FORMULATION AND EVALUATION OF THE HYPOGLYCEMIC EFFECTS OF A SOY-FORTIFIED DIABETIC DIET

BY

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A MASTERS DEGREE

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MAY 2005.

DEDICATION

This work is dedicated to My Brother, UMAR SANDA ADAMU who became a diabetic patient at the age of forty years.

CERTIFICATION

This thesis entitled "Formulation and Evaluation of the hypoglycemic effects of a soy-fortified diabetic diet" was carried out by ALIYU MOHAMMED PAIKO M.Tech/SSSE/2000/2001/671 under my supervision and has been examined, read and found to meet the regulations governing the award of the degree of Masters of Technology in Biochemistry of Federal University of Technology, Minna and is approved for its contribution to knowledge and literary presentation.

Hakamya.	28th March, 2006
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Ohuaka 25/7/06	
Dean Post graduate School	Date
External Supervisor	Date

DECLARATION

I hereby declare that this Thesis is my original work and has never, to the best of my knowledge, been submitted before for the award of any degree. All literature used in the course of this study has been duly cited in the references.

_____ _____

ALIYU MOHAMMED PAIKO DATE

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Allah (SWT) deserves abundant praises, for His mercy, His wisdom and above all, His Knowledge. ALHAMDU LILLAH!

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ABSTRACT

A high protein-energy diabetic diet named Soya Diabetic Diet (SDD) was formulated and prepared using Soya beans, sorghum and unripe plantain. Its quality was evaluated in terms of its nutritive value, microbial load, physiological characteristics and hypoglycemic properties. SDD had physical and nutritive characteristics similar to a very popular commercial baby weaning food in Nigeria (CERELAC) but was of higher protein content. It also had high acceptability by the animals fed the diet. The protein content was 28.0%, 26.2% fat and 33.5% carbohydrate but calcium and phosphorous levels as well as vitamins were however low. Microbial load of the diet indicated that bacterial and fungal levels in the diet were very low and insignificant ($p>0.05$). There was good growth and development in animals fed the diet, with mean body weight recording significant ($p<0.05$) increase in the 21 days period of the experiment. SDD also possess hypoglycemic properties, significantly ($p<0.05$) lowering the mean blood glucose levels of normal, adult experimental albino rats from 82.44mg/ml and 70.6mg/ml for random blood sugar and fasting blood sugar to 55.8mg/ml and 51.7mg/ml respectively. The blend can therefore be recommended for use as a good diabetic diet in Nigeria if properly fortified with minerals and vitamins as most dietary therapy techniques being adopted for diabetics elsewhere are yet to be understood by Africans; as such most of the potential food sources to be used for this purpose have not been properly exploited, despite World Health Organization's (WHO) expert committee on diabetes recommendation for this to be done. The committee goes further to state that much of the increase in diabetes being forecasted will occur in developing countries as a result of population growth, aging, unhealthy diets, obesity and sedentary lifestyles.

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Most of these oral hypoglycemic agents are largely unavailable in Africa or where they are available, they are expensive and unaffordable to most of the African diabetics. Moreover, approximately 80% of people in the rural Africa communities still rely on the use of plant remedies to treat and/or manage diabetes mellitus, even though only a few of the Africa medicinal plants used in folklore medicine as herbal remedies for treatment of diabetes mellitus have yet received science scrutiny despite WHO recommendation for medical and scientific examinations of such plants to be conducted [7]. Therefore, it is at present difficult to rely on any particular herbal remedy of Africa origin to cure diabetes mellitus for lack of reliable scientific evidence.

As a result of the problems associated with the available sources of prevention, cure and/or management techniques of the disease, research potential of the kind that follow the recommendation of WHO expert committee on diabetes are necessary. The WHO committee states that much of the increase in diabetes being forecasted will occur in developing countries as a result of population growth, aging, unhealthy diets, obesity and sedentary lifestyles. The expert report goes further to suggest the primary prevention/management strategy for diabetes to be healthy diet and regular physical activity. [8]

Though the "Ideal diabetic diet" is yet to be formulated, yet according to the American diabetic Association "All diabetics should receive nutrition counseling, by a registered dietitian when possible". [9]

Diet therapy is key in not only controlling blood glucose levels, but in the overall well being of the diabetic. Major changes in dietary recommendation for diabetics were introduced in the 1980s in the UK, continental Europe and USA. Following experimental studies, which showed that a high carbohydrate diet does not have significant deleterious effect on diabetic control, carbohydrate restrictions, which were earlier practiced, were abolished for diets high in carbohydrates, low in fat and average in protein. Those diets recommended for diabetic control and management are quite similar to diets adopted by the general population in terms of provision of calories and the ratios of the food nutrients. [10] [11]

Going by the above dietary recommendations, which have been proved to work for diabetics in areas of maintenance of good general health. alleviation of symptoms and prevention of long-term complications, the aim of this study is to formulate a diabetic diet from cheap and available raw materials of African origin (e.g. Soya beans, Sorghum and unripe Plantains) that would be readily available, posses hypoglycemic properties and yet provide the caloric needs of diabetics to

sustain growth and development without necessarily exposing the patient to the complications of the disease. The diet targeted is to be served either alone as a main meal or taken blended as part of a meal, which could be eaten as breakfast, lunch or dinner. The objectives of this study are:

i To formulate a diabetic diet.

ii Evaluate the chemical composition of the formulation.

iii Determine the microbial load of the formulated diet

iv Carry out animal experiment using albino rats to test for the hypoglycemic effects of the blend formulation.

It is hoped that the experiment would serve as a pilot study for the industrial production of the formulated diet since commercial diabetic foods are still uncommon in Africa.

CHAPTER TWO

2.0 LITERATURE REVIEW.

2.1 HISTORICAL BACKGROUND OF DIABETES

Although the symptoms of diabetes mellitus disease were described over 3,500 years ago in a compendium of medical diseases, the Egyptologist George Ebers in 1872 also described it fully and gave the name *papyrus Ebers* [12]. The Roman physician Celsus in 10 AD was the first to describe it as a disease of excess urination and wasting [13].

The word **diabetes** is derived from a Greek word meaning "going through" or "Siphon" while **mellitus** is the Latin word for honey or sweet. [14] Diabetes mellitus is thus literary translated as 'siphoning of honey or sweet from the body in urine'.

From its name, taking origin from Greeks and Latin, and its description by Egyptians and Romans at different times, it is apparent that the disease, from onset was universal in spread. However, a proper direction for the diabetes mellitus solution involved a lot of researchers from diverse fields and using different criteria. In 1857, Claude Bernard described glycogen as a product of glucose metabolism in liver and set forth the concept that altered the belief that glucose metabolism is the cause of diabetes [15]. In 1869, the islets of Langerhans were discovered by Paul Langerhans [16]. The major conceptual break through came when Dr. Minkowski, a German researcher made a firm connection

between the pancreas and diabetes mellitus in 1889 [17]. The isolation of insulin from the pancreas by the two Canadian Nobel Prize winners, Frederic Banting and Charles Best in 1921 [1] became the landmark in finally concentrating the attention of researches on diabetes to the pancreas. To date, efforts are still on in the search for the ideal, reliable cure, although great strides have been made in different directions.

The major objectives of each treatment and/or management process adopted or targeted as solution is aimed at achieving all or most of the following: -

i. Alleviation of symptoms of the disease.

ii. The maintenance of good general health.

iii. Prevention of long term complications [18].

With different treatment regimes, it is relatively easy to achieve the first of these objectives, and depending on patients, reasonable or good general health can be maintained even though on short or medium term basis without necessarily achieving diabetic control.

The major question, which has generated a lot of research, has to do with the relationship between glycemic control and long term complications of diabetes [18].

So far, researches have indicated that diet, oral hypoglycemic agents and insulin, in that order of importance and either

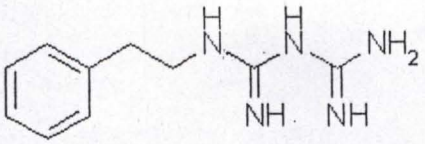
applied singly or in some cases, in combination remain the cornerstones to treatment of diabetes mellitus [18].

2.2 TREATMENT/MANAGEMENT TECHNIQUES OF DIABETES

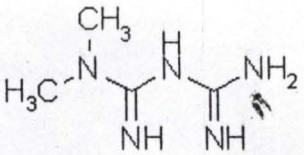
2.2.1 **Oral hypoglycemic drugs**

Oral hypoglycemic agents rely on residual insulin secretion for their action; therefore their use is almost exclusively restricted to type 2 diabetics. Two categories of drugs have been developed; biguanides and sulphonylureas [19]

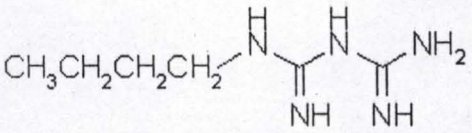
Fig 1: The structures of some common Biguanides



Phenformin
(Phenyl-ethyl biguanide)



Metformin
(1,1 - dimethyl - biguanide)

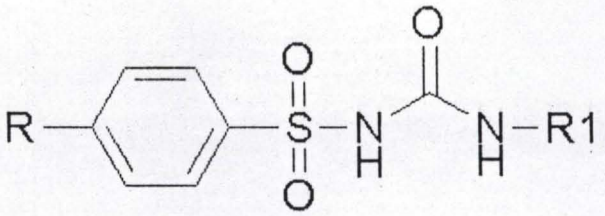


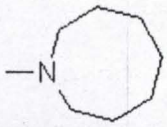
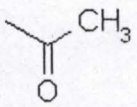
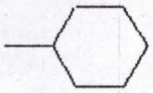
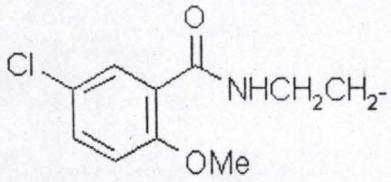
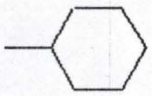
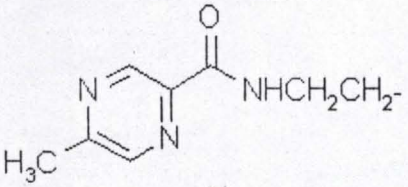
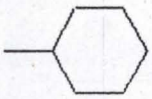
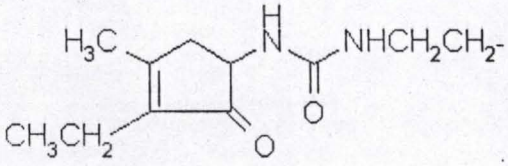
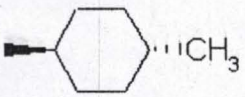
Buformin **(1 - butyl - biguanide)**

Biguanides are marketed either as phenformin or metformin. Phenformin has now been withdrawn from circulation in most countries because of the generation of side effects associated with severe and fatal lactic acidosis. Metformin reduces both fasting and postprandial blood glucose. It's precise mode of action is largely unknown, but seems to be associated with increase in peripheral uptake of glucose by muscle by possibly increasing insulin receptor binding [19].

Sulphonyureas are more common and are sold under different trade names, from the first generation types called tolbutamide, chlorpropamide to the latest types glibenclamide, glibornuride, glipizide, gliclazide and gliquidone. Their major effect is to stimulate insulin secretion, which can be demonstrated, following acute administration either intravenously or orally.

