due to the effect of hydrolysis during the fermentation which increased the content of the crude protein (CP), a process often associated with microbial activities. Yashim *et al*. (2009) and Tamburawa (2010) reported that crude protein increased progressively with increasing fermentation. The increase in protein content agrees with earlier reports (Vlavonou, 1988; Raimbault, 1998; Akindahunsi *et al*., 1999) that fermentation with certain fungi such as *Aspergillus niger* would increase the protein content of plant sample. The increase in the protein content may be because some of the microorganisms which degrade plant sample readily (Vlavonou, 1988; Reade and Gregory, 1975; Akindahunsi *et al*., 1999), could have secreted some extracellular enzymes (protein) in the pulp of the plant (Lehninger, 1987; Akindahunsi *et al*., 1999; Okafor and Ejiofor, 1990). The lower crude protein value in the heat treatments may be related to denaturation of proteins on exposure to high temperature. Lower protein values were obtained when the seeds of mucuna and parkia were roasted as reported by Vadivel and Pungalenthi (2007) and Tamburawa (2010). This may be due to unintended adverse effect which might have taken place as a result of denaturation of proteins during heat processing which alters the chemical nature and decreases the nutritional content of proteins (Liener, 1980; Van der Poel, 1989; Norton, 1991).

The decrease in lipid content of the fermented seed when compared to the raw sample in this study might be attributed to the increased activities of the lipolytic enzymes during fermentation which hydrolyses lipids into fatty acids and glycerol (Chinma *et al*., 2008). Fermentation significantly decreased oil content of African oil bean seed and this was attributed to the

production of glycerol, one of the hydrolytic products of lipids, which is not fat soluble (Landers and Rathmann, 1981; Akubor and Chukwu, 1999). The concentration of lipid reduced progressively from raw to toasted, but reduced slightly with fermentation. Similarly Tamburawa (2010) reported a decline in lipids with toasting. The lowest value of fat obtained in the toasted seed could be attributed to loss of volatile lipid compounds.

The low moisture content of the seed indicates that it would store well and nutrients would be preserved. It has been reported that higher moisture content leads to food spoilage through microbial actions (Onyeike *et al*., 1995). Reduced moisture content ensures the inhibition of microbial growth, hence is an important factor in food preservation (Chew *et al*., 2011). Toasted *Senna obtusifolia* seeds sample had lower moisture content in comparison with fermented seeds; this is expected as seeds are subjected to higher temperatures during toasting. The result indicates that toasting may favor keeping quality of *Senna obtusifolia* seeds.

The result of the ash content revealed that the fermented seeds of *Senna obtusifolia* recorded the lowest ash content, this may be attributed to the effect of leaching. This finding agreed with the report of Pugalenthi *et al*. (2004) who documented that micro and macro minerals can leach out into the soaking medium through the enhanced permeability of the seed coat.

The low value of ash in the raw sample may be as a result of the effect of antinutrients on the mineral contents of the food sample where antinutrients like phytate chelates mineral elements and makes them metabolically unavailable, previous work had reported that antinutrients could interfere with the bioavailability of minerals (Alonso *et al*., 2001; Anigo *et al*., 2009); however, some antinutrients like lectin and protease inhibitors are heat labile, processing method such as toasting had reduced the levels of the antinutrients, thereby improving the bioavailability of the

minerals as seen in the resultant increase in the ash values of the toasted and toasted/fermented seeds. Toasting was found to increase the fibre contents, these increase might be due to conversion of insoluble dietary fibre to short length chains or units (Yuan *et al*., 2005). High fibre contents have been found to bind trace elements, thereby preventing their absorption and consequently leading to deficiencies of iron and zinc (Siddhuraju *et al*., 1996).

Fermentation decreased the nitrogen free extract of *Senna obtusifolia* seeds slightly. The apparent decrease in nitrogen free extract could be attributed to increase activity of amylolytic enzymes which hydrolyze starch and other complex carbohydrates to simple sugars. The sugars then provide energy for the fermenting microorganisms and carbon skeleton for possible synthesis of other compounds thereby increasing nutrient availability (Kazanas and Fields, 1981). In this study, the nitrogen free extract of the toasted and toasted/fermented seeds of *Senna obtusifolia* increased, this is associated with softening and breaking down of the cellulose making the starch more available (Agiang *et al*., 2010).

It has been reported that some antinutrients are heat labile and therefore will be reduced to a great extent by the application of heat to the food (Apata and Ologhobo, 1994). It was observed that toasting reduced the antinutrients to very low levels. As thermal processing, toasting of legumes could enhance tenderization of the cotyledons thereby increasing palatability and nutritional value by inactivating endogenous toxic factors (Nzewi and Egbuonu, 2011).

Reduction in tannins due to toasting and fermentation might have been caused by the activity of polyphenol oxidase or fermented microflora on tannins (Reddy *et al.*, 1984). Applying heat was the most effective processing technique that reduced the tannins content of the seed. Also, reduction in tannins content of fermented seed was observed by Afolabi *et al.* (2011). The

observed decrease in tannins with fermentation agrees with the report of Onweluzo and Nwabugwu (2009). The higher percentage reduction of tannins in toasted seeds showed that tannins is heat labile, thereby destroyed during toasting as reported by Oladele and Aina (2007). The decrease in phytate content of the fungi fermented plant products could possibly be attributed to the secretion of the enzyme phytase by the fungi. This enzyme is capable of hydrolyzing phytate (Oboh *et al*., 2003), thereby decreasing the phytate content of the plant products. Toasting also reduced the phytate content significantly this was because more moisture was lost during toasting. This agrees with the trend reported for roasted maize Khan *et al*. (1990) and toasted pigeon pea Onimawo and Akpojowo (2006). This could be attributed to the type of heat treatment and the moisture content of the samples. Khan *et al*., (1990) stated that the higher the moisture content, the higher the phytate loss.

The result pointed out that fermentation method was effective in reducing saponins content, this agrees with an earlier report of Akande and Fabiyi (2010) who reported that germination has been documented to be an effective treatment to remove some antinutritional factors in legumes by mobilizing secondary metabolic compounds which are thought to function as reserve nutrients.

Fermentation reduced oxalate significantly while toasting was most effective in reducing oxalate. Obasi and Wogu (2008) observed a significant reduction in the oxalate content of yellow maize soaked in water for 12h. Soaking at higher temperature had a significant effect on oxalate reduction. Toasting on the other hand reduced oxalate content significantly especially when toasted in open pan for 30min. The higher loss of oxalate in toasted *Senna obtusifolia* seeds could be attributed to the destruction of the oxalate at high temperature. This result shows that oxalates are water soluble and heat labile.

Fermentation is the most effective processing technique to reduce trypsin inhibitor activity in the seed flours. The results obtained in this work agrees with the observations of the previous workers (Paredez-Lopez and Harry, 1989; Roozen and De Groot 1985) on cooked and fermented soybean, and common beans where reduction in trypsin inhibitor activity is between 91.4-99.9% and 52% were reported for fermented soybean and hot soaked cowpea respectively. Investigation of the parameter for bread nut, cashew nut and fluted pumpkin (Fagbemi *et al*., 2005) shows similar trend. Previous workers indicated significant reductions in trypsin inhibitor activity content (Mbithi-Mwikya *et al.* 2001; Ojokoh *et al.* 2012) of cereal and legumes. Also, during tempeh fermentation *R. oligosporus* had been reported to reduce or eliminate trypsin inhibitors (Fadahunsi and Sani, 2010).

As reported by Kiers *et al.* (2000) that digestibility of cereals and legumes increased during cooking and fermentation. Microflora may produce proteolytic enzymes during fermentation which may be responsible for the increase in protein digestibility (Heseltine, 1983). Also, the elimination of phytic acid contributes to the improvement in protein digestibility of fermented millet (Khetarpaul and Chauchan, 1991). The results obtained in this study agrees with that of Mohiedeen *et al.* (2010) who reported that fermentation was found to improve the *in vitro* protein digestibility of the two maize cultivars and this could be attributed to the partial degradation of complex storage proteins into more simpler and soluble products. It has been reported that the reduction in pH during fermentation plays an important role in enhancing native proteolytic enzymes activity and consequently promotes the breakdown of proteins to smaller polypeptides which are easily digested by enzymes (Khaterpaul and Chauhan, 1989; Yagoub and Abdalla, 2007). Murwan and Ali (2011) reported an increasing trend in *in vitro* protein digestibility of Dabar and Taba as period of fermentation increases. It is well known that severe

heating can limit protein digestibility and amino acid availability caused by denaturation of protein. However, according to Siddhuraju and Becker (2001), heating may cause formation of insoluble complexes between protein and phytate.

The decrease in minerals agrees with the findings of (Alemu, 2009; Afify *et al*. 2012; Difo *et al*. 2015) who attributed it to the leaching of these minerals in fermenting water. It also might be as a result of utilization of these minerals by fermenting microorganisms (Zamora and Fields, 1979). The decrease in minerals seen after heat application agrees with the findings of (Saharan *et al*. 2001; Alemu 2009 and Afify *et al*. 2012). This could be attributed to increase in solubility of these minerals during application of heat (Saharan *et al*., 2001)

The calculated phytate/ zinc molar ratios for raw and processed *Senna obtusifolia* were above the suggested critical level (<15 regarded as favorable for zinc absorption) (Morris and Ellis, 1989). Ratios ≥15 are associated with low zinc bioavailability (Morris and Ellis, 1989). According to WHO cut-offs phytic acid to zinc mole ratio ≥15, 5–15, and <5 is equal to zinc bioavailability as low (10–15%), moderate (30–35%), and high (50–55%), respectively (Goicoechea and W.H.O, 1996). However, the critical phytate:zinc molar ratio may also depend on dietary calcium levels because of the kinetic synergism between calcium and zinc ions resulting in Ca : Zn : Phy complex which is less soluble than phytate complexes formed by either of the ions alone (Lopez *et al*., 2002), suggesting that Ca : phy/Zn molar ratio is a better predictor of zinc bioavailability than Phy : Zn molar ratio alone.

Molar Ratio of Calcium × Phytate/Zinc. Calcium × phytate/ zinc molar ratios of raw and processed *Senna obtusifolia* were above the critical level (0.5mol/Kg) as indicated in Table 4.7 suggesting that calcium interference was more likely to affect zinc bioavailability.

Molar Ratio of Phytate to Iron as indicated in Table 4.7, phytate/iron molar ratios were >1 (indicative of poor iron bioavailability) for all raw and processed *Senna obtusifolia*. This might be due to the reported higher phytate content and insufficient phytic acid degradation after heat application as reported by Akin-Idowu *et al*. (2009).

Molar Ratio of Calcium to Phytate*:* The calcium/phytate molar ratios in all the raw and processed *Senna obtusifolia seeds* were <6, which is regarded as not favorable for calcium absorption Akin-Idowu *et al*. (2008), predicting that there is a poor calcium bioavailability.

In addition to phytic acid, oxalic acid in insoluble form is responsible for interference of divalent metals absorption particularly calcium by forming insoluble salts (Hassan *et al*., 2011). The oxalate/calcium molar ratios of all the selected raw and processed vegetables were above the critical level of 2.5 known to significantly impair calcium bioavailability suggesting that they are weak calcium bioresources for the local populace.

The importance of essential amino acids is well known and their intake is crucial in our diet since human bodies are unable to synthesize it. The non-essential amino acids are amino acids that can be produced and they are equally important as the essential amino acids in development of growth. For examples, alanine helps in the metabolism of glucose and organic acids while arginine helps to maintain a proper nitrogen balance and aid in the excretion of excess nitrogen. Cysteine and proline aids in the production of collagen and promotes proper elasticity and texture of the skin, besides reducing the loss of collagen through the aging process (Brennan *et al*. 2002; McConell 2007).

On overall, the results showed that the fermented seeds contain the highest amount of total amino acids content, followed by toasted seeds and then toasted combining with fermentation. The results obtained showed that fermentation increases the amino acids content. Significant

increment in total amino acids content was observed due to fermentation. The hydrolytic breakdown of the nutrient components during fermentation may have caused the increase in amino acids content observed. Study by Ferial and Esmat (2011) also showed that soaking for 12 h increases the essential amino acids content in chickpea seeds. Other researchers have also reported similar finding as in this study. Baumann and Bisping (1995) found that certain *Rhizopus* strains with high proteolytic activity were able to release nearly 5 times more amino acids than other strains. Ferial and Esmat (2011) observed increases in essential amino acids in fermented chickpea with *Rhizopus* at 48 h of fermentation time. Similarly, Angulo-Bejarano *et al*. (2008) also studied the nutritional value of chickpea fermented with *R. oligosporus* at 34.9°C for 51.3 h. According to Baumann and Bisping (1995), a biochemical mechanism such as transamination took place during fermentation that causes the high amount of amino acids release.