Fig 2: The structures of some common sulphonylureas



name	R	R1
Tolbutamide	$-CH_3$	$-CH_2CH_2CH_2CH_3$
Chlorpropamide	$-Cl$	$-CH_2CH_2CH_3$
Tolazamide	$-CH_3$	
Acetohaxamide		
Glyburide		
Glipizide		
Glimepiride		

The major adverse effects of sulphonylureas are however, hypoglycemia both profound and prolonged [18].

Until recently, insulin was extracted from either cow or pig pancreas. The resulting insulin differed from human insulin by two (2) and one (1) amino acid(s) respectively. The majority of insulin used for treatment of mostly juvenile onset diabetes and other forms of diabetes are now bioengineered using recombinant DNA technology. The proinsulin produced is thus cleaved to give insulin, which is identical to human insulin in its amino acid sequence [18]. The most successful of insulin used in treatment of diabetes were made into several formulations e.g. crystalline zinc, amorphous, crystalline mixed, protamine premixed and biphasic. Most of these formulations are taken by subcutaneous injection either using pen injector or a new advanced technique called continuous subcutaneous insulin infusion (CSII) where insulin is infused continuously via an in-dwelling subcutaneous cannula from a pump, which is worn or carried permanently by the patient. These techniques are however complex and need a lot of supervision [19].

As earlier discussed, the oral hypoglycemic agents and insulin treatment regimes generate a lot of side effects and are too expensive for the African diabetic. They are largely unavailable to the majority of Africans and where available, they are unaffordable. These reasons and many more have driven researches in Africa to other alternative sources for diabetic cure.

2.2.2 PLANT EXTRACTS

All over Africa and else where, increasing attention is being paid to extracts from plants either made into concoctions, decoctions or concentrated and made into powdery remedies and used as anti-diabetic agents as encouraged by WHO [20]. It is hoped that if a break-through is achieved in this research direction, a lot of African diabetics would be saved since approximately 80%of the people in the rural African communities still rely on the use of plant remedies to treat and/or manage diabetes mellitus and many other ailments. More over, most African countries are blessed with a rich flora comprising plants from different species, many of which have been used and are still in use for the treatment of many ailments either singly or in combination [24]. To cite some recent examples, Penchom *et al*, 1997 [21] in Thailand evaluated the hypoglycemic effects of water extract of the whole plant of *Piper sarmentosun roxb.* on normal and streptozotocin-diabetic rats. 0.125 and 0.25g/kg body weight dose of the water extract significantly lowered the plasma glucose levels in the normal rats in single dose application. Also, a reference drug, glibenclamide at a dose of 5mg/kg, for 7 days produced a significant hypoglycemic effect in normal rats, however, a single dose oral administration of the water extract and reference drug individually did not significantly lower plasma glucose level in the streptozotocin-diabetic rats. Repeated oral administration of

the extract at a dose of 0.125g/kg for 7days however, produced a significant effect.

In Iraq, Jamal *et al*, 1997 [22] evaluated the hypoglycemic and anti-hyperglycemic effects of *Trigonella foenum-graecum* leaf in normal and alloxan-induced diabetic rats. Graded amounts of the water and alcoholic extracts of the leaf of the plants were tested for hypoglycemic activity in normal and diabetic rats. The leaf of the plant extracts was discovered to be both nontoxic and possesses hypoglycemic activities, confirming the success of the use of this traditional plant for the management of diabetes in the Middle East.

Kathryn Janis Sullivan, 2000 [23], worked on the effect of C*occina indica,* a relatively abundant weed in wastelands of Africa and Asia, on blood glucose levels in alloxan-induced diabetic mice, compared to a test drug tolbutamide and concluded that the water extract of the plant significantly lowered the blood glucose of the diabetic rats just like the reference drug.

Africans too are not left out of the researches in this direction. S'bahle *et al* in South Africa, 2000 [24] evaluated the hypoglycemic effects of methanol extract of *Hypoxis hemerocallidea* corm (African potato) in rats. Graded doses of the African potato methanol extracts were administered to rats and their blood glucose levels were monitored compared to a test drug glibenclamide. Although less potent than

glibenclamide, the extracts of African potato have been proved to have anti-diabetic properties, and are non-toxic.

2.2.3 MANAGEMENT 0F DIABETES WITH EXTRACTS FROM GRAINS AND LEGUMES

Groundnut

Here in Nigeria, Bilbis *et al*, 2002 [25] have demonstrated the hypoglycemic and hypolipidemic effects of aqueous extracts of *Arachis hypogea* (groundnuts) in normal and Alloxan-induced diabetic rats. Extracts from groundnut soaked overnight, when administered to normal and diabetic rats, significantly reduced their fasting blood glucose, lending credence to the use of groundnuts (whole) or its extracts for anti-diabetic therapy.

In food research institute Ghana in 1994, Nana Tekyiwa and Wisdom A. plahar [28] developed and made a quality evaluation of Soy-fortified Ghanaian weaning food from locally available raw material and it compared favorably with a popular commercial weaning food, CERELAC from Nestle.

Ekpeyong *et al*, 1977 [26], fortified maize floor based diets with blends of cashew nut meal, African Locust bean and sesame oil meal with high degree of success. Bressani *et al*, 1966 [27] have formulated an all-

vegetable protein mixture for human feeding and it was discovered to be nutritionally sound.

Soya beans

The soybean (*Glycine max*.) is a member of the family leguminosea. There are ten species and several thousand varieties. The word Soy/Soya/soja is derived from the Japanese word ***Shoyu*** ***(Sho***=salted beans, yu=oil) meaning soy sauce. It is a summer annual plant that reaches a mature height of between 60-130cm. The soybean seeds are contained enclosed in pods that grow in clusters of 3-5, with each pod usually containing 1-4 seeds. Most commercial varieties have 2 or 3 seeds per pod. The seed coat of most varieties is yellow, however varieties of soybean native to Asia also have green, brown and other combination of colored seed coats.

Growing time from planting to maturity range from 90-180days depending on the variety and environmental conditions. These conditions also affect the yield of the plant. Average yield in the USA is put at 2tonnes per hectare (Ha.) while in Zimbabwe, yields could be as low as 0.65tons/Ha. The world's record of soybean yield is 7.9tons/Ha. Produced at Rudgers University, USA in 1983.

The soybeans are a major source of vegetable proteins and oil for humans and animals consumption. Depending on variety, the soybeans contain about 40% protein and 20% oil. The main food products are

soysauce, bean sprouts, cooking oil, soybean flour/concentrate/isolates, soybean milk, soybean curd (*Tofu, tempeh and miso in Japanese).* In East Asia, soy foods are a regular part of the human diet as a source of protein and oil.

Soybean is Asiatic in origin, linguistic, literary and genetic evidence seem to indicate that the soybean emerged as a domesticated plant in the Eastern part of North China in the early Chou dynasty, about 11century BC. There seem to have been no interest in the production of soybean for commercial purpose outside East Asia until early 19th century. It is now cultivated around the world between latitude 50 degrees North and 50 degrees south. By 1986, the world's production of soybeans is estimated to be about 95million metric tones, including Africa, [29] by 2004 the world production of Soya beans had risen to 229million metric tones [41]

Maize

Maize (*Zea Mays L)*, or corn is the most important cereal crop in sub-Saharan Africa and, with rice and wheat, one of the three most important cereal crops in the world. In developed countries, maize is used mainly as livestock feed and as a raw material for industrial products while it is for human consumption in low-income countries. In Sub-Saharan Africa, it serves as staple food for an estimated 50% of the population. It serves as an important source of carbohydrate, Iron,

vitamin B and minerals. Africans consume maize as a starchy base in a wide variety of porridges, pastes, grits and beer. Green maize (fresh on the cob) is eaten parched, baked, roasted or boiled and serves to fill the hunger gap after the dry season.

According to Food and Agricultural organization, FAO data, 589million tones of maize were produced worldwide in the year 2000 on 138 Ha. The United States of America, USA was the largest maize producer (43% of world production) followed by Asia (25%) and Latin America and Caribbean (13%). Africa produced 7% of world's maize. The world's average yield in 2000 was 4255Kg/Ha. The average yield in the USA was 8600Kg/Ha while Sub-Saharan Africa was 1316Kg/Ha.

Through out the Tropics and sub-tropics, small-scale farmers grow most maize, generally for subsistence as part of agricultural systems that feature several crops and sometimes livestock production. The system often lacks inputs such as fertilizer, improved seeds, irrigation and labor. However, most maize producing developed countries employ intensive input and highly mechanized monocropping production systems. Hybrid maize varieties are commonly used. [30]

In Africa, where maize is commonly used for human consumption, dietary preferences, processing and mode of preparation affect the contribution of maize in human nutrition. Nutritional factors, although

important in determining quality, are often overlooked since no premium in the market for maize varieties with superior nutritional value.

Sorghum & millet

Sorghum (or guinea-corn) and millets have been important staples in the semi-arid tropics of Asia and Africa for centuries. These crops are still the principal sources of energy, protein, vitamins and minerals for millions of the poorest people in these regions.

Sorghum and millets are grown in harsh environments where other crops grow or yield poorly. They are grown with limited water resources and usually without application of any fertilizers or other inputs by a multitude of smallholder farmers in many countries. Therefore, because they are mostly consumed by disadvantaged groups, they are often referred to as "coarse grain" or "poor people's crops". They are not usually traded in the international markets or even in local markets in many countries. The farmers seldom have an assured market in the event of surplus production.

No one legume or cereal can provide adequate amounts of all nutrients to meet the nutritional requirements of an individual. However, even before knowledge on protein content, protein quality, digestibility and the nutrient requirements of humans became available, it was recognized that mixing legumes with cereals in the diet could improve

overall nutrition. The present and newly derived knowledge in these areas makes it possible to blend, mix or fortify one food material with others so that the resulting fortified mix has not only better nutritional quality but also the necessary attributes for consumer acceptance.

The nutritional quality of sorghum and millets, especially the former, is poor. The sorghum bran is low in protein and ash and rich in fiber components. The germ fraction in sorghum is rich in ash, protein and oil but very poor in starch. Over 68 percent of the total mineral matter and 75 percent of the oil of the whole kernel is located in the germ fraction. Its contribution to the kernel protein is only 15 percent. Sorghum germ is also rich in B-complex vitamins. Endosperm, the largest part of the kernel, is relatively poor in mineral matter, ash and oil content. It is, however, a major contributor to the kernel's protein (80% of kernels protein), starch (94% of kernel's starch) and B-complex vitamins (50-75% of kernel's B-complex vitamins). [40]

Therefore attempts have been made to fortify these cereals with legumes or other cereals to make them nutritionally superior and acceptable products. Cost, availability of ingredients and marketability must be taken into consideration if fortification is to be implemented successfully on a sustained basis. [40]

Sorghum and pearl millet have been successfully used in feeding programs after fortification with legumes. Vimala, Kaur and Hymavati

(1990) [40] described various infant mixes based on sorghum and pearl millet and fortified with soybean, green gram, red gram or Bengal gram flour, as they are called in India. They were evaluated through rat feeding trials and nitrogen balance studies in children.

It is possible to fortify malted finger millet (rug)) weaning food with green grams. The food has the advantage of having low cooked paste viscosity and has high energy density when mixed in the proportion of 70 percent malted ragi flour and 30 percent green gram flour. The NPU of this food was observed to be 52 percent and was comparable to that of a commercially available weaning food according to Malleshi, Desikachar and Venkat Rao (1986). [40]

Okeiyi and Futrell (1983) [40] evaluated the protein quality of various combinations of sorghum with cereals and legumes. These included (dehulled) sorghum, wheat and soy flours; sorghum, wheat, cowpeas and soy flours; sorghum, wheat and cowpeas flours plus peanut butter; sorghum and wheat flours plus peanut butter; and sorghum, wheat and soy flours plus peanut butter. A diet of sorghum, wheat and soy flours met the FAO recommendations for required amino acids. Over 25 percent of the energy of this diet was provided by fat and 10 percent of the energy was provided by protein as recommended by the United Nations Protein Advisory Group for the formulation of high-protein foods

for children. The diet had the same high protein efficiency ratio (PER) as casein.

Brookwalter, Warner and Anderson (1977) [40] evaluated the stability of sorghum fortified with soy and cottonseed flour in different proportions. The various formulations were stored at -18°C (control), 49°C for two months, 37°C for six months and 25°C for 12 months. All combinations displayed adequate stability as measured by change in available lysine, acidity and flavor. The flavor of all blends was acceptable.

In Burundi, sorghum fortified with maize and soy flours, locally known as musalac, has been used as a baby and adult food. It has the following composition: 35 percent sorghum flour, 30 percent maize flour, 20 percent soybean flour, 10 percent sugar and 5 percent milk powder. This combination has about 16 percent protein, with 3.76 percent of protein contributed by lysine and 440 kcal per 100 g of product. Musalac is very popular; 60 tonnes/month were sold commercially in 1989, and production is expected to reach 9 000 tones by the year 2000.

Sorghum and millets will continue to be major food crops in several countries, especially in Africa (and in particular in Nigeria and the Sudan, which together account for about 63 percent of Africa's sorghum production). These grains will be used for traditional as well as novel foods. However, there is a need to look into the possibilities of alternative

uses. Though sorghum and millets have good potential for industrial uses, they have to compete with wheat, rice and maize. Sorghum in particular could be in great demand in the future if the technology for specific industrial end uses is developed. Although pearl millet has some potential for industrial use, other millets have limited potential because of their small grain size and the associated difficulties of adopting a suitable dehulling technology. However, they can be considered for animal and poultry feed. There is a need to compare their performance as feed in comparison with maize. [40]

Plantains

Plantains and a wide range of other dessert banana varieties provide a staple food for millions of people in the developing countries of the tropics, while exported bananas are ranked as the world's most valuable fruit crop. Humans have been gaining nutrients from Bananas and plantains for several thousands of years. For the last hundred years, it has been one of the few food crops to be enjoyed globally, on the tables of families, from the highest-income to the lowest in all parts of the world. Its texture, taste, its convenience, ease to eat and low cost to grow have all contributed to this success. The energy value of food is derived from its carbohydrate, fat and protein content. In the case of Plantain and especially for the purpose for which it is intended for use in this work, the carbohydrate fraction is far the most important. Sugars and starches that

make up this fraction are present in varying concentrations according to the fruit ripeness. In unripe plantains, starch comprises over 80% of the dry weight of the pulp. The two main components of the starch are amylose and amylopectin in the ratio 1:5. Major changes usually occur during the process of ripening, especially the conversion of these starches to sugars, raising the sugar content to as high as 17% of the dry matter weight from as low as 1.3% when unripe. [38] It is for this reason that the unripe fruit was considered for this study. Moreover, certain compounds in bananas and plantains behave like angiotensin-converting enzyme (ACE) inhibitors. ACEs govern the release of angiotensin-2, a substance that has the effect of causing a rise in blood pressure through the constriction of blood vessels. Indian researchers have reported the ACE-inhibiting properties in six varieties of bananas confirming earlier studies in USA suggesting that potassium rich foods like bananas and its species help reduce blood pressure. In the Indian experiments, 2 bananas per day resulted in a drop of up to 10% in blood pressure within a week. [39]As earlier mentioned, high blood pressure is one of the complications of diabetics of whatever kind, and any therapeutic drug or food item that would ultimately reduce this symptom is highly recommendable.

FOOD FORMULATION

Human beings require certain nutrients in their diet for maintenance of good general health, repair and replacement of dead tissues and for management of disease conditions. Since animals cannot produce energy through photosynthesis, or take up nutrients from the soil, they must rely ultimately on plant sources to provide essential nutrients. The minimum requirements must be met for six groups of nutrients: -

i. Carbohydrates

ii. Lipids (fats and oils)

iii. Proteins

iv. Vitamins

v. Minerals

vi. Water

Digestion and metabolism of carbohydrates, lipids and protein provide the basic energy needs. The body stores energy in fats, which can be utilized when calorie intake is insufficient to meet demand. [31]

These nutrients are found in nature either in individual sources in large quantity or combined with other nutrients in ratios that are not proportionate to satisfy the human body needs. In such situations, mixing the nutrient sources during food preparation serve to provide the caloric needs and in case of metabolic disorders like diabetes, to manage the

disease condition such that symptoms are alleviated and complications are averted.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 **Experimental diets** (Purchased from cereal and fruit hawkers in Minna market, Nigeria).

-Unripe plantain

-Soya beans

-Sorghum (Guinea-corn)

3.1.2 Chemicals (Analytical grade)

-Sodium sulphide, Sodium hydroxide (BDH Chemicals Ltd, Poole England).

-Boric acid, Hydrochloric acid (conc.), Ethyl alcohol, Hexane, Sulphuric acid (conc. 98%), Kjedahl catalyst(Oxoid Ltd, Basingstoke, Hamphire, England).

3.1.3 Agar (Oxoid Ltd, Basingstoke, Hamphire, England)

-Nutrient agar (NA)

-MacConkey agar (MCA)

-Saboriend dextrose agar (SDA)

steamed Soya beans grains were transferred into a drying tray and dried just below 100°C for about 4 hours. Using the dry milling machine, the dried Soya beans grains were milled as fine as possible and allowed to cool to room temperature. The milled grains were then sieved using the flour sieve to separate out the fine powder, which was then packaged and labeled instant Soya powder (ISP).[32]

3.2.2 **Production of Plantain powder**

The digester unit was also set up with washed and sliced unripe Plantain as in 3.2.1a and b above. The system was also evacuated and steam was let in and allowed to run for about 60minutes. The steamed sliced plantain was then transferred into a drying tray and oven dried just below 100°C for 4 hours to make plantains chips. The chips were then milled into fine powder as much as possible. The milled powder was sieved, packaged and labeled Plantain Powder. [32]

3.2.3 **Production of Sorghum powder**

The digester unit was also set up with exact quantity of cleaned sorghum as in 3.2.2a and b above. The system was evacuated and the steam let in and allowed to run for about 60minutes. The steamed sorghum grains were then oven dried just below 100°C for about 4 hours, milled as finely as possible and then sieved. The sieved fine sorghum was then packaged and labeled sorghum powder. [32]

3.2.4 Formulation of Soy-Fortified Diabetic diet

Using a stepwise calculation procedure for blend formulation, [33] Soy-fortified Diabetic Diet (SDD) was prepared to contain 40% ISP, 30% plantain powder and 30% sorghum powder on %weight basis. Maximum complementation of amino acids and protein content of about 20% was targeted, to satisfy the minimum protein content of 15% required under the FAO/World Health Organization (WHO) Codex Alimentarius Standards, and to meet the recommendations of the United Nations Protein Advisory Group (PAG) for the formulation of high-protein foods [34]. The final blend formulation, SDD was stored in aseptic waterproof, polythene bags.

3.2.5 Determination of Moisture content

Samples of the SDD and the raw ingredients before blend formulation were analyzed for their moisture content as follows;
With the aid of a weighing balance, tarred, porcelain dishes were weighed and their weight recorded (W1). 5grammes each of either the raw samples or SDD was measured into the porcelain dish and their weights recorded (W2). The porcelain bearing the samples was then transferred into an oven maintained at between 80-100°C for about 24 hours. After this period, the porcelain bearing the samples was then transferred into a

3.1.4 Experimental animals (From the animal facility, National Pharmaceutical research Institute and development, Iddu-Abuja)

-Weanling albino rats (adult, Healthy)

3.1.5 Apparatus, Kits & other laboratory equipments

Weighing balance, Beakers, Test tubes, Porcelain dishes (tarred), Heating Mantle, Desiccators, Filter paper (Whatman), Spatula, Soxhlet Extraction apparatus, Kjedahl apparatus, Pumice chips, Fume chamber, Measuring cylinder (graduated), Buckner funnel, Burette, Suction pump, Spectrophotometer, Petri- dishes, Autoclave, One-Touch Glucometer® kit (All diabetic, 2255 Glades Rd, Suite 324A Boca Raton, Fl.33431. USA), Theatre scissors, Flour sieve, Drying tray, Soya digester (Laboratory apparatus, FUT. Minna-Nigeria)

3.2 METHODS

3.2.1 Production of Instant Soya Powder (ISP)

The Soya digester was assembled and heated until the system began to steam freely. Exact quantity of Local Soya beans was washed, cleaned and transferred into the digester. The digester system was evacuated and steam was let in and left to run for about 90minutes, to remove most of the anti-nutritional agents. After the time period, the

desiccators to cool, and then weighed again (W3). The moisture content was then calculated using the formula below: -

$$\text{Moisture content (\%)} = \frac{(W2 - W1) - (W3 - W1)}{W2 - W1} \times 100 \quad [35]$$

The experiment was performed in triplicates for each of the samples determined and the mean of the three results was taken, as recorded in Appendix 1 and shown on *fig. 1*

3.2.6 **Determination of Ash content**

With the aid of a weighing balance, tarred porcelain dishes were weighed and their weights recorded (W1). Gradually, 5grammes each of either the raw sample or SDD was measured into the porcelain dishes and the dishes were weighed again (W2). The porcelain dish bearing the sample was then placed on top of a heating mantle, inside a fume chamber, set to about 250°C, to partially burn off the organic matter until the sample stopped smoking. The dish was then transferred into an oven set at 600°C for 6hours for the organic matter to complete combustion. After this time, the porcelain dish bearing the sample was then transferred into a desiccator to cool, and weighed again (W3). The Ash content of the samples was calculated using the formula below: -

$$\text{Ash content (\%)} = \frac{W3 - W1}{W2 - W1} \times 100 \qquad [35]$$

The Ash content of the samples was determined in triplicates and the mean of the determination was taken, as recorded in Appendix 1 and *fig. 4*

3.2.7 **Determination of Crude Fat Content**

With the aid of a weighing balance, filter paper was weighed and the weight recorded (W1). Using a spatula, 5grammes of the samples each was measured unto the filter paper and the filter paper bearing the sample was weighed again and recorded (W2). The filter paper was then folded to hold the sample intact and stapled. The stapled filter paper bearing the sample was then dropped into a round bottom flask on the Soxhlet Extraction apparatus. Beneath the extraction thimble, a dry tarred solvent flask was placed into which 300mls of hexane were poured. The apparatus was fitted to a condenser set connected to a tap to supply water. The heating temperature of the apparatus was adjusted to 60°C to give a condensation rate of about 10-12 drops per second and extraction was continued for about 4hours. After this time, the extraction was discontinued, the apparatus dismantled and the stapled filter paper bearing the remains of the sample was then put into an oven maintained at 80°C to dry off the remaining hexane, then dropped into a desicator to

cool and then weighed again and the weight recorded (W3). The Crude Fat content was then calculated using the formula below: -

$$\text{Crude Fat Content (\%)} = \frac{W3 - W1}{W2 - W1} \times 100 \qquad [35]$$