While toasting followed by fermentation on the other hand, causes decrease in the amino acids content of the sickle pod. On overall, the total amino acids content in the toasted/fermented seeds was reduced due to denaturation of protein during application of heat. Other researchers have also reported similar observations on the reduction effects of amino acids content due to application of heat (Alajaji and El-Adawy 2006; Ferial and Esmat 2011; Pedrosa *et al*. 2011). It is well known that severe heating can limit protein digestibility and amino acid availability caused by denaturation of protein. However, according to Siddhuraju and Becker (2001), heating may cause formation of insoluble complexes between protein and phytate.

While toasting on the other hand, causes increase in the amino acids content of the sickle pod. On overall, the total amino acids content in the toasted seeds were improved due to destruction of antintrients during application of heat. The findings are in good agreement with the reported

values by Vijayakumari *et al*. (1997) and Abd El-Hady and Habiba (2003). Improvements in dry sickle pod amino acids might be attributed to the removal of antinutrients and the inactivation of enzyme inhibitors by heating.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

1. The present study depicted the potential of *Senna obtusifolia* seeds as a good source of plant protein and energy. *Senna obtusifolia* seeds appear to be superior in the essential amino acids content to sorghum and millet grains.
2. The processing by fermentation and toasting is suitable and effective in reducing various antinutritional compounds most especially the heat labile antinutrients, without significantly affecting the nutrient content of *Senna obtusifolia* seeds. Toasting followed by fermentation is suitable for use since it increased source of calcium, magnesium, potassium and iron of the seeds. Fermentation and toasting could have led to the decrease of some of the minerals by leaching and subsequent volatility of other elements during heating while increment of some minerals might be associated with the conversion of the insoluble reserve foods by enzymes during fermentation.
3. The various processing methods impacted some chemical changes in the nutritional composition of *Senna obtusifolia* seeds. The fermentation and toasting methods are suitable for use after it was established that such processing methods reduced the antinutrients content to a greater extent and had better amino acid contents and *in vitro* protein digestibility compared to the raw and toasted/fermented seeds. As such, the potential for nutritional exploitation was further enhanced by the fact that these seeds would not require prolonged and expensive heat treatment prior to use.
4. Hence this economic and potential processing method could be adopted for utilization of *Senna obtusifolia* seeds as a protein and energy source. Incorporation of these seeds in

food/feed may obviously reduce the present day over-dependence on common legumes for rapidly increasing protein requirements for man and livestocks industries, especially in developing countries like Nigeria.

6.2 Conclusion

The result in this research indicated that *Senna obtusifolia* seeds had low fat and moisture content, indicating that it could be stored for a long period. The processed *Senna obtusifolia* seeds are good sources of micronutrients and macronutrients particularly protein, and could be used in areas where accessibility and affordability for other legumes are inadequate. The different processing treatments used in this study were found to be effective in reducing the antinutritional components of the raw seeds with toasting/fermentation being the most effective.

However, the inactivation of enzyme inhibitors by toasting may cause formation of insoluble complexes between protein and tanninss which probably will be the reason for the low value of *in vitro* protein digestibility. Fermentation greatly enhanced the amount of all amino acids in the *Senna obtusifolia* as well as crude protein content. The inclusion of *Senna obtusifolia* seeds as a blend in recipe development may help to alleviate the problem of malnutrition prevalent in rural areas of Nigeria, particularly during the period of food scarcity.

6. 3 RECOMMENDATIONS

1. Based on these findings, microbial fermentation improved protein contents and greatly reduced antinutrient contents of the seeds and the fermented food products obtained from these seeds are superior to other processing methods and so is it recommended.

2. However a significant reduction of antinutrients by toasting was observed and an increase in amino acid profile, carbohydrate, ash and dietary fibre contents were recorded.

3. Other heating conditions (boiling, steaming, microwave and autoclaving) should be investigated to determine their effect on the nutrients and antinutrients composition of *Senna obtusifolia* seeds.

4. Animal studies should be carried out in order to investigate the safe utilization of *Senna obtusifolia* seed in feed formulation.

REFERENCES

Abbey, B.W and Berezi, P.E. (1988). Influence of processing on digestibility of African yam bean (*Shnenostylis stenocarpa*) flour. Nutrition Reports International, 32:819-827.In: *Research Journal of Biological Sciences* 2(1): 41-44.

Abd El-Hady, E.A.A. and Habiba, R.A. (2003). Effects of soaking and extrusion condition on antinutrients and protein digestibility of legume seeds. *Journal of Food Science and Technology*, 36, 285-293.

Abdelseed, B.H., Abdelwahab, H.A., Abu ElGasim, A.Y., Mohamed, I A. and Babiker, E.E. (2011). Some nutritional attributes of selected newly developed lines of sorghum after fermentation. *Journal of Agricultural Science and Technology*., 13: 399-409.

Abeke, F.O. and Out, M (2008). Antinutrients in poultry feeds: Concerns and options. Proceedings of the 13th Annual Conference of the Animal Science Association of Nigeria (ASAN), 15th - 19th Sept., 2008, A.B.U Zaria, Nigeria, pp: 396-398.

Abu-Tarboush, H.M. (1998). Irradiation inactivation of some anti-nutritional factors in plant seeds. *Journal of Agriculture and Food Chemistry*, 46:2698-2702.

Addisu, S. (2016). Effect of dietary tannins source feeds on ruminal fermentation and production of cattle: *A Review Online Journal of Animal and Feed Research* 6(2),45-56.

Adeniji, C.A, Fakoye K.A and Omamohwo V.R. (2005). Utilization of *Amaranthus sponosus* in the diet of nile tilapia *Oreochromis niloticos*. *Tropical Journal of Animal Science* 9: 79-83.

Adeniyi S. A, Orjiekwe C.L and Ehiagbonare J.E (2009). Determination of alkaloids and oxalates in some selected food samples in Nigeria. *African Journal of Biotechnology*. 8(1): 110-112.

Afify AMR, El-Beltagi H. S, Abd El-Salam S. M and Omran A. A (2012). Effect of Soaking, cooking, germination and fermentation processing on proximate analysis and mineral content of three white sorghum varieties (*Sorghum bicolor* L. Moench). *Not Bot Horti Agrobo*, 2012, 40 (2), 92-98

Afolabi, I. S; Marcus, G. D., Olanrewaju, T.O. and Chizea, V. (2011). Biochemical effect of some food processing methods on the health promoting properties of underutilized *Carica papaya s*eed. *Journal of Natural Products* 4:17-24.

Agiang, M.A., Umoh, I.B., Essien, A.I. and Eteng, M.U. (2010). Nutrient changes and antinutrient contents of beniseed and beniseed soup during cooking using a Nigerian traditional method. *Pakistan Journal of Biological Sciences,* 13(20): 1011–1015.

Agwunobi, L.N., Angwukam, P.O., Cora, O.O. and Isika, M.A. (2002). Studies on the use of *colocasia esculenta* (Taro cocoyam) in the diets of weaned pigs. http:www.springerlinks.com.lapp/home/ contribution.asp?. Retrieved on 02/09/2010.

Ahamefule, F.O. and V.U. Odemelam, (2008). Effect of soaking duration on the proximate composition, gross energy, mineral content and some antinutritional properties of *Canavalia plagiosperma* seed. Proceedings of the 13th Annual conference of the Animal Science Association of Nigeria (ASAN), 15th-19th Sept., 2008, ABU, Zaria, Nigeria, pp: 491- 494.

Akande, K. E and Fabiyi, E. F. (2010). Effects of processing on some antinutritional factors in legume seeds for poultry. *International Journal of Poultry Science* 9(10):996-1001.

Akindahunsi A. A, Oboh G and Oshodi A. A (1999). Effect of fermenting cassava with *Rhizopus oryzae* on the chemical Composition of its flour and gari. *La Rivista Italiana Delle Sostanze Grasse,* 76: 437– 440.

Akindahunsi A.A and Salawu S.O (2005). Phytochemical screening and nutrient and anti-nutrient composition of selected tropical grean leafy vegetables. *African Journal of Biotechnology*. 4: 497-501.

Akindumila F. and Glatz B. A (1998). Growth and oil production of *Apiotrichum curvatum* in tomato juice. *Journal of Food Production* 61(11): 1515–1517.

Akin-Idowu P. E, Odunola O. A., Asiedu R., Maziya-Dixon B. and Uwaifo A. O.(2008) "Variation in nutrient and antinutrient contents of tubers from yellow yam (*Dioscorea cayenensis*) genotypes grown at two locations," *Journal of Food, Agriculture and Environment*, vol. 6, no. 3-4, pp. 95–100.

Akin-Idowu P. E, Asiedu R, Maziya-Dixon B, Odunola A and Uwaifo A. (2009) "Effects of two processing methods on some nutrients and anti-nutritional factors in yellow yam (*Dioscorea cayenensis*)," *African Journal of Food Science*, vol. 3, no. 1, pp. 022–025,.

Akpan, E.J. and Umoh, I.B. (2004). Effect of heat and tetracycline treatments on the food quality and acridity factors in cocoyam (*Canthosom sagittifolium*) (L). *Pakistan Journal of Nutrition*., 3: 240-243.

Akpodiete, O.J., Okagbare, G.O., Omeje S.I. and Orogun, J.A. (2001). Effect of roasting, boiling and boiling with potash on soyabean and its utilization by finishing broiler chickens. Proceedings of the 6th Annual Conference of Animal Science Association of Nigeria (ASAN), 17th - 19th September 2001, University of Maiduguri, Nigeria, pp: 50-52.

Akubor, P.I. and Chukwu J.K. (1999). Proximate composition and selected functional properties of fermented and unfermented African oil bean (*Pentaclethra microphylla)* seed flours. *Plant Foods Human Nutrition*, 54: 227-228.

Alajaji, S.A. and El-Adawy, T.A. (2006). Nutritional composition of chickpea (*Cicer arietinum* L.) as affected by microwave cooking and other traditional cooking methods. *Journal of Food Composition and Analysis* 10: 1016-1023.

Alemu M.K (2009). The effect of natural fermentation on some antinutritional factors, minerals, proximate composition and sensory c*haracteristics in sorghum based weaning food M.S.c Thesis Addis Ababa, University*., Ethiopia

Aletor V.A. and Fetuga B.L (1987). Pancreatic and intestinal amylase (EC 3.2.1.1) in the rat fed haemagglutinin extract.II Evidence of impaired dietary starch utilization. *Journal of Animal Physiology and Animal Nutrition*. 57(3):113-117.

Aletor V.A (1991). Antinutritional factors in some Nigerian feedstuffs, herbage byproducts, crop residues and browse plants. A monographprepared for the Presidential task force on alternative formulation of livestock feeds; Product Development, Quality Evaluation and Health Implications.

Aletor V.A (1993). Allelochemicals in plant foods and feeding Stuffs. Part I. Nutritional, biochemical and physiopathological aspects in animal production. *Vet. Human Toxicol.* 35(1): 57-67.

Al-Kahtani, H. A. (1995). Some anti-nutritional factors in *Moringa pregrina* (Al-Yassar or Al-ban) and soybean products. *Journal of Food Science,* 60:395-398.

Alonso R, Rubio LA, Muzquiz M and Marzo F (2001). The effect of extrusion cooking on mineral bioavailability in pea and kidney bean seeds meals. *Anim. Feed. Sci. Tech.* 94: 1-13.

Angulo-Bejarano, P.I., Verdgo-Montoya, N.M., Cuevas-Rodriquez, E.O., Milan-Carillom, J., Mora-Escobedo, R., Lopez-Valenzuela, J.A., Garzon-Tiznado, J.A. and Reyes-Moreno, C. (2008). *Tempeh* flour from chickpea (*Cicer arietinum* L.) nutritional and physicochemical properties. *Food Chemistry* 105: 106-112.

Anigo K.M, Ameh D.A, Ibrahim S, and Danbauchi SS (2009). Nutrient composition of commonly used complementary foods in North Western Nigeria. *African Journal of Biotechnology*. 8(17): 4211-4216.

AOAC (1990). Official methods of analysis, Vol.1,14th (Eds.) Association of Official Analytical Chemists, Arlington, VA, 1102 pp.

AOAC (2000). Association of Official Analytical Chemists Official Methods of Analysis.17th edition, Washington, D.C. USA.

AOAC Official (2003) Official methods of analysis of AOAC international. 17th Edition, AOAC International, Gaithersburg.

AOAC (Association of Official Analytical Chemists) (2006) Official Method of Analysis of the AOAC (W. Horwitz Editor Eighteen Edition, Washington: D.C., AOAC.

Apata D.F and Ologhobo, A.D (1994). Biochemical evaluation of some Nigerian legume seeds. *Journal of Food Chemistry*., 49, 333-338.

Austin, J.,. Beck, C.B., Kalbfleish, G., Kagal, L.C. and R.H. Mathews, (1981). Variation in the vitamin and mineral content of raw and cooked commercial *Phaseolus vulgaris* classes. *Journal of Food Science*., 46: 1701-1706.

AVRDC (2008). The World Vegetable Center. *Point of Impact: Healthy Urban Fast Food:A New Maasai Enterprise*; AVRDC - The World Vegetable Center: Tainan, Taiwan.

Ayenor, G.S., (1985). Effect of retting of cassava on product yield and cyanide detoxification. *Journal Food Technology*., 20: 89-96.

Balogun, J.K., Abdullahi, S.A., Auta, J. and Ogunlade, O.P. (2004). Feed conversion, protein efficiency, digestibility and growth performance of *Oreochromis niloticus* fed *Delonix regia* Seed meal. *Proceedings of the National Conference of Fisheries Society of Nigeria* (FISON). Pp23-29.

Balogun, T.F., Kaankuka, F.G and Bawa, G.S. (2001). Effect of boiling full -fat soyabean on its amino acid profile and on performance of pig. *Nigerian Journal of Animal Production*., 28: 45-51.

Barbour, E.K., Kallas M. and Farran, M.T. (2001). Immume response to Newcastle disease virus in broilers: A useful model for the assessment of detoxification of ervil seeds. *Rev. Sci. Tech. Off. Int.* Epiz, 20: 785- 790.

Banergee, G.C., 2004. *A Text Book of Animal Husbandry*. 8th Edn., Oxford and IBH Publishing Co. Pvt Ltd. New Delhi.

Barker, C.J., and Berggren, P. (1999). Inositol hexakisphosphate and beta-cell stim secretion coupling. *Anticancer Research*, 19: 3737–3742.

Barry, T.N. and Manley, T.R. (1984). "The role of condensed tannins in the nutritional value of *lotus pedunculatus* for sheep.2. " Quantitative digestion of carbohydrates and proteins. *British Journal of Nutrition*, 51:493-504

Baumann, U. and Bisping, B. (1995). Proteolysis during *tempe* fermentation. *Food Microbiology* 12: 39-47.

Bate Smith., E.C. (1973).“Haemanalysis of tannins” The concept of relative astringency. *Phytochemistry* 12:907-912

Benitez, L. V. (1989). Amino acid and fatty acid profiles in aquaculture nutrition studies p. 23-35. In: S.S Desilva (Ed). Fish Nutrition Research in Asia. Proceeding of the third Asian fish Nutrition Network meeting. *Asian Fish Society Special Publication*, 4, 166p. Manila Philippines.

Bennett, J. W. (1985). Molds, manufacturing and molecular genetics. In *UCLA symposia on molecular and cellular biology (USA)*.

Blood D.C, and Radostits O.M (1989). *Veterinary Medicine*, 7th Edition, Balliere Tindall, London, pp. 589-630.

Borchers, R. and Ackerson, C.W. (1950). The nutritive value of legume seeds. Effects of autoclaving and trypsin inhibitor test for 17 species. *Journal of Nutrition*., 41: 339-345.

Brennan, L., Shine, A., Hewage, C., Malthouse, J.P.G., Brindle, K.M., McClenaghan, N., Flatt, P.R. and Newsholme, P. (2002). A nuclear magnetic resonance-based demonstration of substantial oxidative L-alanine metabolism and L-alanine-enhanced glucose metabolism in a clonal pancreatic β-cell line. *Diabetes Journals* 81: 1714-1721.

Bressani, R. and Elias, L.G. (1980). The nutritional role of polyphenols in beans. In: Polyphenols in cereals and legumes. (Hulse, J.H. Edn.) International Development Research Center. Ottawa, Canada, pp: 61.

Bressani, R. and Sosa, J.L. (1990). Effects of processing on the nutritive value of *Canavalia* jackbean (*Canavalia ensiformis*). *Plant Food Human Nutrition*., 40: 207-214.

Bressani, R., (1993). Grain quality of common beans. *Food Rev. Int*., 9: 217-179.

Bressani, R., (2002). Factors influencing nutritive value in food grain legumes: *Mucuna* in comparison to other grain legumes. In: *Mucuna* as a Food and Feed: current uses and the way forward. Edited by M. Flores, M. Eilitta, R. Myhrman, L. Carew and R. Carsky, Workshop held April 26th - 29th, 2000 in Tegucigalpa, Honduras. CIDICCO, Honduras, pp: 164-188.

Buba, W., Duru, S. and Shehu, B.M. (2010). The effects of cooking duration on the chemical and antinutritional composition of sickle pod (*Cassia tora*) seeds. Proceedings of the 15th Annual Conference of Animal Science Association of Nigeria (ASAN), 13th-15th Sept, 2010. University of Uyo, Akwa Ibom State, Nigeria, pp: 560-561.

Bvochora, J. M., Reed, J. D., Read, J. S., and Zvauya, R. (1999). Effect of fermentation processes on proanthocyanidins in sorghum during preparation of Mahewu, a non-alcoholic beverage. *Process Biochemistry*, *35*(1), 21-25.

Carlini, C.R. and Udedibie, A.B.I (1997). Comparative effects of processing methods on haemagglutmating and antitryptic activities of *Canavalia ensiformis* and *Canavalia braziliensis* seeds. *Journal of Agriculture and Food Chemistry*., 45: 4372-4377.

Charles, O.W. and Muller, H.D. (1975). *Cassia obtusifolia* toxicity response of laying hens (Abstract*). Poultry Science*, 54: 1745.

Chatterjea, M.N. and Shinde, R. (2007). *Textbook of Medical Biochemistry*. 7th Edn., New Delhi: Jaypee Brothers Medical Publishers (P) Ltd. p: 799.

Cheeke P.R and Shull L.R (1985). *Natural toxicants in feeds and livestock.* AVI Publishing Inc., West Port, Connecticut.

Chew S.H.K, Bhupinder N.H, Karim A.A, and Fazilah A (2011). Effect of fermentation on the composition of *Centellasaitica*teas. *American Journal of Food Science and Technology*. 10: 1-13.

Chinma, C.E., Alemede, I.C. and Emelife, I.G. (2008). Physiochemical and functional properties of some Nigerian cowpea varieties. *Pakistan Journal of Nutrition,* 7:186-190.

Cotula, L., Dyer, N. and Vermeulen, S., (2008). Fueling Exclusion? The Biofuels Boom and Poor People's Access to Land International Institute Environment Development, London. ISBN: 978-1-84369-702-2, pp: 72

Crowhurst A., (1972). *The Weed Cook book*. Lancer Books, Inc. New York, pp: 190.

D'Mello, J. P. F. (1982). Toxic factors in some tropical legumes. *World Review of Animal Production*, *18*(4), 41-46.

D'Mello, J. P. F., and Walker, A. G. (1991). Detoxification of jack beans (*Canavalia ensiformis*): studies with young chicks. *Animal Feed Science and Technology*, *33*(1-2), 117-127.

Das, T.K., Banarjee, D., Chakravorty, D., Pakhira, M.C., Shirivastava, B. and Kuhad, R.C. (2012). Saponins: Role in Animal system. *Veterinary World,* 5(4), 248-254.

David, G. C. (2002). Cognition and cultural transmission of Tzetal Maya medical plant knowledge.

Delgado, C., Rosegrant M., Steinfeld H., Ehui, S. and Courbois, C., (1999). Livestock to 2020 the next food revolution. IFPR/FAO/ILRI, International Food Policy Research Institute, Washing, DC.

Desphande, S. and Cheryan, M. (1984). Changes in the phytic acid, tanninss and trypsin inhibitor activity on soaking of dry beans *(Phaseolus vulgaris* L.). *Nutrition Report International*., 27: 371-577.

Deshpande, S.S. (2002). Handbook of Food Toxicology, *Food Science and Technology*, CSC Press, Amazon. com., pp 920.

Difo, V. H., Onyike, E., Ameh, D. A., Njoku, G. C. and Ndidi, U. S. (2015). Changes in nutrient and antinutrient composition of *Vigna racemosa* flour in open and controlled fermentation. *Journal of Food Science and Technology*, *52*(9): 6043-6048.

Dirar, H.A. (1993). Flavours and substitutes of plant origin: in the indigenous fermented foods of the Sudan. A study in African Food and Nutrition. CAB Int. Editor, Wallingford UK, pp. 412 447.

Edwards, D. G., and Duthie, I. F. (1973). Processing to improve nutritive-value of field beans. In *Journal of the Science of Food and Agriculture* (Vol. 24, No. 4, pp. 496-497).

Eicher, N. J., and Saiterlee, L. (1988). Nutritional quality of Great Northern bean proteins processed at varying pH. *Journal of Food Science*, *53*(4), 1139-1143.

Elbert, A.W. (2014). Potential of underutilized traditional vegetables and legume crops contribute to Food and Nutritional Security, income more sustainable production, *Sustainability*, 6: 319-335.

El-Boushy, A.R.Y. and Vander Poel, (2001). Poultry Feed from Wastes, Processing and Uses. 1st Edn.,Chapman and Hall, pp: 377.

Elkhalifa, A.O., Schiffler, .B. and Bernhardt, R. (2004). Effect of fermentation on the starch digestibility, resistant starch and some physicochemical properties of sorghum flour. Nahrung/ *Food*, 48: 91-94.

El-Morsi, E. A., Abdel-Salam, H.S., Ismail, H.A., Aboul-Fetouh, S.A and Fawzay, A.M. (1992) Effect of gamma irradiation on trypsin inhibitor and subunit structure of proteins in Field bean (*Vicia faba*) seeds during germination. *Journal of Agricultural Resources Development*, 14: 849-871.

Esonu, B. O., Udedibie, A. B. I. and Carlini, C. R. (1998). The effect of toasting, dry urea treatment and sprouting on some thermostable toxic factors in the jackbean seed. *Nigerian Journal of Animal Production*, *25*(1), 36-39.

Eyo, A. A. (2003,). Fundamentals of fish nutrition and diet development-An overview. In *Eyo, A. A (ed). Proceedings of the National Workshop on fish feed development and feeding practices in Aquacuiture. FISON/NIFFR/FAO-NSPFS* (pp. 1-33).

Fadahunsi, I. F. and Sanni, A. I. (2010). Chemical and biochemical changes in bambara nut (*Voandzeia subterranea* (L) Thours) during fermentation to'Tempeh'. *Electronic Journal of Environmental, Agricultural & Food Chemistry*, *9*(2).

Fagbemi, T.N., Oshodi, A. A . and Ipinmoroti, K.O. (2005) .Processing effects on some antinutritional factors and *in vitro* multi enzymes protein digestibility (IVPD) of three tropical seeds: bread nut, cashew nut and fluted pumpkin. *Pakistan Journal of Nutrition* 4(4): 250-256.

Falayi, B.A. (2003). Techniques in fish feed manufacture. In: A.A. Eyo (Ed.), *Proceeding of the Joint Fisheries Society of Nigeria*/National Institute for Freshwater Fisheries Research/FAO-National Special Programme for Food Security National workshop on Fish feed development and Feeding Practices in Aquaculture held at National Institute for Freshwater Fisheries Research, 15-19 September. New-Bussa: 43-55.

F.A.O (2001). Assessment of the world food security situation. Committee on world food situation. 24th Session.

FAO, (2004). Assessing quality and safety of animal feeds. Food and Agriculture Organization of the United Nations, Rome, pp:36-52

FAO/WHO/UNU (2007). Protein and Amino Acid Requirements in Human Nutrition. Report of a Joint WHO/FAO/UNU Expert Consultation.WHO Technical Report Series no. 935. WHO.

FAO, (2008). The state of food insecurity in the world 2008: High food prices and food insecurity-threats and opportunities. Food and Agriculture of United Nations, Rome, pp:8

Farag, M. D. E. H. (1989). Radiation deactivation of anti-nutritional factors: trypsin inhibitor and hemagglutinin in soybeans. Egypt. *Journal of Radiation Science Application,* 6: 207-215.

Farag, M. D. E. H. (1998). The nutritive value for chicks of full-fat soybeans irradiated at up to 60 kGy .*Animal Feed Science Technology.* 73:319-328.

Farran, M.T., Dakessian, P.B., Darwish, A.H., Uwayjan, M.G., Dbouk, H.K., Seliman F.T. and Ashkarian, V.M. (2001). Performance of broilers production and egg quality parameters of laying hens fed 60% raw and treated common vetch (*Vicia ervilia*) seeds. *Poultry Science*., 80: 203-208.

Fernandez, N.M., Aranda, P., Lopez-Jurado, M., Urbano, G., Estrella, I., Sotomayor, C., Diaz, C., Prodanov, M., Frias, J. and Vidal-Valverde, C. (1993). Effect of processing on some antinutritive factors of faba beans: Influence on protein digestibility and food intake in rats. In: *Recent advances of research in antinutritional factors in Legume seeds*; Wageningen Per: Wageningen, The Netherlands, pp: 467-471.

Fernando R. (1987). Plant poisoning in Sri Lanka. In: Progress in venom and toxin research. Proc. of the 1st Asia-Pacific Congress in Animal, Plant and Microbial toxins, pp. 624-627.

Ferial, M.A.S. & Esmat, A.A.A. (2011). Physico-chemical properties of *tempeh* produced from chickpea seeds. *Journal of American Science* 7(7): 107-118. fermentation of peanut flour: effect on chemical composition and nutritive value. *Journal of Food Science* 40: 470-474.

Finkelestein, D. B. (1989). Protein secretion in Aspergillus niger. *Genetics and Molecular Biology of Industrial Microorganisms*, 295-300.