The experiment was performed in triplicates for all the samples and the mean of each was taken, as recorded in Appendix 1 and *fig. 3*

3.2.8 **Determination of Crude Protein Content**

With the aid of a weighing balance, 1gramme of each of the samples was weighed into a digestion flask and 1 tablet of Kjedahl catalyst was added. 20mls of concentrated Sulphuric acid (98%) with some pumice chips also added to reduce bumping. The digestion flask was placed in a fume chamber and was heated gently at first, then vigorously until the solution became very clear in the Kjedahl apparatus inside a fume chamber. When the solution became clear, heating was stopped and the solution allowed to cool. On cooling, about 90mls of distilled water was added, then 25mls of sodium sulphide solution (4%) and mixed thoroughly. 80mls of Sodium hydroxide solution (40%) were also added while tilting the flask such that two layers of solutions formed. This was then rapidly connected to the condenser unit of the apparatus,

heated to about 100°C and the distilled ammonia released was collected in 50mls of boric acid/indicator solution. 50mls of distillate were then collected. On completion of distillation, the receiver was removed and titrated against hydrochloric acid standard solution (0.1N). The amount of crude protein in each of the samples was calculated from the Nitrogen content (**N**) using the formula below: -

$$\text{N Content (\%)} = \frac{\text{ml acid} \times \text{Normality of acid} \times 0.014 \times 100}{\text{Wt. Of sample (g)} \quad 1}$$

Crude protein content (%) = Nitrogen content X 6.25 [36]

The experiment was carried out in triplicates for each of the samples and the mean of the three were taken, as recorded in Appendix 1 and *fig. 2*

N.B: - 0.014 is the atomic mass of Nitrogen while 6.25 is the correction factor to convert amount of Nitrogen to crude Protein.

3.2.9 **Determination of Crude Fiber Content**

With the aid of a weighing balance 2grammes of the dried, fat free sample was measured into a 600mls-beaker (W1). 200mls of hot sulphuric acid were added; the beaker was brought under the condenser and brought to boiling within a minute. This was boiled gently for about 30minutes, using distilled water to maintain the volume and wash the residues adhering to the sides of the beaker. This was then filtered with

Whatman No. 541 paper in a Buckner funnel using suction and washing well with boiling water. The residue was transferred back to the beaker and 200mls of hot sodium hydroxide solution were added. This was replaced under the condenser and boiled for 30 minutes. After this period, the residue was filtered through porous crucible and washed with boiling water, 1-% hydrochloric acid and again boiling water. It was washed twice with Ethyl alcohol and dried overnight in an oven maintained at 100°C. This was cooled and weighed (W2). The residue was then ashed at 500°C for 3hours. The residue was then cooled in a desicator and weighed again and recorded (W3). The Fiber content of the samples was determined using the formula below: -

$$\text{Crude Fiber content (\%)} = \frac{W2 - W3}{W1} \times 100 \qquad [35]$$

The experiment was performed in triplicates and the mean of the three results was taken, as recorded in Appendix 1 and *fig. 6*

3.2.10 Determination of Carbohydrate content (Plus nucleic acids)

The content of Carbohydrates can be determined by the difference after obtaining the values of organic matter, proteins and lipids as follows: -

Amount of protein = a%

Amount of Lipid = b%

Protein + Lipid = (a + b) %

Organic matter = c%

Therefore, Carbohydrate and Nucleic acid = c – (a + b)%

The result is as recorded in Appendix 1 and *fig. 5*

3.2.11 Energy Value (Caloric Value)

The energy value of a food is usually expressed in calories. A calorie is defined as the amount of heat required to raise the temperature of 1liter of water by 1°C. In nutrition, kilocalories are commonly used, which refer to 1000 calories and refers to the total calories of energy that the food preparation contributes to the body when it is consumed. The amount of calories of energy released when 1 gram of the food is combusted in a Bomb or Adiabatic calorimeter is termed the energy value of the food. Using this method, it has been shown that burning 1 gram of carbohydrates or 1gram of protein, 4kilocalories of energy is produced while burning 1gram of fat yields 9kilocalories of energy respectively. These values are known as *Atwater* factor and are useful in estimation of the energy values of food after chemical analysis of food. Thus by this

process of physical scoring, the energy value of SDD and the raw materials is as calculated and recorded in Appendix 1 and *fig. 7*

3.2.12 Minerals content (Calcium and Phosphorous)

Calcium: -

With the aid of a weighing balance, 2.5g of the samples were weighed into a porcelain dish and ashed (as in ash content determination). 40mls of hydrochloric acid and a few drops of nitric acid (70%) were added and boiled. The mixture was then cooled and transferred to a 250mls volumetric flask, diluted to the volume with distilled water and mixed thoroughly.100mls aliquots of the solution was pippeted out into a beaker and 2 drops of methyl red indicator (1g in 200mls alcohol) was added. Ammonium hydroxide was then added dropwise until a brownish orange coloration was obtained, then 2 drops of hydrochloric acid was added to give pink color. This was then diluted with 50mls of distilled water, boiled and while stirring 10mls of 4.2% ammonium oxalate was also added. The pH was adjusted to bring back the pink color with hydrochloric acid. A precipitate formed which settled and was filtered out with filter paper while washing it with ammonium hydroxide solution (about 25mls). The filter paper bearing the precipitate was placed in a beaker and a mixture of 125mls of water and 5mls of sulfuric acid (98%) were added, heated to 70°C and then titrated against a standard potassium

permanganate solution (0.05N). The amount of Calcium in the sample was calculated using the formula below: -

$$\text{Calcium (\%)} = \frac{\text{ml permanganate soln}}{\text{Wt of sample}} \times \frac{\text{aliquot used (ml)}}{250} \times 0.4 \quad [37]$$

The results are as summarized and recorded in Appendix 1 and *fig. 8*

Phosphorous: -

Aliquot of the sample solution from the Calcium determination was pippeted out into a 100mls volumetric flask and 20mls of molybdovanadate reagent were added and made to the volume with distilled water, mixed and allowed to stand for 10minutes. The aliquots of the working standard containing 0.5, 0.8, 1.0 and 1.5mg phosphorous was transferred into 100mls flasks each and treated like the test sample above. The samples were read in a spectrophotometer at 400nm, setting the 0.5mg standard at 100% transmission. Milligram phosphorous in each of the samples was determined from the standard curve as recorded in Appendix 1 and *fig. 9* [37]

3.2.13 Microbial load determination

To test that the diet preparation procedure was asceptic, the total microbial load of the dietary preparation was undertaken as follows: -
Three different types of media were used and these are Nutrient agar (NA) for total viable count, MacConkey agar (MCA) for total coliform

count and Saborend dextrose agar (SDA) for total fungal count (yeast and moulds). These media are commercially available in readily prepared form, they are only purchased, weighed out according to standard practices, dissolved in distilled water and sterilized using autoclaves.

Serial dilution: 9.0mls of distilled water was dispensed each in 6 clean test tubes and corked. These were then sterilized in an autoclave at 121 degrees Celsius for 15 minutes, after which they were allowed to cool. 1g of the sample was then dissolved in number one test tube aseptically; disposable sterile syringes and needles were used for the serial dilution from test tube number one to six, taking 1ml of solution from tube one into tube two etc until the process was completed, after mixing thoroughly.

After the serial dilution, 1ml each of the solutions of the 10^{-3}, 10^{-4}, 10^{-5} dilution test tubes were plated out in 3 separate Petri dishes each containing NA, MCA and SDA respectively. This was then incubated at 37°C for 24-48 hours for bacteria and coli forms while that for SDA was incubated at room temperature for 72 hours.

After incubation, the macro-culture was observed. For bacteria, gram staining was also performed to isolate and identify the specific microorganism found in the sample. All work was performed under aseptic conditions. The results of microbial counts are as recorded in Table 1.

3.2.13.1 Preparation of Nutrient Agar (NA)

For NA, 28g of the powdered agar was weighed out using a weighing balance and dissolved in 1 liter of distilled water in a volumetric flask. This was sterilized in an autoclave at 121°C for 15 minutes (moist heat sterilization). This was then poured into Petri dishes to cool and set.

3.2.13.2 Preparation of MacConkey Agar (MCA)

For MCA, 56g of the MCA powder was weighed out into 1liter of distilled water and the same procedure as above were repeated.

3.2.13.3 Preparation of Sobrend Agar (SDA)

For SDA, 62g of the SDA powder were weighed out into 1 liter of distilled water. 0.5g of chloranphenicol injection powder was also added and the same procedure for NA was also repeated. The addition of chloranphenicol was to inhibit bacterial growth since the process was for the determination of fungus. The choice of chloranphenicol was because of its heat resistance.

All the petri dishes were sterilized at 160°C for 1 hour using an oven (dry heat sterilization). This was to kill all the microorganisms present in them so that only the ones in the sample would be evaluated.

3.2.14 Glucose determination

For the blood glucose measurements, blood was obtained from the tail of each of the rats using a theatre scissors to clip the tail (tail-clip method) and blood was then dripped unto the glucose test strip paper of the Glucometer® kit, which was then inserted into meter and the results then read electronically as recorded in Appendix 3 and 4 and *figs. 11 and 12*

3.2.15 Animal studies

Adult albino rats (*Rattus norvegicus*), obtained from the Animal facility of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja-Nigeria were randomly divided into two (2) groups; the random sugar group (RSG) and the fasting sugar group (FSG).

All the albino rats in the 2 groups were fed on regular laboratory rat feeds for 7 days. This was to quarantine and acclimatize them to the laboratory conditions and to ascertain their suitability for the experiment and record their basal weights and blood glucose levels. After quarantine, their weights were taken and recorded as basal weight, also by means of a One-Touch Glucometer® the basal blood glucose level of all the rats was taken and recorded.

All the rats in the 2 groups were then progressively fed the formulated SDD *ad libitum* with free access to clean drinking water for 21days (3 weeks). The weights and blood glucose levels (whether random

or fasting) of the rats were monitored and recorded on weekly (7 days) basis. The rats in the FSG group were normally fasted for 14hours (overnight) prior to their blood glucose and body weight measurements.