Foo, L., Karschesy, Y., and Joseph, J. (1989). Chemical nature of phlobaphens. Chemistry and significance of condensed tannins, p109-118 doi:10.1007/978

Fowler, D. G. (2006). Traditional fever remedies: a list of Zambian plants. *http://www. giftshealth. org/ritam/news/Traditional_Fever_remedies 1. pdf). Accessed April*, *20*, 2010.

Freiberger, C. E., Vanderjagt, D. J., Patsuzyn, A., Glew, R. S., Mounkaila, G., Milson, M., Glew, R. H. (1998). Nutrient content of seven wild plants from Niger. *Plant Food Human Nutrition*, 53(1): 57-69

Frolich, W. (1995). Bioavailability of micronutrients in a fibre-rich diet, especially related to minerals. *European journal of clinical nutrition*.

Fu, P. P., Xia, Q., Lin, G., and Chou, M. W. (2002). Genotoxic pyrrolizidine alkaloids—mechanisms leading to DNA adduct formation and tumorigenicity. *International Journal of Molecular Sciences*, *3*(9): 948-964.

Gabriel G. B, Ibrahim Y and Suleiman O. E. S. (2015): Evaluation and nutrient quality of toasted flamboyant seed (*Delonix regia*) meal in the diet of *Clarias gariepinus* Fingerlings. *Journal of Agriculture and Ecology Research International., 5(2): 1-9*

Ganiyu, O. (2005). Nutritional and safety evaluation of some tropical green leafy vegetables. *Journal of Food Technology* 3: 389-392.

Gemede, H.G., and Ratta. N. (2014). Antinutritional factors in plant foods: potential health benefit and adverse effects. *Global Advanced Research Journal of Food Science and Tehnology*, 3(4): 102-117.

Ghadge, P.N. Shewalkar, S.V and Wankhede, D.B. (2008). Effect of processing methods on qualities of instant whole legume: pigeon pea (*Cajanus cajan* L.) *Agricultural Engineering International*: The CIGR journal.

Gharaghani, H., Mojtaba, Z., Gholamreza, S. and Hossein, M. (2008). Effect of Gamma Irradiation on Anti Nutritional Factors and Nutritional Value of Canola Meal for Broiler Chickens. *Asian-Australian Journal of Animal Science*, 21(10):1479-1485

Ghazy, M. A. (1990). Effect of gamma-irradiation on some antinutritional factors in kidney bean (*Phaseolus vulgaris* L.) seeds. *Mania Journal of Agricultural Resources and Development*, 12:1965-1980.

Gohl, B.O. (1975). Tropical Feeds, *FAO Agric. Studies No. 96. Rome. Italy.*

Goicoechea, J, W.H.O and Regional Office for Europe. (1996) *Primary Health Care Reforms*, World Health Organization, Geneva, Switzerland.

Graf, E. and Eaton, J. W. (1993). Suppression of colonic cancer by dietary phytic acid. *Nutrition and cancer* 19: 11-19.

Grases, F., March, J. G., Prieto, R. M., Simonet, B. M., Costa-Bauza, A. and Garcia-Raja, A., (2000). Urinary phytate in calcium oxalate stone formers and healthy people– Dietary effects on phytate excretion. *Scandinavian Journal of Urology and Nephrology*, 34: 162 164.

Gupta, B.S., Satapathy, N., Chabbra, S.S. and Ranjhan, S.K. (1970). Effects of various levels of 'Chakunda' leaf meal on growth and egg production. *Indian Veterinary Journal*, 47: 1094-1101.

Gustavson, K. H. (1956). *Chemistry of tanning processes*.

Hagerman, A.E and Butler, L.G. (1978). Protein precipitation method for the quantitative determination of tannins. *Journal of Agricultural Food Chemistry* 26(4): 809-812.

Hagerman, A. E., and Butler, L. G. (1981). The specificity of proanthocyanidin-protein interactions. *Journal of Biological Chemistry*, *256*(9): 4494-4497.

Haslam E. (1966). *Chemistry of vegetable tannins*. Academic press, New York, USA 179pp.

Harborne, J. B. and Mabry, T. J. (2013). *The flavonoids: advances in research*. Springer.

Hassan, L. G., Bagudo, B. U., Aliero, A. A., Umar, K. J., & Sani, N. A. (2011). Evaluation of nutrient and anti-nutrient contents of Parkia biglobosa (L.) flower. *Nigerian Journal of Basic and Applied Sciences*, *19*(1).

Hedwig A., Wilma H. Oldewage-Theron and Abdulkadir A.E. (2012). Dietary diversity, micronutrient intake and their variation among black women in informal settlements in South Africa. A cross-sectional study. *International Journal of Nutrition and Metabolism* vol. 4(2) 24.

Herbert, G.D. and Flory, W. (1983). Determination of the oral toxicity of *Cassia obtusifolia* seeds in chickens. *Veterinary and Human. Toxicology*, 25: 164-166.

Heseltine, C.W. (1983). The Future of Fermented Foods. Nutrition Review. 41: 293-301.hick peas (Cicer arietinum). *Journal of Food Science,* 44(1), 234-236.

Holm, L. G., Pancho, J. V., Herberger, J. P. and Plucknett, D. L. (1979). *A Geographical Atlas of World Weeds*. John Wiley and Sons, New York. 391 pp.

Horvath, P.J. (1981). The nutritional and ecological significance of acer-tanninss and related polyphenols. *MSc Thesis*, Cornell University, Ithaca, New York USA.

Hughes, J.d'A.and Ebert, A.W. (2013). Research and Development of Underutilized Plant Species: The Role of Vegetables in Assuring Food and Nutritional Security. In *Proceedings of the 2nd International Symposium on Underutilized Plant Species: Crops for the Future—Beyond Food Security*; Massawe, F., Mayes, S., Alderson, P.,Eds.; International Society for Horticultural Sciences (ISHS): Korbeek-Lo, Belgium, 2: 79–91. human health. *Phytochemistry*, 65: 1199-1221.

Hurrell, R.F. (2004). Phytic acid degradation as a means of improving iron absorption. *International Journal of Vitamins Nutrition Resources,* 74:445-52.

Idris, W. H., AbdelRahman, S. M., ELMaki, H. B., Babiker, E. E., and Tinay, A. H. E. (2005). Sorghum (*Sorghum biocolor*) Cultivars. *Journal of Food Technology*, *3*(3), 410-416.

Ingweye, J.N., Kalio,G.A.,Ubua, J.A. and Umorem, E.P. (2010). Nutritional Evaluation of Wild Sickle Pod (*Senna obtusifolia*) Seeds from Obanliku South-Eastern Nigeria. *American Journal of Food Technology*, 5(1): 1-12.

Irwin, H.S., and Barneby, R.C. (1982). The American Cassinae. A synoptical revision of Leguminosae tribe *Cassieae* subtribe *Cassiinae* in the New World. Memoirs of the New York *Botanical Garden* **35**: 252-255.

Iwuoha, C. I. and Aina, J. O. (1997). Effects of steeping condition and germination time on the alpha-amylase activity, phenolics content and malting loss of Nigerian local red and hybrid short Kaura sorghum malts. *Food Chemistry*, *58*(4), 289-295.

Iyayi, A. E., & Losel, M. D. (1999). Changes in nutritional status of cassava products for livestocks feeding following solid substrate fermentation by fungi. *Report of Research Undertaken at the Department of Animal and Plant Science, University of Sheffield, UK*, *9*.

Jaffe, W.G., (1949). Toxicity of raw kidney beans. *Experienti*a, 5: 81.

Jain, S. M. and Gupta, S. D. (2013). *Biotechnology of neglected and underutilized crops*. Springer.

Jariwalla, R. J., Sabin, R., Lawson, S., and Herman, Z. S. (1990). Lowering of serum cholesterol and triglycerides and modulation of divalent cations by dietary phytate. *Journal of Applied Nutrition*, 42: 18–28.

Jayne-Williams, D. J. (1973). Influence of Dietary Jack Beans (*Canavalia ensifortnis*) and of *Concanavalin A* on the Growth of Conventional and Gnotobiotic Japanese Quail (*Coturnix coturnix japonica*). *Nature, 243*(126), 150-151.

Johnson, I. T., Gee, J. M., Price, K., Curl, C. and Fenwick, G. R. (1986). Influence of saponinss on gut permeability and active nutrient transport *in vitro*. *The Journal of Nutrition*, 116(11): 2270 -2277.

Jong, S.C. and M.J. Gantt, (eds.) (1987). *Catalogue of fungi and yeasts*, 17th edition. American Type Culture Collection, Rockville, MD.

Kadam, S.S.,. Smithard, R.R, Eyre, M.D. and Armstrong, D.G. (1987). Effects of heat treatments on antinutritional factors and quality of proteins in wingedbean. *Journal of the Science of Food and Agriculture*., 39: 267-275.

Kakade, M.L. and R.J. Evans, (1965). Nutritive value of navy beans (*Phaseolus vulgaris*). *British Journal of Nutrition*., 19: 269.

Kazanas, N. and Fields, M.l. (1981). Nutritional improvement of sorghum by fermentation. *Journal of Food Science*, 46(3): 819-821.

Kessler, M., Belmar, R., and Ellis, N. (1990). Effects of autoclaving on the nutritive value of the seeds of *Canavalia ensiformis* (jackbean) for chicks. *Tropical Agriculture, 67*(1), 16-20.

Khan, N., R. Zaman and M. Elahi, (1990). Effect of heat treatments on the phytic acid content of maize products. *Journal of the Science of Food and Agriculture*., 54: 153-156.

Khetarpaul, N. and Chauhan, B. M. (1989). Effect of fermentation by pure cultures of yeasts and lactobacilli on phytic acid and polyphenol content of pearl millet. *Journal of food Science, 54*(3), 780-781.

Khetarpaul, N. and Chauhan, B.M. (1991). Effect of Natural Fermentation on Phytate and Polyphenol Content and *In vitro* Digestibility of Starch and Protein of Pearl Millet (*Pennisetum typhoideum*) . *Journal of Science and Food Agriculture*, 55: 189-195.

Kiers, J. L., Van Laekan, A.E.A., Romboults, F.M. and Nout, M.J.R. (2000). In vitro Digestibility of Bacillus Fermented Soybean. *International Journal of Food Microbiology*, 60: 163-169.

Kingsley, M. O. (1995). Effect of processing on some antinutritive and toxic components and on the nutritional composition of the African oil bean seed (*Pentaclethra macrophylla* Benth). *Journal of the Science of Food and Agriculture, 68*(2): 153-158.

Kubicek, C. and Rohr, M. (1986). Citric acid fermentation. CRC *Critical Reviews* in Biotechnology 3(4):331-373.

Kumar R, (1992). Antinutritional factors, the potential risks of toxicity and methods to alleviate them. Proceedings of the FAO Expert Consultation held at the Malaysian Agricultural Research and Development Institute (MARDI) in Kuala Lumpur, Malaysia, 14-18 October, 1991. Andrew Speedy and Pierre-Luc Puglise (eds).

Kumar, R., and Horigome, T. (1986). Fractionation, Characterization, and protein precipitating capacity of the condensed tannins from *Ribiwa Pseuda acacia* (L.) leaves. *Journal of Agriculture and Food Chemistry*, 34: 487-489.

Lacassagne, L., Francesch, M., Carré, B., and Melcion, J. P. (1988). Utilization of tannins-containing and tannins-free faba beans (*Vicia faba*) by young chicks: effects of pelleting feeds on energy, protein and starch digestibility. *Animal Feed Science and Technology*, *20*(1): 59-68.

Lehninger AL (1987). Bioenergetics and Metabolism, *Principle of Biochemistry*, 2nd Preprint, CBS Publisher and Distribution, pp. 313–356.

Liener, I. (1980): Toxic factors in edible legumes and their elimination. *American Journal of Clinical Nutrition.* 2: 281.

Loewus, F. (2002). Biosynthesis of phytate in food grains and seeds. *Food Phytates,* 53-61.

Landers, R.E. and Rathmann, D.M. (1981). Vegetable oils: effects of processing,storage and use on nutritional values. *Journal of American Oil Chemists Society*, 58:255-259.

Loomies, W.D and Battaile, J. (1966). Plant polyphenolic compounds and the isolation of plant enzymes."*Phytochemistry,* 5:423-438.

Lopez, H. W., Leenhardt, F., Coudray, C. and Remesy, C. (2002). Minerals and phytic acid interactions: is it a real problem for human nutrition?. *International Journal of Food Science and Technology*, 37(7): 727-739.

Lucas, G.M. and Markakas, (1975). Phytic acid and other phosphorus compounds of bean (*Phaseolus vulgaris*) *J. Agric. Edu. Chem.*, 23: 13- 15.

Maenz, D.D., Irish, G.G. and Classen, H.L. (1999). Carbohydrate-binding and agglutinating lectins in raw and processed soybean meals. *Animal Feed Science Technology*. 76: 335-343.