At the end of the test period, the results obtained were analyzed statistically, using paired T-test for the standard error, mean and standard deviation to determine the significant variation as recorded in Appendix 3 and 4

CHAPTER 4

4.0 RESULTS, DISCUSSION AND CONCLUSSION

4.1 RESULTS

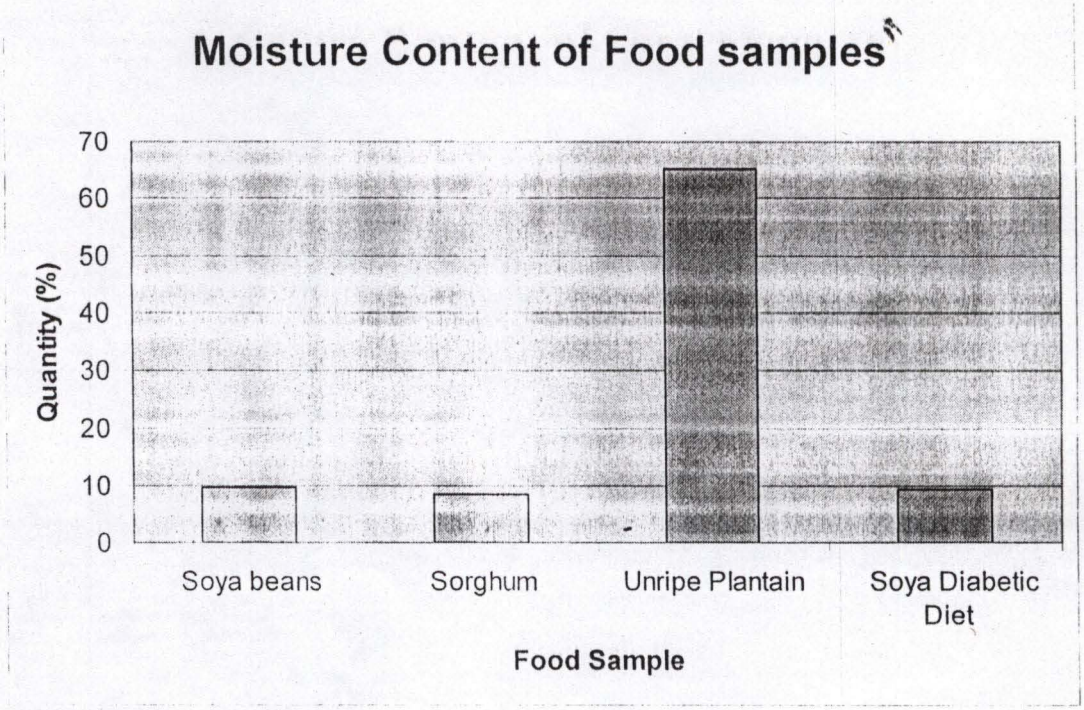
1.The results of the Moisture, Protein, Fat, Carbohydrate, Ash, crude fiber contents and Energy Values of the raw food samples used in the formulation of the Soya diabetic diet (SDD) and the Chemical composition of the SDD are given: (*See figures 1-9*)

From the figures shown, for the moisture content, unripe plantain has the highest content of 65.3% followed by Soya Beans with 9.7%, then SDD with 9.4%, then Sorghum is the least with 8.6%. As for the protein content, Soya beans has the highest content of 36.6% while unripe plantain has the least content of 1.3%.

Soya beans also has high content of fat of 19.5% but lower than SDD which has 26.2% with unripe plantain recording the lowest content of 0.4%. Percentage Ash content is higher in Soya beans with 5.6% while unripe plantain has the lowest of 1.1%. Sorghum has the highest content of carbohydrates of 73.8% while Soya beans have the lowest value of 28.6%. Energy value in kilocalories is higher in SDD with 522kcal/g while unripe plantain has 136kcal/g as the lowest value.

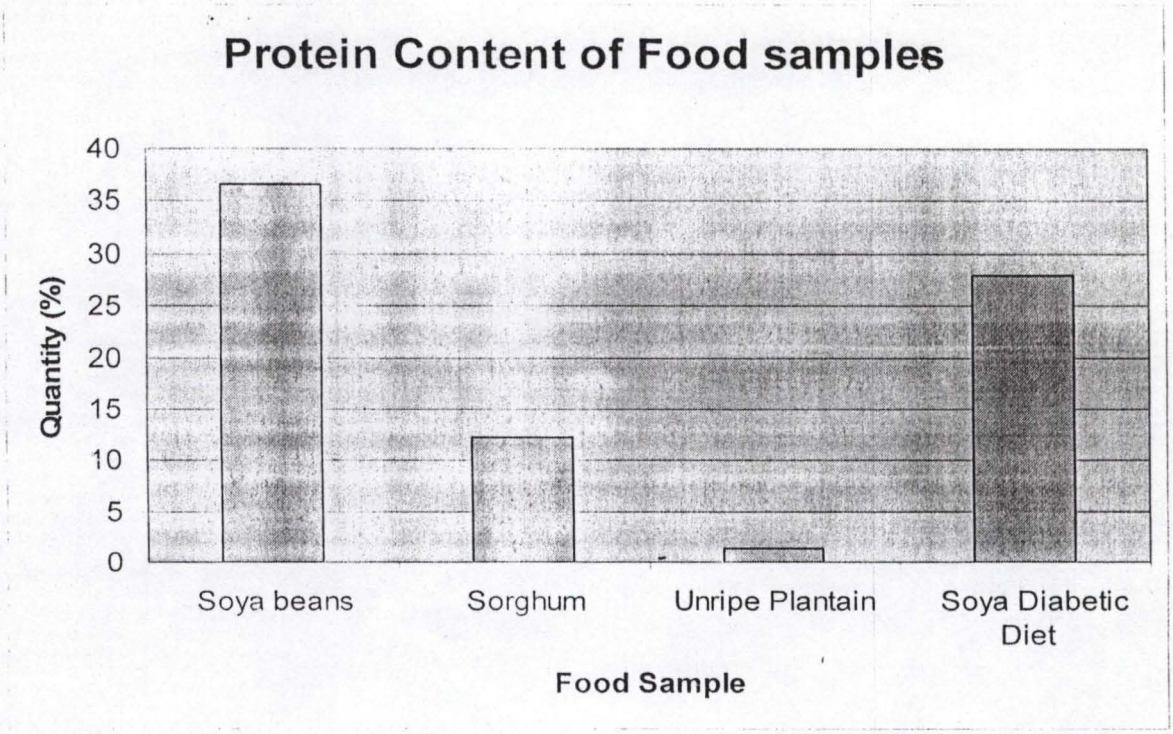
Both Calcium and Phosphorous values are highest in Soya beans and lowest in Sorghum while crude fiber is more in SDD with a value of 14.1%

Fig1: Moisture Content of Soya beans, Sorghum, Unripe Plantain and Soya Diabetic Diet



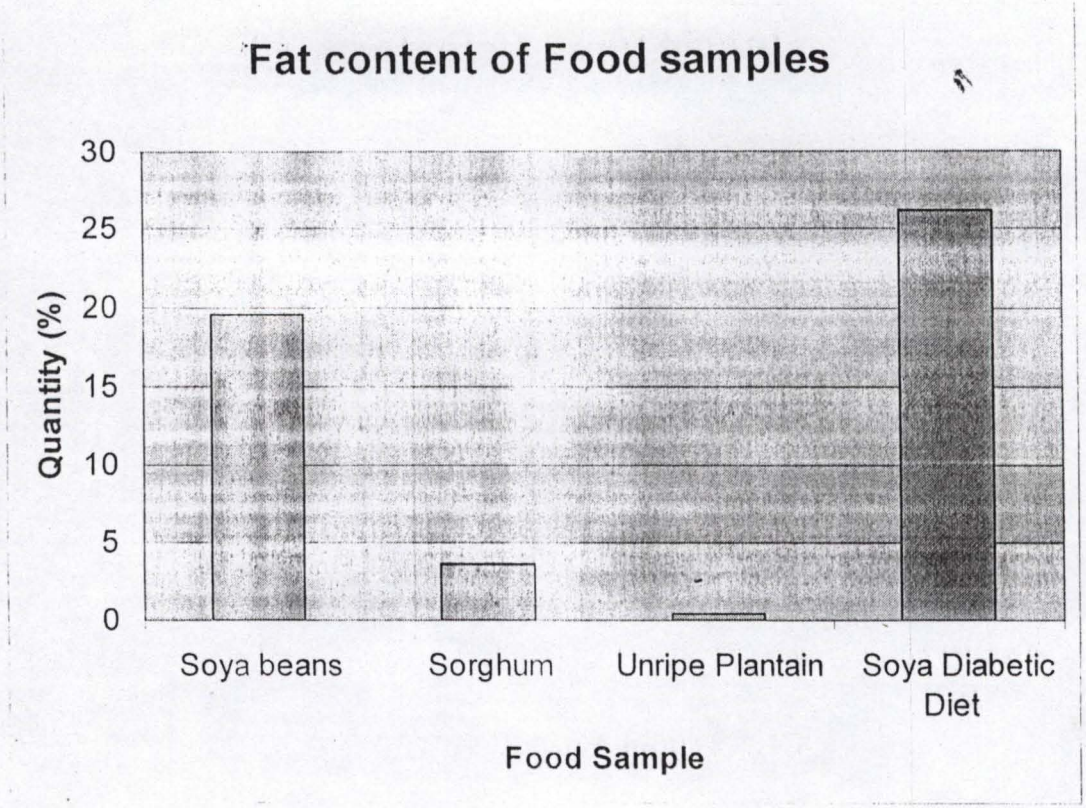
See Appendix 1 for details

Fig. 2: Protein Content of Soya beans, Sorghum, Unripe Plantain and Soya Diabetic Diet



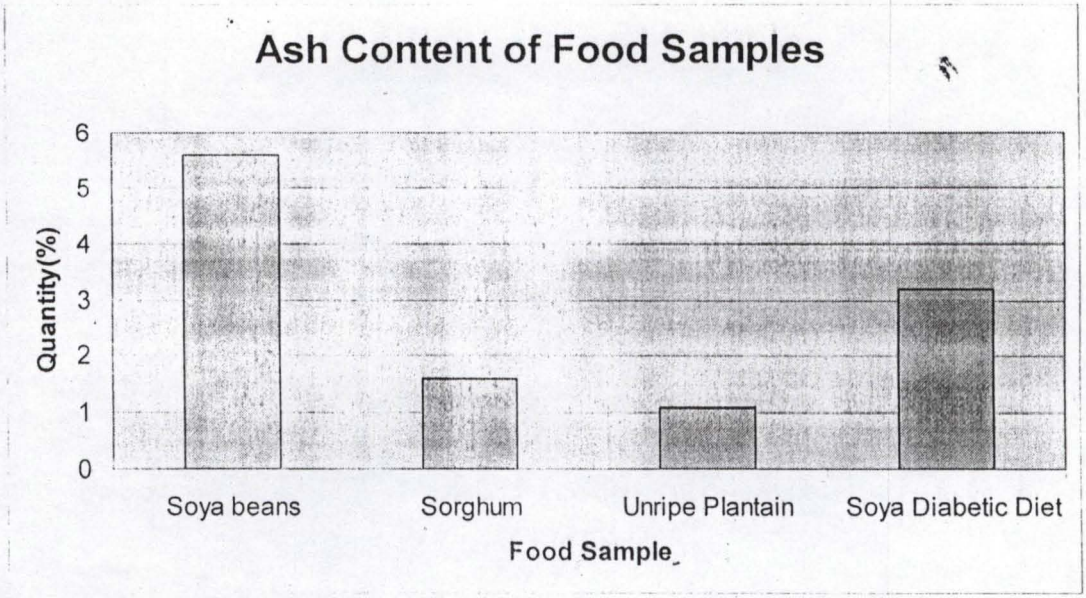
See Appendix 1 for details

Fig 3: Fat Content of Soya beans, Sorghum, Unripe Plantain and Soya Diabetic Diet