Makkar, H.P.S., Blummel, M. Borowy, N.K. and Becker, K. (1993). Gravimetric determination of tanninss and their correlations with chemical and protein precipitation methods. *Journal of Science and Food Agriculture* 61: 161- 165.

Martín-Cabrejas, M. A., Sanfiz, B., Vidal, A., Mollá, E., Esteban, R., and López-Andréu, F. J. (2004). Effect of fermentation and autoclaving on dietary fiber fractions and antinutritional factors of beans (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry*, *52*(2): 261-266.

Marfor, E.R. and Oke, O.L. (1988). Phytate. FAO Corporate Document Repository. In: Introduction to Animal Science, pp: 146-196.

Marklinder, I., Haglund, Å., Johansson, L.(1996). Influences of lactic acid bacteria on technological, nutritional and sensory properties of barley sour dough bread. *Food Quality Preference*, 7:285-292.

Mbithi-Mwikya, S.,Van-came, J., Rodriguez, R. and Hyhebeart, A. (2001). Effects of Sprouting on Nutrient Composition of Kidney Beans. *European Food Resource Technology*, 121: 181-191.

McConell, G.K. (2007). Effect of L-arginine supplementation on exercise metabolism. *Current Opinion in Clinical Nutrition and Metabolic Care* 10: 46-51.

McCue, P. P. and Shetty, K. (2004). Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase *in vitro*. *Asia Pacific Journal of Clinical Nutrition*, *13*(1).

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. and Morgan, C.A. (1995). *Animal nutrition*. 5th Edn, Essex: Pearson Education Publishers, 49-127.

Mcloed, M.N. (1974). Plant tanninss: their role in forage quality. *Nutrition Abstracts and Reviews*, 44: 803-815.

McNaughton, J. M., and Reece, F. N. (1980). Effect of moisture content and cooking time on soybean meal urease index, trypsin inhibitor content, and broiler growth. *Poultry Science*, 59:2300–2306.

Mehansho, H., Butler L.G., and Carlson D.M. (1987). Dietary tannins and salivar proline-rich proteins interactions, induction and defence mechanisms. *Annual. Review of Nutrition*, 7:423-430

Mensah, P. P. A., Tomkins, A. M., Drasar, B. S. and Harrison, T. J. (1990). Fermentation of cereals for reduction of bacterial contamination of weaning foods in Ghana. *Lancet* 336 (8708): 140 – 143.

Mertz, E. T., Hassen, M. M., Cairns-Whittern, C., Kirleis, A. W., Tu, L. and Axtell, J. D. (1984). Pepsin digestibility of proteins in sorghum and other major cereals. *Proceedings of the National Academy of Sciences*, 81(1): 1-2.

Minihane, A. M. and Rimbach, G. (2002). Iron absorption and the iron binding and antioxidant properties of phytic acid. *International Journal of Food Science and Technology*, 37(7): 741–748.

Moelijopawiro, S., Field, M. T. and Gordon, D. (1998). Bioavailability of zinc in fermented Soya beans. *Journal of Food Science*, 53:460-463

Mohamed Nour, A.A., Mohamed Ahmed, I.A., Babiker, E.E. and Yagoub, A.E.A. (2010) Investigations on winter season Sudanese sorghum cultivars: effect of sprouting on the nutritional value. . *International Journal of Food Science and Technology, 45*(5): 884-890.

Mohammed, N. A., Ahmed, I. A. M. and Babiker, E. E. (2011). Nutritional evaluation of sorghum flour (*Sorghum bicolor* L. Moench) during processing of injera. *World Academy of Science, Engineering and Technology, 51*, 72-76.

Mohiedeen, E., Tinay, A.H.E., Eikhalya, A.E.O., Babiker, E.E. and Mallasiy, L.O. (2010). Effect of Fermentation on *In vitro* Protein Digestibility, Protein Fractions And Amino Acids Composition of Maize (*Zea mays Linnaus*) cutlivars. *Electronic Journal of Environmental Agriculture and Food Chemistry*, 9: 838-847. Monosperm, Food Chemistry 55:173-177.

Morales, A.E., Cardenete, G., Sanz, A. and de la Higuera, M. **(**1998). Re-evaluation of crude fibre and acidinsolubleash as inert markers, alternative to chromic oxide, in digestibility studies with rainbow trout (*Oncorhynchus mykiss*). *Aquaculture,* 179(14):71-79.

Morris E. R. and Ellis R. (1989) "Usefulness of the dietary phytic acid/zinc molar ratio as an index of zinc bioavailability to rats and humans," *Biological Trace Element Research*, vol. 19, no. 1-2, pp. 107–117,.

Muhammad I.R., Muh, S., Kallah, S.A.S., Olorunju, J.O., Bale, U.S., Abdullahi and R. Lawal (2002). Response of Nutrients and anti-nutritional constituents in the seeds of *Cassia tora* to treatments. *Journals of Agriculture and Environment* 3(2)2002: 225-234.

Mukhopadhyay, N. and Ray, A.K. **(**1999). Improvement of quality of sesame *Seasamum indicum* seed meal protein with supplemental amino acids in feeds for rohu *Labeo rohita* (Hamilton) fingerlings. *Aquaculture Research*, 30(8): 549–557.

Murwan, K.S. and Ali, A.A. (2011). Effect of Fermentation Period on the Chemical Composition, *In Vitro* Protein Digestibility and Tannins Content In Two Sorghum Cultivars (Dabar and Tabat) in Sudan. *Journal of Applied Biosciences* 39: 2602-2606.

Nnam, N. M. (1999). Chemical and organoleptic evaluation of complementary foods formulated from maize, cowpea and plantain flours. *Nigerian Journal of Nutritional Sciencce 20,* 35-40.

Norton, G. (1991); Proteinase inhibitors. In: D'Mello, F.P.J., Duffus, C.M., Duffus, J.H. (Eds.). Toxic substances in Crop Plants. The Royal Society of Chemistry, Thomas Graham House, Science Park, Cambridge CB4 4WF, Cambridge, pp 68-106. nutritional attributes of selected newly developed lines of sorghum after fermentation. *Journal of Agricultural Science and Technology*., 13: 399-409.
NY.

Nzewi D.C and Egbuonu A.C.C. (2011). Effect of boiling and roasting on some anti-nutrient factors of asparagus bean (*Vignases quipedalis*) flour.*African Journal of Food Science and Technology* 2(3): 075-078.

Obasi, N.E. and Wogu, C.O. (2008). Effect of soaking time on proximate and mineral compositions and anti-nutritional factors of yellow maize (*Zea mays*). *Nigerian Food Journal*, *26*(2).

Oboh G and Akindahunsi A.A (2003). Biochemical changes in cassava products (flour & gari) subjected to S*accharomyces cerevisae* solid media fermentation. *Food Chemistry*. 82(4): 599-602.

Oboh, G., Akindahunsi, A. A. and Oshodi, A. A. (2003). Dynamics of phytate-zinc balance of fungi fermented cassava products (gari and flour). *Plant Foods for Human Nutrition 58*(3), 1-7.

Obun, C. O. and Ayanwale, B. (2006). Utilization potential of *Afzelia africana* seed meal in the diet of starter broiler chicks. *Tropical Journal of Animal Science, 9,* 55-61. *Review Online Journal of Animal and Feed Research* 6 45-56.

Ogundipe, S.O., Damang, P.J., Dafwang, I.I. and Abu, E.A. (2008). Biochemical evaluation of raw and cooked African locust bean seeds (*Parkia spp*) for poultry feeding. Proceedings of the 13th Annual Conference of the Animal Science Association of Nigeria (ASAN), 15th-19th Sept., 2008. ABU, Zaria, Nigeria, pp: 586-589.

Oh, H. I., Hoff, J. E., Armstrong, G. S and Haff, L. A. (1980). Hydrophobic interaction in tannins-protein complexes. *Journal of Agricultural and Food Chemistry, 28*(2): 394-398.

Ojiako, O. A and Igwe, C. U. (2008). The nutritive, antinutritive and hepatotoxic properties of *Trichosanthes anguina* (snake tomato) fruits from Nigeria. *Pakistan Journal of Nutrition*, *7*(1), 85-89.

Ojokoh, A. O., Abiola, A. B. and Lawal, R. T. (2012). Changes in Nutrient and Antinutrient Composition of Popcorn and Groundnut Composite Flour Subjected to Solid Substrate Fermentation. *African Journal of Agricultural Research* 7(23): 3439-3445.

Ojokoh, A. O. (2014). Proximate composition and antinutrient content of pumpkin (*Cucurbita pepo*) and sorghum (*Sorghum bicolor*) flour blends fermented with Lactobacillus plantarum, *Aspergillus niger* and *Bacillus subtilis*. *Ife Journal of Science*, *16*(3), 425-435.

Okafor N. and Ejiofor, A.O. (1990): Rapid detoxification of cassava mash by a yeast simultaneously producing linamarase and amylase. *Process Biochem*. 25: 82–86.

Oke, O. L. (1965). Chemical Studies on some Nigerian Foodstuffs-" Lafun". *West African Journal of Biology & Applied Chemistry.*, *8*(3), 53-6.

Oke, O. L. (1969). Oxalic acid in plants and in nutrition. In *World Review of Nutrition and Dietetics* Vol. 10, pp. 262-303. Karger Publishers.

Okojie, J.A., (1999). The role of government and universities of agriculture in improving animal production and computations in Nigeria. *Trop. J. Anim. Sci*., 2: 1-7.

Oladele K.A., Osundahunsi F.O. and Adebowale A.Y. (2009). Influence of Processing Techniques on the Nutrients And Antinutrients of Tiger nut (*Cyperus esculentus L.*). *World Journal of Diary and Food Sciences* 4 (2): 88-93

Oladele, A. K and Aina, J. O. (2007). Chemical composition and functional properties of flour produced from two varieties of tigernut (*Cyperus esculentus*). *African Journal of Biotechnology*, *6*(21).

Ologhobo, A. D., Apata, D. F and Oyejide, A. (1993). Utilisation of raw jackbean (*Canavalia ensiformis*) and jackbean fractions in diets for broiler chicks. *British Poultry Science*, *34*(2), 323-337.

Omoikhoje, S.O., Etebha, E.T.E., Bamgbose, A.M. and Osifi, B.A. (2009). Performance indices of staring broiler chickens fed dietary levels of roasted fluted pumpkin pod Husk waste meal. Proceedings of the 14th Annual conference of Animal Science Association of Nigeria (ASAN), 14th- 17th Sept., 2009, LAUTECH. Ogbomoso, Nigeria, pp: 459-461.

Onimawo, I. A. and Akpojovwo, A. E. (2006). Toasting (dry heat) and nutrient composition, functional properties and antinutritional factors of pigeon pea (*Cajanus cajan*) flour. *Journal of Food Processing and Preservation*, *30*(6), 742-753.

Onimisi, H. U., Oniye, S. J. and Bolorunduro, P. I. (2017). Nutritional evaluation of boiled *Senna obtusifolia* (linn) seed meal for fish nutrition. *Journal of Animal Production Research*, *29*(1), 312-320.

Onweluzo, J. C. and Nwabgwu, C.C. (2009). Fermentation of Millet (*Pennisetum americana*) and Pigeon pea (*Cajanus cajan*) Seeds to Flour Production: Effects on Composition and Selected Functional Properties. *Pakistan Journal of Nutrition* 8(6): 737- 744.

Onwuka, G. I. (2005). *Food analysis and instrumentation*: theory and practice. Naphthalic prints, Surulere, Lagos, Nigeria, 219-230.

Onyango, C., Noetzold, H, Ziems, A, Hofmann, T, Bley, T and Henle, T. (2005). Digestibility and antinutrient properties of acidified and extruded maize-finger millet blend in the production of Uji. LWT, *Food Science and Technology* 38: 697-707.

Onyeike E.N, Olungwe T. and Uwakwe AA (1995). Effect of heat treatment and defatting on the proximate composition of some Nigerian local soup thickeners. *Food Chemistry*. 53: 173-175.

Osagie AU (1998). Antinutritional Factors. In: *Nutritional Quality of Plant Foods*. Ambik Press Ltd, Benin City, Nigeria, pp. 1-40; 221-244.

Osuntokun, B. O. (1970). Cassava diet and cyanide metabolism in Wistar rats. *British Journal of Nutrition*, *24*(3), 797-800.

Ousman, A., Ngassoum, M. and Kamga, C. (2005). Chemical composition of *Senna obtusifolia* L. *Journal of Food Technology* 3: 453-455.

Page, R.K., Vesey, S., Charles, O.W. and Hollifield, T. (1977). Effect on food consumption and egg production of coffee bean seed (*Cassia obtusifolia*) fed to White Leghorn hens. *Avian Diseases*, 21: 90-96.

Pant G., Panwaar MS, Negi DS. and Rawat MS (1989). Spermicidal activity of triterpenoid glucosides of *Pentapanax leschenaultii*, Ibid. 54: 477.

Paredes-Lopez, O. and Harry, G.l. (1989). Changes In Selected Chemical and Anti-Nutritional Components During Tempeh Preparation Using Fresh and Hardend Common Beans. Journal of Food Science, 54: 968-970.