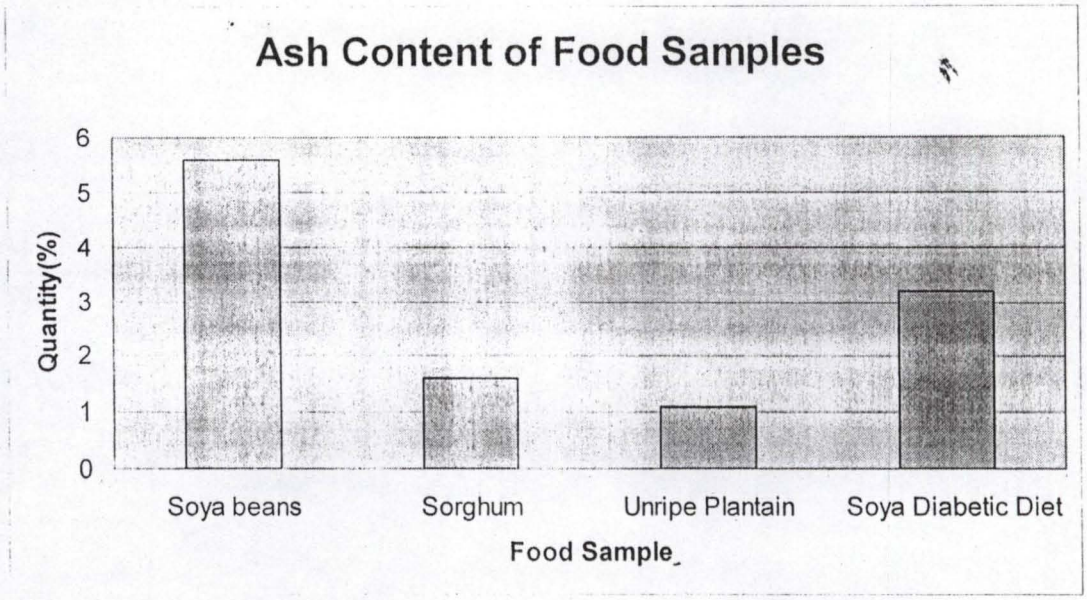
See Appendix 1 for details

Fig 4: Ash Content of Soya beans, Sorghum, Unripe Plantain and Soya Diabetic Diet



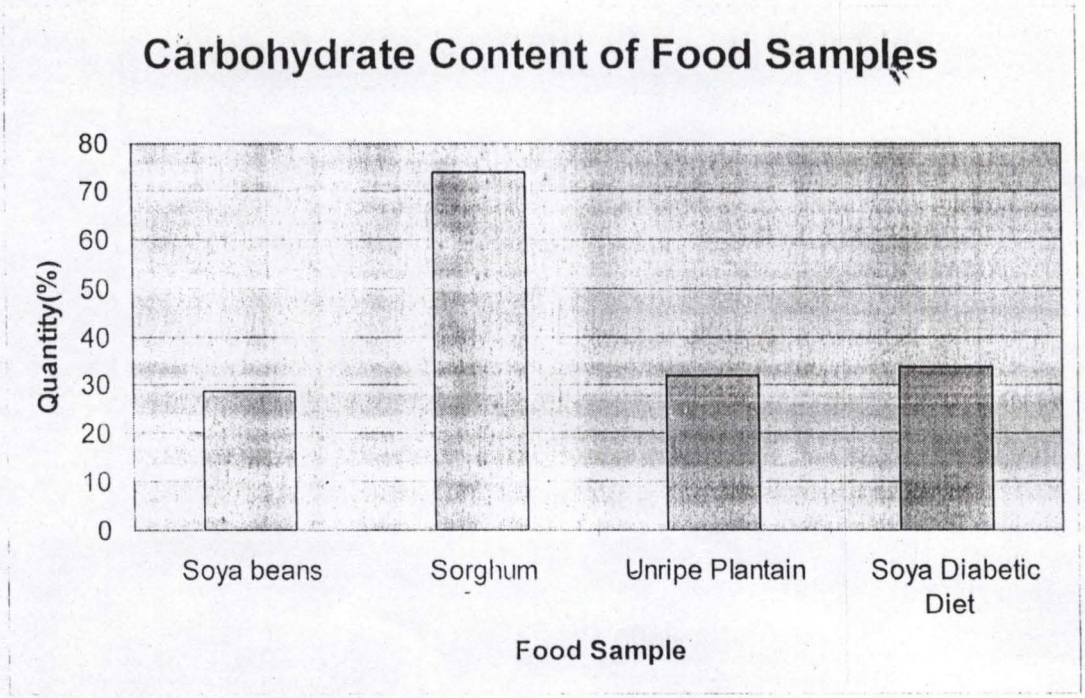
See Appendix 1 for details

Fig 4: Ash Content of Soya beans, Sorghum, Unripe Plantain and Soya Diabetic Diet



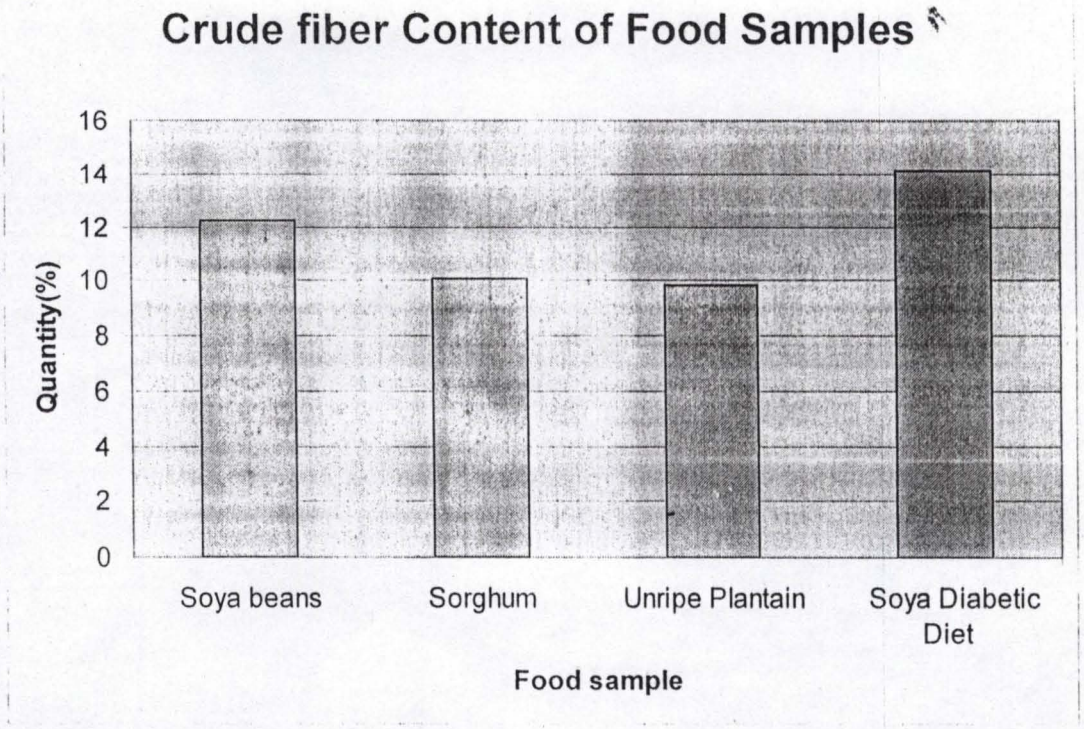
See Appendix 1 for details

Fig 5: Carbohydrate Content of Soya beans, Sorghum, Unripe Plantain and Soya Diabetic Diet



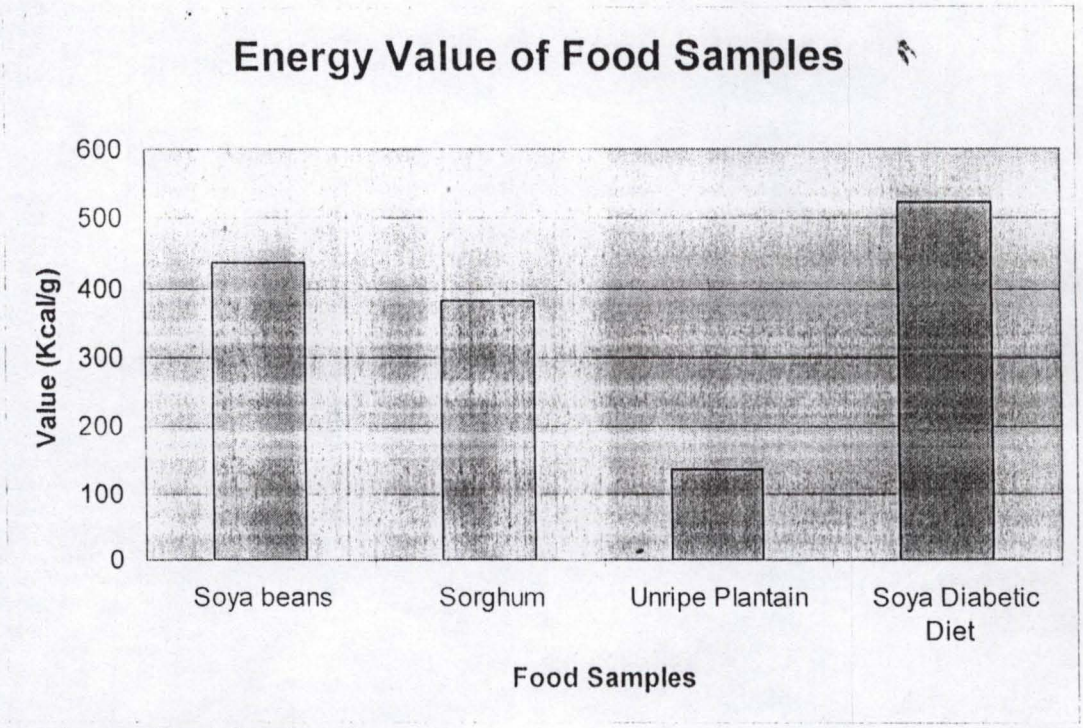
See Appendix 1 for details

Fig 6: Crude Fiber Content of Soya beans, Sorghum, Unripe Plantain and Soya Diabetic Diet