Parsons, W. T. and Cuthbertson. E. G. (1992). Noxious Weeds of Australia. Inkata Press, Melbourne. 698pp.

Pedrosa, M.M., Cuadroda, C., Burbano, C., Allaf, K., Haddad, J., Gelencser, E., Takacs, K., Guillamon, E. and Muzquiz, M. (2011). Effect of instant controlled pressure drop on the oligosaccharides, inositol phosphates, trypsin inhibitors and lectins contents of different legumes. *Food Chemistry* 10: 1016-1023.

Pinto, M.C., L.C. Trugo, L. Cabral and C.W.P. Carvalho, 1997. Effect of malting and extrusion-cooking processes on nutrient composition of soybean. Book of Abstracts, 26th Annual Conference; *Brazilian Society of Biochemistry and Molecular Biology*: Caxambu, Brazil, May 3rd-6th, pp: 27.

Pugalenthi, M., Vadivel, V., Gurumoorthi, P. and Janardhanan, K. (2004). Comparative nutritional evaluation of little known legumes, *Tamarindus Indica*, *Erythrina Indica* and *Sesbania bispinosa*. *Tropical and Sub- Tropical Agro ecosystem*. 2004 (4):107-123.

Pusztai, A., (1989). Biological effects of dietary lectins. J.Huisman, T.F.B.Poel and I.E.Liener, Recent Advances of Research in Anti-nutritional Factors in Legumeseeds: *Animal Nutrition, Feed Technology,* Analytical Methods. Wageningen (Netherlands), Pudoc, pp.17-29.

Quinn, M. R., Beuchat, L. R., Miller, J., Young, C. T. and Worthington, R. E. (1975). Fungal fermentation of peanut flour: Effects on chemical composition and nutritive value. *Journal of Food Science*, *40*(3), 470-474.

Raboy, V. (2001). Seeds for a better future: Low phytate grains help to overcome malnutrition and reduce pollution. *Trends in Plant Science* 6: 458–462.

Raboy, V. (2003). Myo-inositol-1,2,3,4,5,6-hexakisphosphate. *Phytochemistry* 64: 1033-1043.

Raimbault, M. (1998). General and microbiological aspects of solid substrate fermentation. *Electronic Journal of Biotechnology*, *1*(3), 26-27.

Rao, V.S. and Vakil, U.K. (1983). Effects of gamma-irradiation on flatulencecausing oligosaccharides in green gram (*Phaseolus aureus*). *Journal of Food Science,* 48: 1791-1795.

Raper, K. B. and Fennell, D. I. (1965). Aspergillus flavus group. *The Genus Aspergillus*, 357-404.

Reade, A. E. and Gregory, K. F. (1975). High-temperature production of protein-enriched feed from cassava by fungi. *Applied microbiology*, *30*(6), 897-904.

Reddy, N.R., Pierson, M.D., Shridhuar, K. and Salunkhe, D.K. (1984). CRC. Critical reviews in food science and nutrition: Legume-based fermented Foods: their Preparation and nutritional quality 17(4): 335-365.

Reddy, N. R. (2002). Occurrence, distribution, content, and dietary intake of phytate. In N. R.Reddy and S. K. Sathe (Eds.), Food phytates (pp. 25–51). Boca Raton, Florida: CRC Press.

Reed, J.D., Horvath, P.J., Allen, M.S and Van Soest P.J (1985). Gravimetric determination of soluble phenolics including tanninss from leaves by precipitation with trivalent Ytterbium. *Journal of Science Food and Agriculture,* 36: 255-261.

Reed, J.D (1986).”Relationship among soluble phenolics, insoluble proanthocyanidins and fiber in East African browse species.” *Journal of Range Management* 39:5-7

Refstie, S., Sahlstrom, S., Brathen, E., Baeverfjord, G. and Krogedal, P. **(**2005). Lactic acid fermentation eliminates indigestible carbohydrates and antinutritional factors in soybean meal for Atlantic salmon (*Salmo salar*). *Aquaculture*, 246(1–4): 331–345.

Reimerdes, E.H. (1990). Improvements of protein functionalities from milk. *Food Biotechnology*, 4: 59-68.

Rhabasa, L and Chiasson, J. L. (2004). Alpha-Glucosidase Inhibitors, **Vol. 1**, 3rd Ed. John Wiley & Sons Ltd., UK p. 901-904.

Riguera R. (1997). Isolating bioactive compounds from marine organisms. *Journal of Marine Biotechnology* 5: 187–193.

Robel, E.J. (1967). Ion-Exchange Chromatograph for the Determination of Tryptophan *Analytical Biochemistry 18: 406-413.*

Roeder, E. (1995). Medicinal plants in Europe containing pyrrolizidine alkaloids. *Pharmazie*, *50*(2), 83-98.

Roozen, J.P. and De Groot, J. (1985). Electrophoresis and assay of trypsin inhibitors in different stages of tempeh production. *Journal of Food Biochemistry*, 9: 37-48.

Sathe, S.K., Deshpande, S.S. and D.K. Salunkhe, (1984). Dry beans of *Phaseolus*: A review: Part 1. Chemical Compsotion: Proteins. CRC, *Crit. Rev. Food Sci. Nutr*., 20: 1-46. *Science* 20: 35-40.

Saharan, K., Kheterpaul, N. and Bishnois (2001). HCL-extractability of minerals from rice bean and faba bean: Influence of domestic processing methods. *Innovation Food Science Emerging Technology* 2(4): 323-325

Saito, K., Horie, M., Hoshino, Y., Nose, N. and Nakazawa, H. (1990). High-performance liquid chromatographic determination of glycoalkaloids in potato products. *Journal of Chromatography A*, *508*, 141-147.

Sandberg, A.S. (1991). The effect of food processing on phytate hydrolysis and availability of iron and zinc, Consequences of food processing. *Nutritional Toxicology*, 33: 499-508.

Selvam, R. (2002). Calcium oxalate stone disease: Role of lipid peroxidation and antioxidants. *Urological Research*, 30(1): 35–47.

Shahidi, F. (1997).Antinutrients and phytochemicals in foods Washington DC, USA. *American Chemical Society*, Symposium, Series 662, 334pp.

Shamsuddin, A. M. (2002). Anticancer function of phytic acid. *International Journal of Food Science and Technology*, 37(7): 769–782.

Siddhuraju, P. Vijayakumari, K. and Janardhanan, K. (1996). Chemical composition and protein quality of the little-known legume, velvet bean (*Mucuna pruriens* (L.) DC). *Journal of Agriculture and Food Chemistry*, 44: 2636-2641.

Siddhuraju, P and Becker, K. (2001). Effect of various domestic processing methods on antinutrients and *in vitro* protein and starch digestibility of two indigenous varieties of Indian tribal pulse, *Mucuna pruriens* var. *utilis*. *Journal Agriculture Food* 49(6): 3058-3067.

Siegel, A. and Fawcett, B. (1978). *Food Legume Processing and Utilization*. International Development Research Centre Technical Studies Series, Ottawa. 10-12pp

Sigh, N. (1964). Leaf protein extract from some plants of North India. *Journal of Food, Science and Technology*, 3: 37-39.

Skrede, G., Storebakken, T., Skrede, A., Sahlstrom, S., Sorensen, M., Shearer, K.D. and Slinde, E. **(**2002). Lactic acid fermentation of wheat and barley whole meal flours improves digestibility of nutrients and energy in Atlantic salmon (*Salmo salar* L.) diets. *Aquaculture*, 210(1–4):305–321.

Smith, K.J. (1986). Legumes chemistry, *Technology and Nutrition.*(Ed). Mathews, R.H. Marcel Dekker, Inc pp22.

Sokrab, A.M., Mohamed Ahmed, I.A. and Babiker, E.E. (2012). Effect of malting and fermentation on sorghum through fermentation in traditional African porridges. *International Journal of Food Science Technology*., 37: 129-138.

Sasson, A. (2012). Food security for Africa: an urgent global challenge. *Agriculture & Food Security*, *1*(1), 2.

Su, H., and Guo, R. (1986). Inhibition of acrosine activity of human spermatozoa by saponinss of Bulbostermma paniculatum Xtian Yike Daxue Xuebae 7, 225. In *Chem. Abstr* (Vol. 1008, p. 49459).

Swain, T. (1979). Tannins and lignin. In G.A. Rosenthal and D.H. Janzen (eds), *Hervibores-their interaction with secondary plant metabolites*. Academic press, New York, USA Pp. 657-682

Tamburawa, M.S. (2010). Effect of locustbean seed meal diets on the performance and carcass charactersitics of broiler chickens a Ph.d research Proposal, presented at the postgraduate seminar series of the Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University (A.B.U.) Zaria. 15pp

Taparia, A.L., Talmale. T.W. and Shamra, V.V. (1978). Utilization of *Cassia tora* seeds in growth rations of buffalo calves. *Indian Journal of Animal Science,* 48: 804-810.

Taylor, J., and Taylor, J. (2002). Alleviation of the adverse effect of cooking on sorghum protein digestibility through fermentation in traditional African porridges. *International Journal of Food Science and Technology*, *37*(2): 129-137.

Teucher, B., Olivares, M. and Cori, H. (2004). Enhancers of iron absorbtion: Ascorbic acid and other organic acids. *International Journal of Vitamin Nutrition Resources*, 74: 403-19.

Thompson, L. U. (1993). Potential health benefits and problems associated with antinutrients in foods. *Food Research International,* 26: 131–149.

Torki, M. and Farahmand-Pour, M (2007). Use of dietary enzyme inclusion and seed germination to improve feeding value of sorghum for broiler chicks. Proceedings of the 16th European symposium on poultry nutrition: Strasbourg. France. In: World poultry Science Association, pp: 643-646.

Trugo, L.C., Ramos, L.A. Trugo, N.M.F. and Souza, M.C.P. (1990). Oligosaccharide composition and trypsin inhibitor activity *of Phaseolus vulgaris* and the effect of germination on the alpha-galactoside composition and fermentation in the human colon. *Food Chemistry*, 36: 53-61.

Uchegbu, M.C., Ibekwe, A.O., Ogbuewu, I.P., Obikaonu, H.O., Nwaodu, C.H. and Okoli, I.C. (2008). Performance of broiler finisher chikens fed diets containing raw and cooked *Napoleon imperialis* seed meal. Proceedings of the 13th Annual conference of Animal Science Association of Nigeria. (ASAN), 15th - 19th Sept., 2008 A.B.U, Zaria, Nigeria, pp: 384-387.

Udedibie, A. B. I. and Nwaiwi, J. (1988). The potential of jackbean (*C. ensiformis*) as animal feed. *Journal of Nigerian Agriculture*, *23*, 118-129.

Udedibie, A. B. I., Esonu, B. O., Unachukwu, C. and Iwouha, N. C. (1996). Two-stage cooking as a method of improving the nutritive value of jackbean (*Canavalia ensiformis*) for broilers. *Nigerian Journal of Animal Production*, *23*(1): 107-110.

Vadivel, V. and Pugalenthi, M. (2007). Biological value and protein quality of raw and processed seeds of *Mucuna pruriens* var. utilis. *Livestock Research for Rural Development* 19 (7) 11pp.

Vadivel, V., Pugalenthi, M. and Megha, S. (2008). Biological evaluation of protein quality of raw and processed seeds of gila bean (*Entada scandens* Benth.). *Tropical and Subtropical Agroecosystems, 8*(2).

Valencia, S, Svanberg, U., Sandberg, A. S. and Ruals, J. (1999). Processing of Quinoa (*Chenopodium quinoa*, Willd). Effects on *in vitro* iron availability and phytate hydrolysis. *International Journal of Food Science and Nutrition* 50: 203-208.

Van der Poel, A. F. B. (1989). Effects of processing on antinutritional factors (ANF) and nutritional value of legume seeds for non-ruminant feeding. In *Recent advances of research in antinutritional factors in legume seeds* (pp. 213-229). Pudoc, Wageningen, Netherlands.

Vasudevan, D.M and Sreekumari, S. (2007). *Textbook of Biochemistry for Medical* Students 5th Edn. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd., pp535.

Verde, C. V. V., Frías, J., and Verde , S. V. (1992). Effect of processing on the soluble carbohydrate content of lentils. *Journal of Food Protection*, *55*(4): 301-303.

Vijayakumari, K., Pugalenthi, M and Vadivel, V. (2007). Effect of soaking and hydrothermal Processing methods on the levels of antinutrients and *in vitro* protein digestibility of *Bauhinia purpurea* L. Seeds *Food Chemistry*. 103: 968-975.

Vin, M. (2009). A hidden danger with nuts, grains, beans and seeds.*Journal of Agriculture and Food Chemistry*, 73:209-215.

Vlavonou BM (1988). Cassava processing technologies in Africa. In Natalie D. Hahn(Ed.), *Praise of cassava* (pp. 25–32). Int. Inst. Trop. Agric. (IITA).

W.H.O. Malnutrition. (2013) The Global Picture. World Health Organization. Available at http://www.who.int/home-page/. Accessed on 1 January.