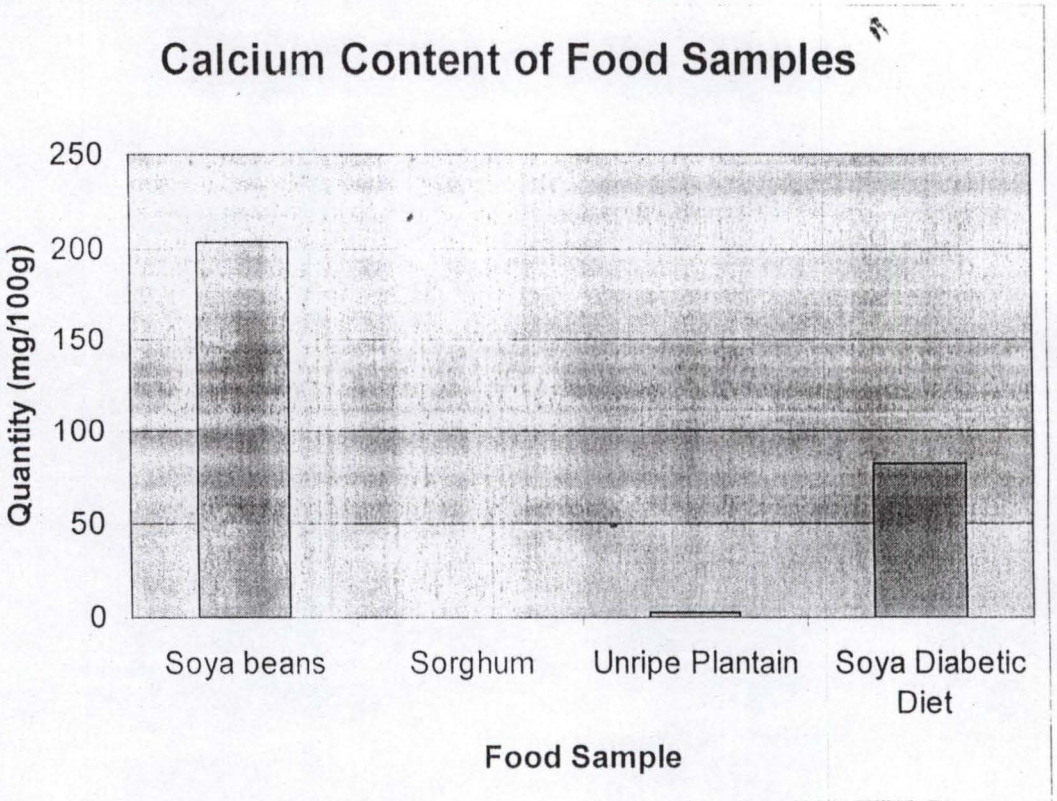
See Appendix 1 for details

Fig 7: Energy Value of Soya beans, Sorghum, Unripe Plantain and Soya Diabetic Diet



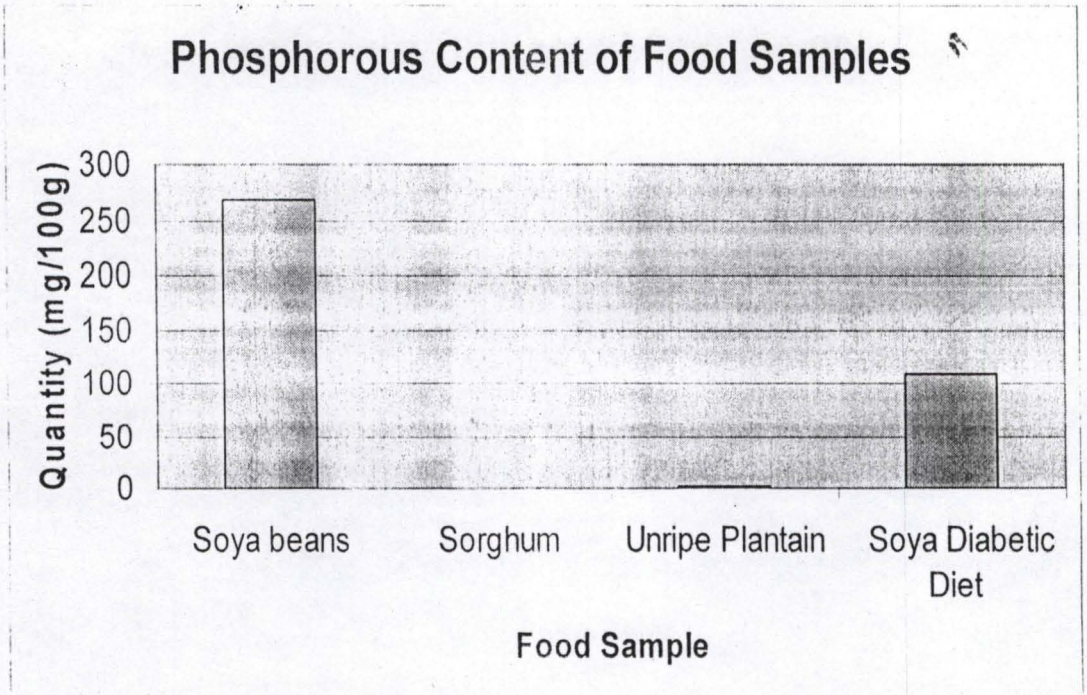
See Appendix 1 for details

Fig 8: Calcium Content of Soya beans, Sorghum, Unripe Plantain and Soya Diabetic Diet



See Appendix 1 for details

Fig 9: Phosphorous Content of Soya beans, Sorghum, Unripe Plantain and Soya Diabetic Diet



See Appendix 1 for details

TABLE 1: Total microbial load of Soya Diabetic Diet (SDD).

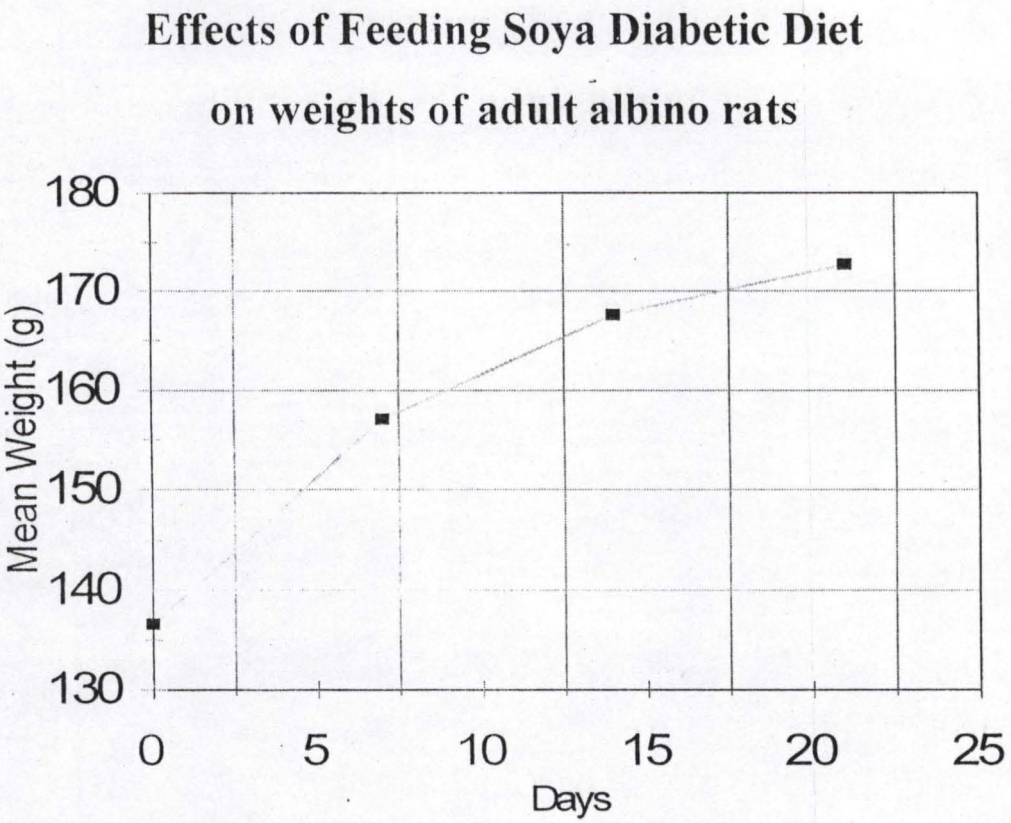
Media type	DILUTION			
	10^{-3}	10^{-4}	10^{-5}	T.C cfu/ml
Nutrient agar	8	1	-	$4x10^3$
MacConkey agar	-	-	-	-
Saboriend dextrose agar	14	6	2	$5x10^3$

TC=Total count, cfu/ml=colony forming units per mls of sample solution

Total Count (TC) = Average count x reciprocal of dilution factor.

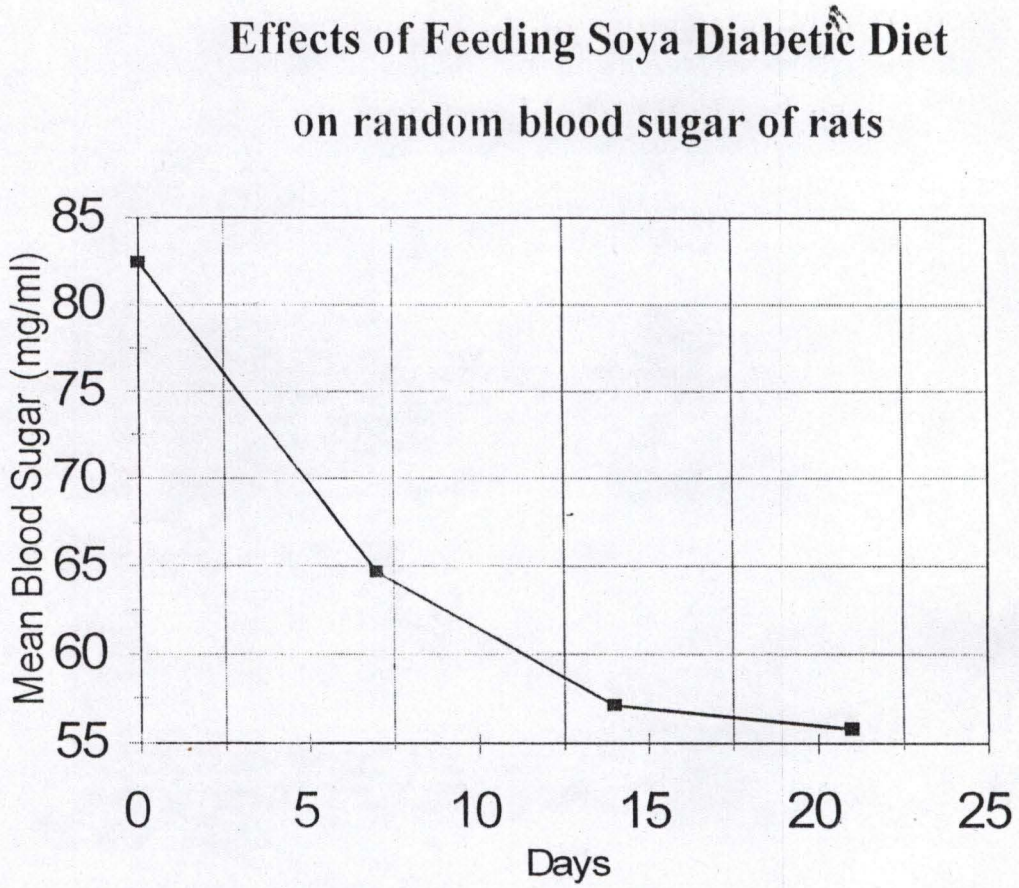
The results of the effects of feeding the Soya diabetic diet on the Weights, random blood sugar (RSG) and Fasting blood sugar (FSG) of the adult albino rats over a period of 21 days of the experiment are as shown on figs. 10, 11 and 12 as follows:

Fig.10: Effect of feeding Soya diabetic diet on weights of adult albino rats over 21 days period.



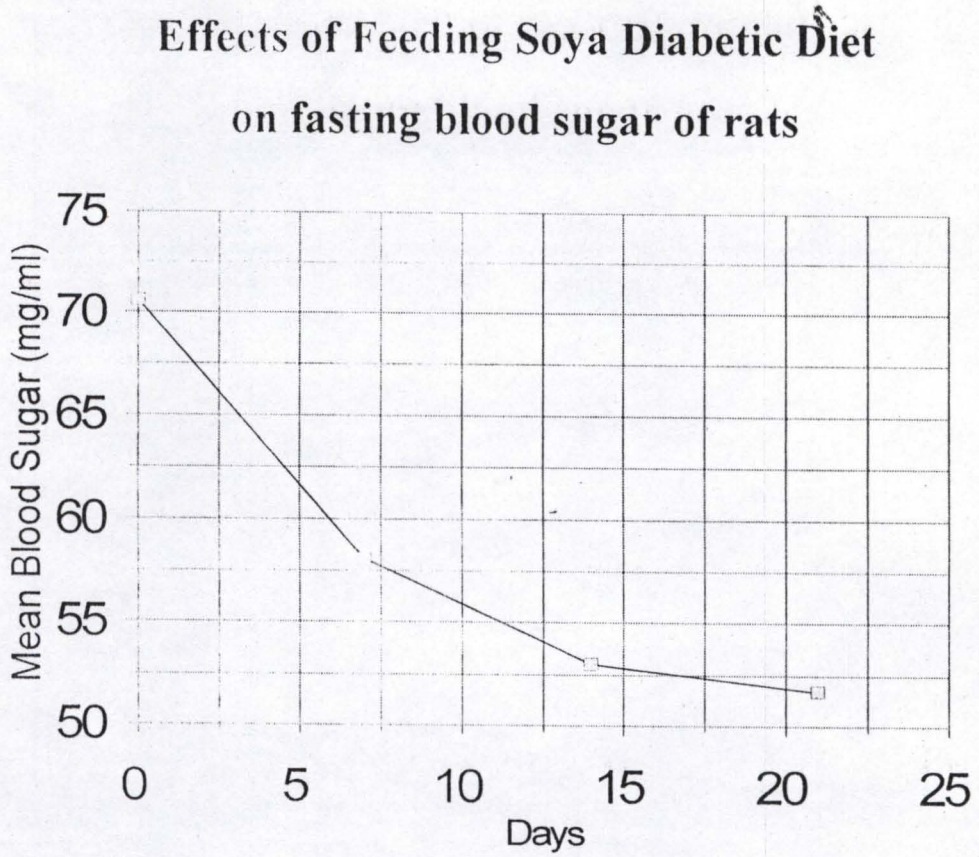
See Appendix 2 for details

Fig 11: Effect of feeding Soya diabetic diet on random blood sugar of adult albino rats over 21 days period.



See Appendix 3 for details

Fig 12: Effect of feeding Soya diabetic diet on fasting blood sugar of adult albino rats over 21 days period.



See Appendix 3 for details

4.2 DISCUSSIONS

Chemical composition of the Formulated SDD

From the results on Table 1 above, it is apparent that mostly sorghum and unripe plantain contribute carbohydrate to the blend while fat and proteins are contributed by Soya beans. The protein content of the formulated blend, though lower than that in the Soya beans is more than twice the amount in sorghum and over twenty times the amount in unripe plantain. According to the recommendations of the protein advisory group (PAG) guidelines for high nutrient foods, protein content should be at least 20% (on a dry weight basis), fat levels up to 10%, moisture of 5-10% and total ash of not more than 5% [34]. SDD certainly meets most of these recommendations as its results fall within acceptable ranges. The amounts of ash in the blend are however lower than 5% as obtained for the ash content of 3.2%. The amount of ash obtained in this study is lower than the lower limit of 5% recommended by PAG. This is an indication that the blend is low in minerals, and suggests that fortification may be necessary.

The energy value of 522kcal of energy derivable from SDD is a clear indication that the formulated diet is a diet of High glycemic index (GI) and can supply the required nutrients of food in adequate

proportions. Foods of high GI are highly recommended for consumption by Diabetics. [9]

Acceptability

No one legume or cereal can provide adequate amounts of nutrients to meet the nutritional requirements of an individual. However, even before knowledge on protein content, protein quality, digestibility and the nutrient requirements of humans became available, it was recognized that mixing legumes with cereals in the diet could improve overall nutrition. The present and newly derived knowledge in these areas makes it possible to blend, mix or fortify one food material with others so that the resulting fortified mix has not only better nutritional quality but also the necessary attributes for consumer acceptance.

Non-starch polysaccharides (collectively called fibers) include crude fiber, cellulose, pectic substances, hemicellulose and other polysaccharides. SDD contains about 14% fiber and will therefore be a good recipe for Diabetics as it enhances good digestibility.

Microbial load of SDD

The quantity of microorganisms in the SDD blend formulation was very low and insignificant to cause any serious deleterious effect upon consumption of the diet. This is suggested by the Total Count (TC) of colony forming units per ml of the test solution (cfu/ml), which was

found to be 4000 cfu/ml for total viable count on NA, 5000 cfu/ml for total fungal count on SDA and no organism was recorded for total coliform count on MCA. Qualitatively, the microorganisms discovered were *Aspergillus flavus*, Muccor (Fungi) and *Bacillus subtilis* (Bacteria). The reason for the low count of microorganisms could be because most of them cannot survive in temperatures above 60°C,the temperature used for the blend formulation. However, the few isolated ones are common environmental organisms, which are found even in the air. This suggests that the formulation procedure is aseptic and is acceptable for human consumption.

Hypoglycemic effect

According to their physical appearances, the rats used in the experiment showed ideal growth and development while on the SDD compared other rats in the laboratory feeding on normal laboratory food. They had neat fur, fine tails and were very agile. Mean weight gain over the days period was progressively significant, suggesting that the diet stimulated their growth. Throughout the test period, the animals did not reject the diet by refusing to consume it, rather the animals' interest in the increased after about 10 days of the experiment. Mean blood glucose levels continued to decrease significantly from the basal reading mg/ml and 70.6mg/ml for the RSG and FSG respectively, as

basal readings before the animals were placed on the SDD, to 55.8mg/ml and 51.7mg/ml for the RSG and FSG respectively after 21 days feeding on SDD. This is a good indication that the diet pocesses hypoglycemic properties and could be used as a recipe for diabetics.

4.3 CONCLUSION

Soya bean, Sorghum and unripe plantain are all traditional foods found in the African sub-continent in abundance and have been staple foods for quite a long time. Soya bean has been used effectively elsewhere as an acceptable protein supplement with high degree of success. Meanwhile, diabetes, as a disease has become of serious significance world wide, and the cure is nowhere in sight. The WHO recommendations that attention should be paid to traditional African plants [20] and foods for diabetic and other ailment cure is more than a motivation for this research. The formulation developed through this study successfully produced a high protein-energy food, which has hypoglycemic properties with acceptable functional characteristics as well as excellent nutritional qualities. The results of the present study demonstrated the beneficial effects adding grain legume to cereal foods. The technique can be easily adopted at both household and village levels or improved and produced on commercial basis to help enhance the nutritional status and manage the disease condition of the Nigerian and indeed African diabetics

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Appendix 1

Chemical composition of Soya beans, sorghum, unripe plantain and Soya diabetic diet.

Composition	Food sample			
	Soya beans	Sorghum	Unripe Plantain	SDD
Moisture (%)	9.7	8.6	65.3	9.4
Protein (%)	36.6	12.3	1.3	28.0
Fat (%)	19.5	3.67	0.4	26.2
Ash (%)	5.6	1.6	1.1	3.2
Carbohydrate (%)	28.6	73.8	31.9	33.59
Energy (kcal)/g	436	381	136	522
Calcium (mg/100g)	204	-	3.0	82.2
Phosphorous(mg/100g)	269	-	2.2	108.1
Crude fiber (%)	12.2	10.1	9.8	14.1

Values are means of triplicate determination expressed as one.

Appendix 2

Effect of feeding Soya diabetic diet on weights of adult albino rats .

Label	Days into the Experiment			
	0	7	14	21
	Weight (g)	Weight (g)	Weight (g)	Weight (g)
H	137.3	163.2	157.5	164.7
T	176.1	215.5	211.3	215.4
LL	119.7	147.1	149.2	153.8
RL	141.9	171.5	165.4	167.2
N	102.3	129.3	136.0	140.8
H&T	157.2	198.6	204.0	211.1
H&BK	126.3	121.2	159.0	163.5
BK	144.3	135.2	179.5	183.9
RL	124.5	132.4	146.2	154.3
Mean	**136.62**	**157.11**	**167.57**	**172.74**
SD	21.76	32.84	25.87	25.78

SD=Standard deviation.

Appendix 3

Effect of feeding Soya diabetic diet on random blood sugar of adult albino rats

Label	Days into the Experiment			
	0	7	14	21
	RBS (mg/ml)	RBS (mg/ml)	RBS (mg/ml_	RBS (mg/ml)
H	80	53	51	53
T	66	59	57	52
LL	86	83	63	57
RL	87	68	62	60
N	104	62	57	56
H&T	68	66	52	61
H&BK	114	61	55	52
BK	66	63	58	55
RL	71	67	59	56
Mean	**82.44**	**64.67**	**57.11**	**55.78**
SD	17.25	8.26	4.04	3.20

RBS=Random Blood Sugar (mg/ml), SD=Standard deviation.

Appendix 4

Effect of feeding Soya diabetic diet on fasting blood sugar of adult albino rats.

Label	Days into the Experiment			
	0	7	14	21
	FBS(mg/ml)	FBS (mg/ml)	FBS (mg/ml_	FBS (mg/ml)
T	99	67	60	58
LL	53	52	46	50
N	92	54	56	51
H	71	59	58	55
T2	72	51	54	51
BK	86	69	64	60
RL	64	50	52	49
LL2	60	63	53	52
N2	51	58	45	49
LL&RL	58	58	42	42
Mean	**70.6**	**58.1**	**53.0**	**51.7**
SD	25.86	16.71	35.17	6.61

FBS=Fasting Blood Sugar (mg/ml), SD=Standard deviation.

silage increase. Ammonization of grains can destroy some mycotoxins, but there is no practical method to detoxify affected forages already in storage. Increasing nutrients such as protein, energy and antioxidant nutrients may be advisable (Brucato *et al.*, 1986, Coffey *et al.*, 1989, Smith *et al.*, 1971).

Adsorbent materials such as clays (bentonites) added to contaminated diets fed to rats, poultry, swine and cattle have helped reduce the effects of mycotoxins (Diaz *et al.*, 1997).

2.3.9 Decontamination of Mycotoxins

Contaminating mycotoxins in foods and feeds should be removed, inactivated or detoxified by physical, chemical and biological means depending on the conditions. However, the treatment has its own limitations, since the treated products should be healthsafe from the chemicals used and their essential nutritive value should not be deteriorated. The following methods are suggested to be applied for effective decontamination of some mycotoxins (Park *et al* 1988).

Physically, fungi-contaminated seeds can be removed by hand picking or photoelectric detecting machines. The method would consume time and labour or expensive.

Organic solvents (chloroform, acetone, hexane and methanol) have been used to extract aflatoxins for agricultural products, but mainly in vegetable oil refining process.

Heating and cooking under pressure can destroy nearly 70% of aflatoxin in rice compared to under atmospheric pressure only 50% destroyed (Coomes *et al.*, 1966). Dry and oil roastings can reduce about 50-70% of aflatoxin B_1 (Feulle, 1966). Only