Ward, A. T., Marquardt, R. R and Campbell, L. D. (1977). Further studies on the isolation of the thermolabile growth inhibitor from the faba bean (*Vicia faba* L. var. minor). *The Journal of Nutrition, 107*(7), 1325-1334.

Ward, O.P (1989). *Fermentation biotechnology*. Prentice Hall, Englewood Cliffs, NJ.

Weinberger, K. (2007). Are indigenous vegetables underutilized crops? Some evidence from Eastern Africa and Southeast Asia. *Acta Hortic*. 752, 29–34.

White, T. (1957). Tannins-their occurrence and significances. *Journal of Science Food and Agriculture* 8:377-385.

Woodward, A., & Reed, J. D. (1989). The influence of polyphenolics on the nutritive value of browse: a summary of research conducted at ILCA.

Wu, Y. V., and Abbott, T. P. (2005). Gum and protein enrichment from sicklepod (Cassia obtusifolia) seed by fine grinding and sieving. *Industrial crops and products*, *21*(3), 387-390.

Yagoub, A. E. and Abdalla, A. A. (2007). Effect of domestic processing methods on chemical composition, *in- vitro* digestibility of protein and starch and functional properties of bambara groundnut (*Voandzeia subterranean*) seed. *Research Journal of Agriculture and Biolological Science*, 3: 24-34.

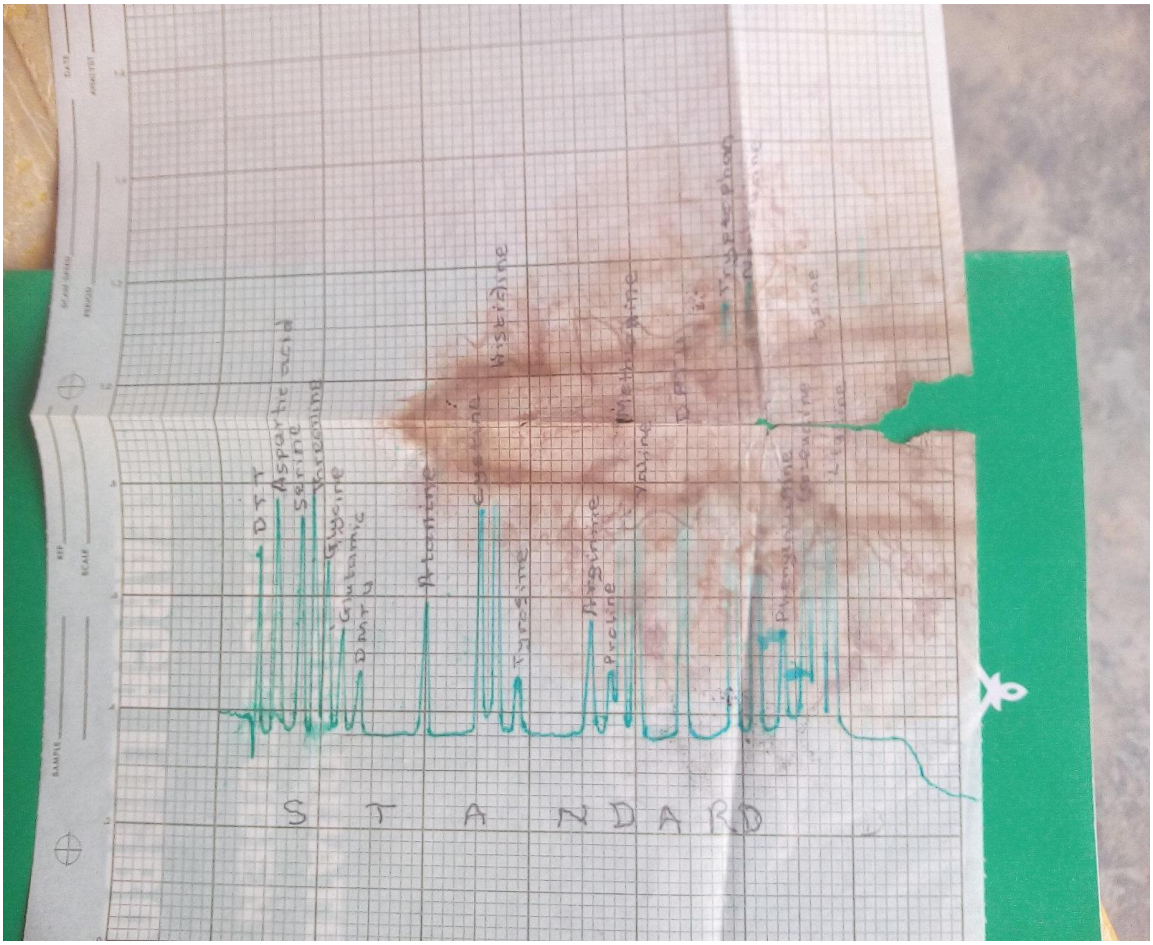
Yashim, S.M.; Segun, A.J.; Abdu, S.B.; Duru, S. and Adamu, H.Y. (2009). Effect of duration of soaking of rattlebox seeds (*Crotalaria retusa* L.) on the level of some chemical composition and minerals In: Proceedings Nigerian Society for Animal Production 34th Annual Conference pp236-238.

Yoshiki, Y., Kudou, S. and Okubo, K. (1998) Relationship between chemical structures and biological activities of triterpenoid saponinss from soybean (Review). *Bioscience Biotechnology and Biochemistry* 62: 2291–2299.

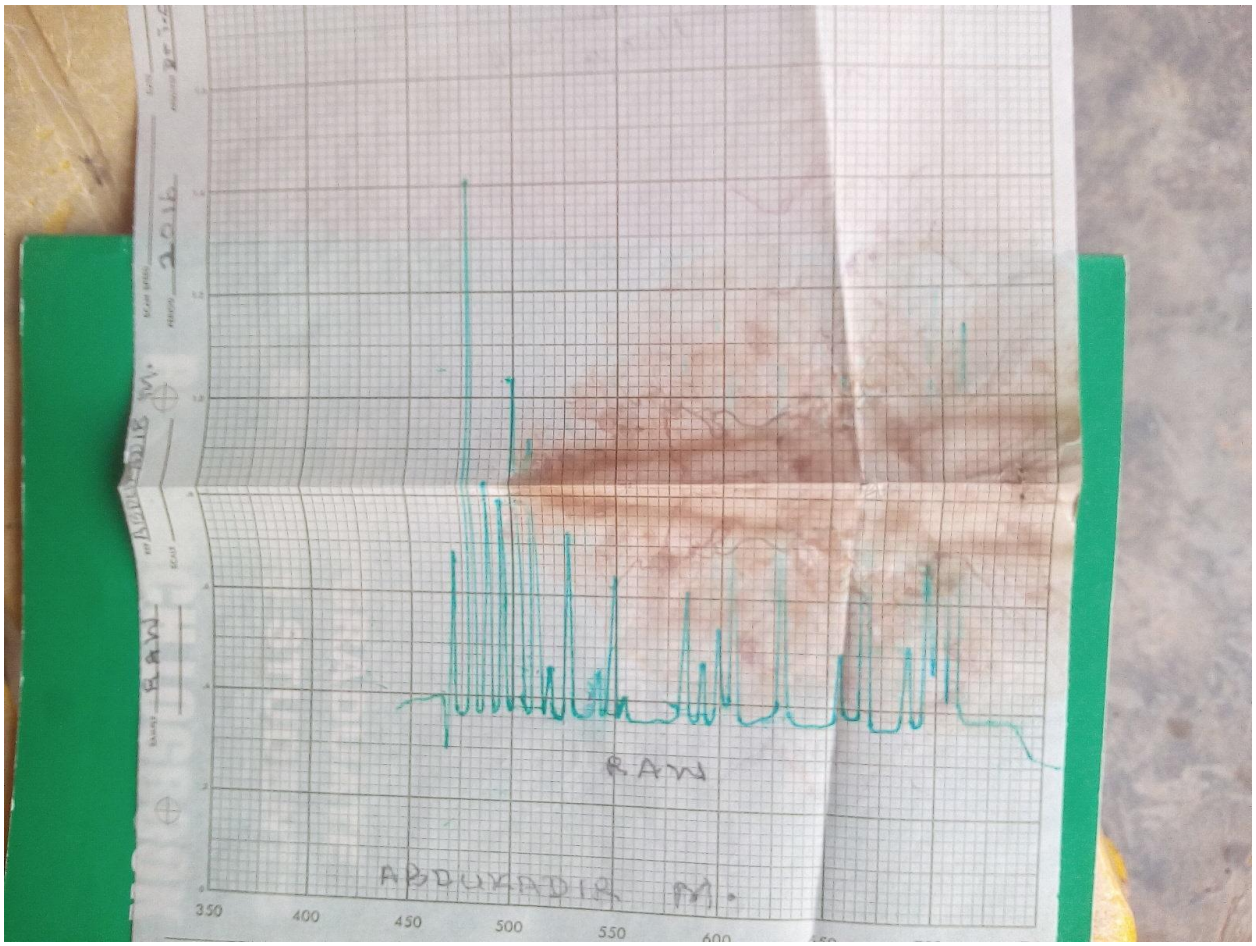
Yuan X., Wang, J., Yao, H. and Venant N. (2005). Separation and identification of endoxylanases from *Bacillus subtilis* and their actions on wheat bran insoluble dietary fibre, *Process Biochemistry*, 40**:** 2339-2343.

Zamora, A. F., and Fields, M. L. (1979). Nutritive quality of fermented cowpeas (*Vigna sinensis*) and chickpeas (*Cicer arietinum*). *Journal of Food Science*, *44*(1), 234-236.

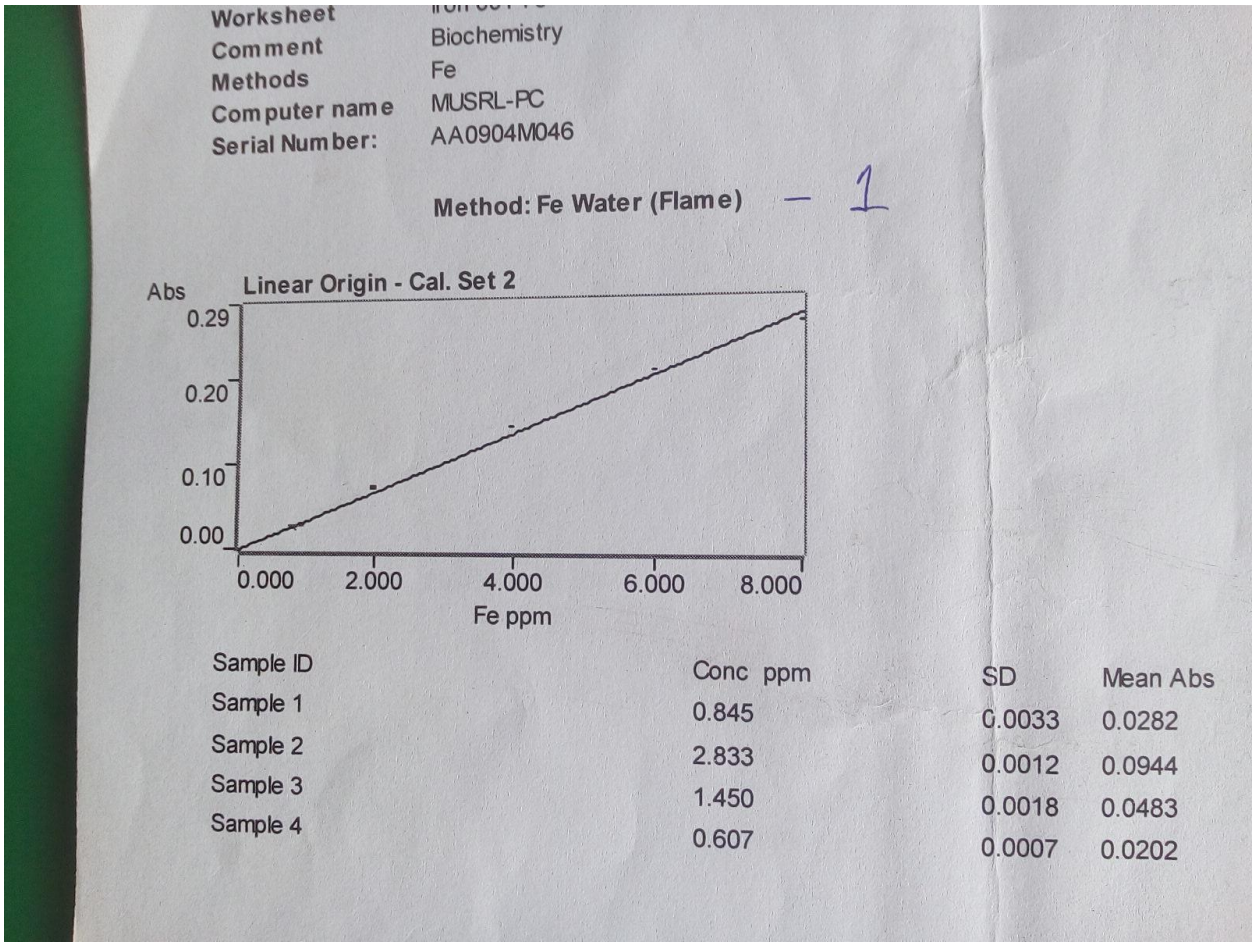
APPENDICES



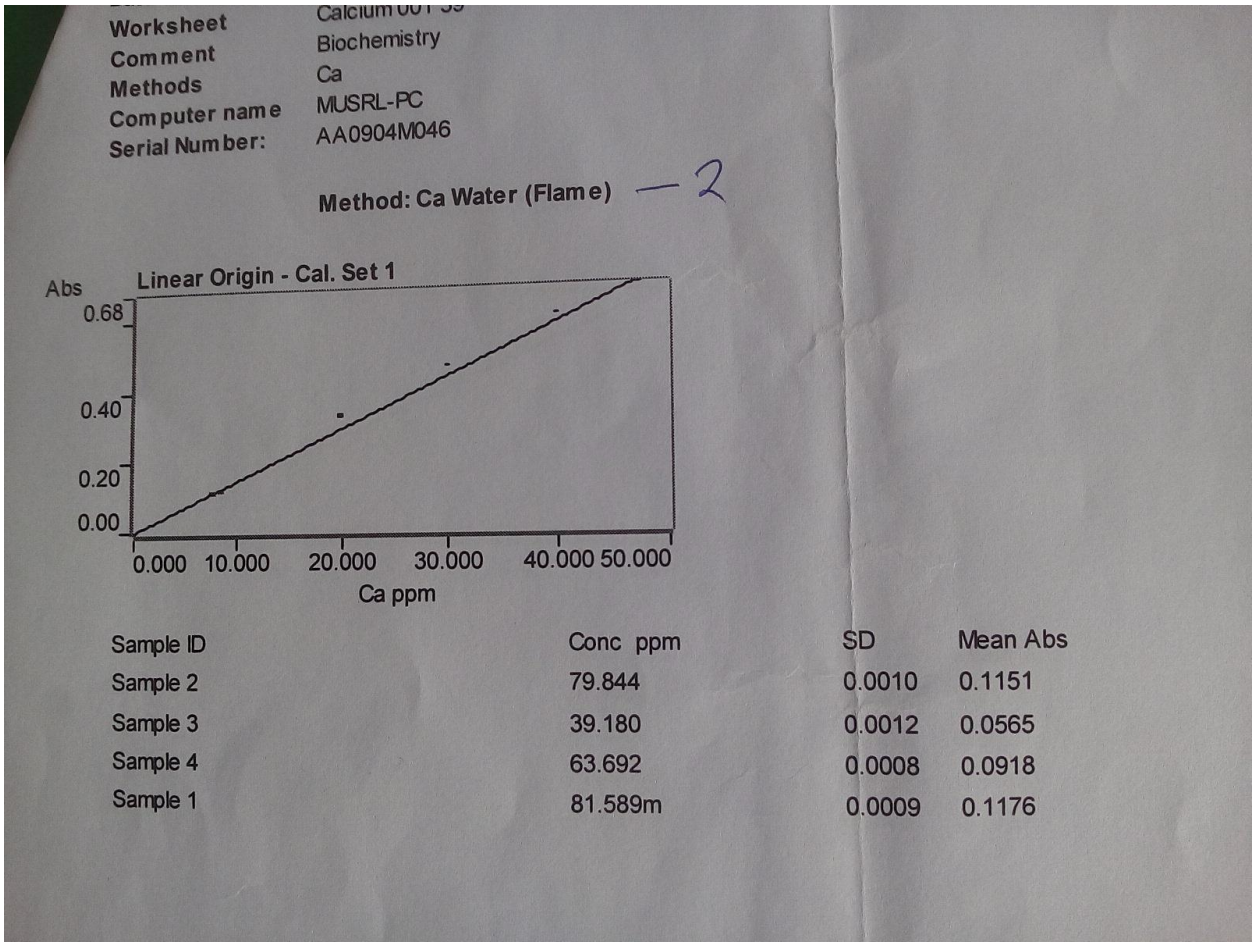
Appendix 1: Standard Chromatogram of Amino Acids Curve



Appendix 2: Chromatogram of Amino Acids Composition of Raw Seeds Sample of *Senna obtusifolia*

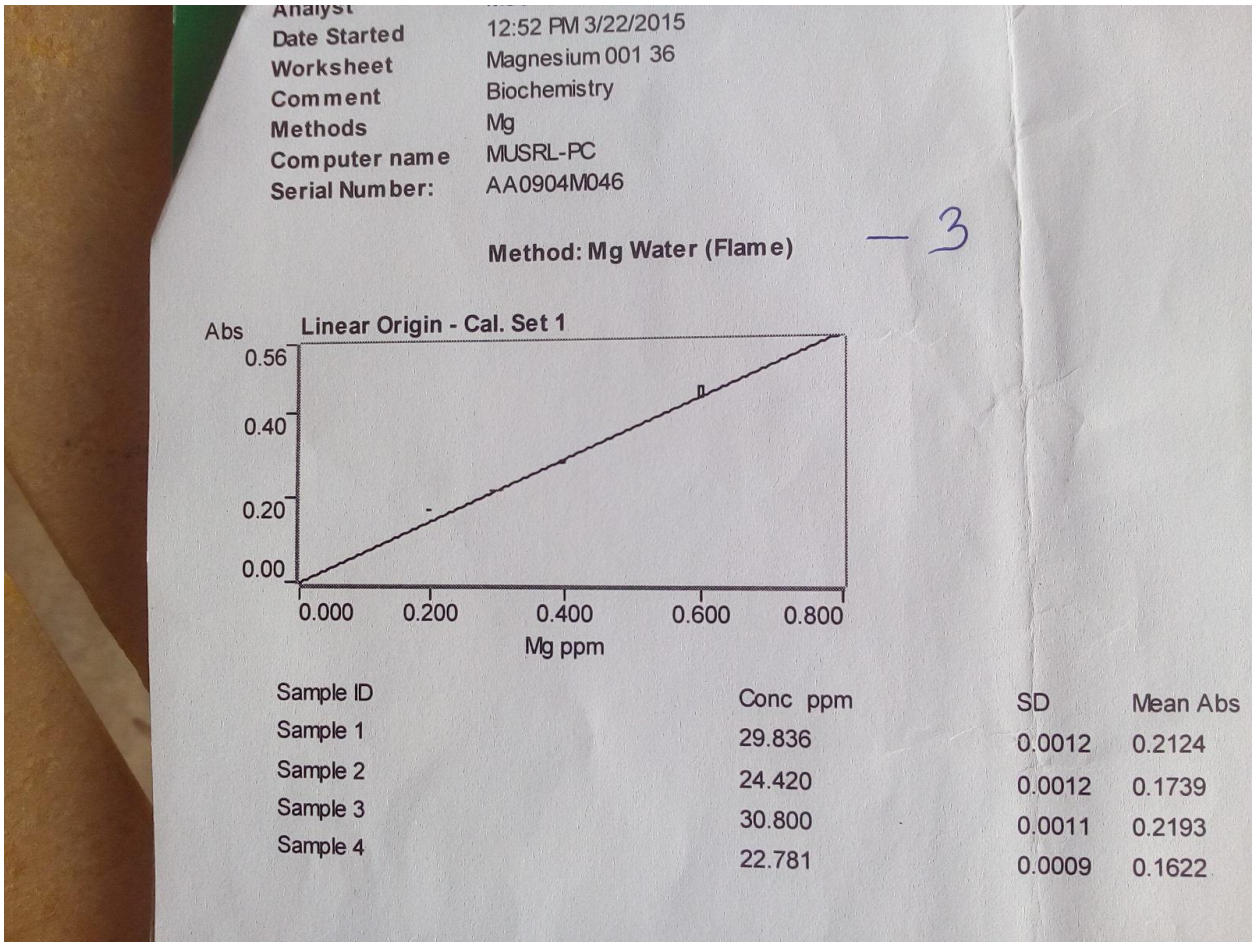


Appendix 3: Calibration Curve for Determination of Fe in Raw and Processed *Senna obtusifolia* Seeds Sample



Sample ID	Conc ppm	SD	Mean Abs
Sample 2	79.844	0.0010	0.1151
Sample 3	39.180	0.0012	0.0565
Sample 4	63.692	0.0008	0.0918
Sample 1	81.589m	0.0009	0.1176

Appendix 4: Calibration Curve for the Determination of Ca in Both Raw and Processed *Senna obtusifolia* Seeds Sample

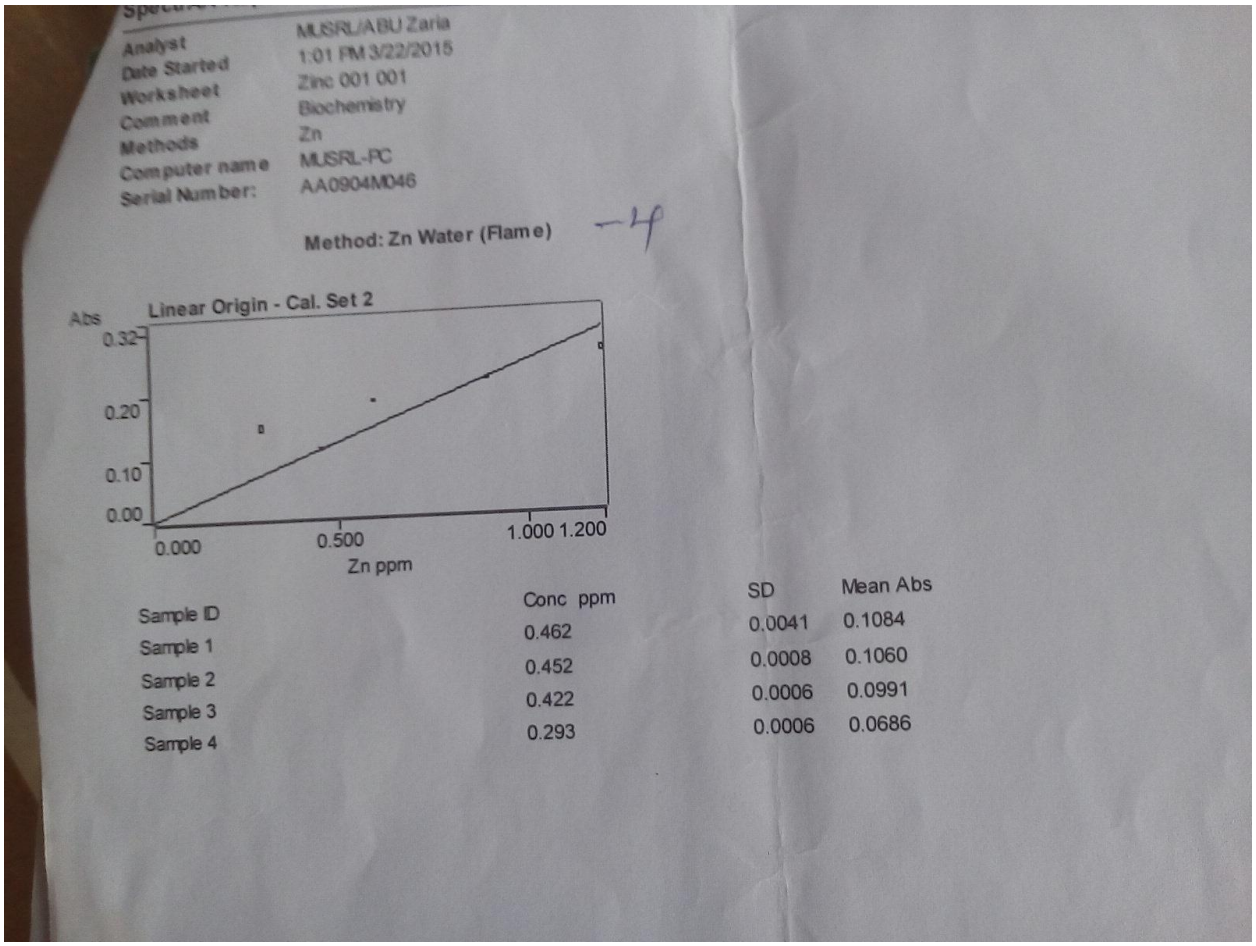


Analyst
Date Started 12:52 PM 3/22/2015
Worksheet Magnesium 001 36
Comment Biochemistry
Methods Mg
Computer name MUSRL-PC
Serial Number: AA0904M046

Method: Mg Water (Flame) — 3

Sample ID	Conc ppm	SD	Mean Abs
Sample 1	29.836	0.0012	0.2124
Sample 2	24.420	0.0012	0.1739
Sample 3	30.800	0.0011	0.2193
Sample 4	22.781	0.0009	0.1622

Appendix 5: Calibration Curve for the Determination of Mg in Both Raw and Processed *Senna obtusifolia* Seeds Sample



Appendix 6: Calibration Curve for the Determination of Zn of Both Raw and Processed *Senna obtusifolia* Seeds Sample