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Sustainable Dietary Supplements: An Analytical Study of African Yam Bean-*Sphenostylis Sternocarpa* and Corn-*Zea Maiz*

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ABSTRACT

African yam bean -Sphenostylis sternocarpa and Corn -Zea maiz are nutritional food sources that are valuable industrially even in consideration for enzyme production purposes. African yam bean Sphenostylis sternocarpa is a leguminous crop grown in West African particularly in Cameroon, Cote d'lvoire, Ghana, Nigeria and Togo. It is used extensively in various dietary preparations and has potential for supplementing the protein requirement of many families throughout the year. In this study, African yam bean seeds was soaked for 12 hours and dehulled after which it was boiled for 1 hours and it was then sieved and poured hot in calabashes laid with plantain leaves. It was covered and wrapped with jute bags and allowed to ferment naturally at room temperature for 72 hours. Bacteria such as Aerococcus viridans and Pediococcus cerevisiae were isolated and used as starter culture for controlled fermentation. The results of the proximate analysis show that protein content and the fat content of the sample fermented with Aerococcus viridans was higher than the one fermented with Pediococcus cerevisiae while moisture content and carbohydrate content decreased when fermented with Aerococcus viridans and increased when fermented with Pediococcus cerevisiae. The result of anti-nutrient content showed that there was an increase in phytic acid, oxalic acid and tannic acid of sample fermented with Aerococcus viridans than the ones fermented with Pediococcus cerevisiae. Corn, another dietary source is a cereal carbohydrate source that is widely cultivated in tropical Africa and the world at large for various domestic and industrial uses. The microbial source of the genus Bacillus used for this study utilized white cornstarch substrate as a sole carbon substrate as well as soluble starch used for comparative purpose. The enzyme production values of test organisms range from 0.022 unit/cfu x 10^2 in B.circulans (WBC) to 0.912 unit/cfu x 10^2 in B.licheniformis (WBL) for cornstarch and 0.01 unit/cfu x 10^2 in both B.megaterium (SBG) and B.licheniformis (SBL) to 0.693 unit/cfu x 10^2 in B.subtilis (WBS) for soluble starch.

Keywords: African Yam Bean-*Sphenostylis Sternocarpa,* Analytical, Corn-*Zea Maiz,* Dietary.

INTRODUCTION

African yam bean *Sphenostylis sternocarpa* and Corn Zea maiz are two major nutritional food sources of different categories that is proteinous and cereal carbohydrate source that are widely consumed or used as food supplements in Africa and even on industrial bases in different parts of

the world. Fermentation is required to improve the quality of this food processes. This involves metabolic process whereby electrons released from nutrients are ultimately used to release energy and generate simpler products or nutrients. In food processing, typically, fermentation can be described as the conversion of carbohydrates to alcohols and carbondioxide or organic acids using yeasts, bacteria or a combination thereof under anaerobic conditions. A more restricted definition of fermentation is the chemical conversion of sugars into ethanol (39). There are three main kinds of fermentation which are alcoholic, acetic and putrefactive. Each is caused by the presence of bacteria, yeasts or molds which produce the particular enzymes responsible for chemical change (18).

Man mostly lived for food and we may call earlier period the survival food age in the 20th century access to food no longer was a problem in most of the affluent societies, but time was short for work and leisure people wanted to save cooking and obtaining food. Consequently convinces food became major item in the food market. Now, we all noticed that the 21st century is the "functional food era". Health oriented food and nutrachemical are major concern or today's consumer (1).

Methods of Fermentation could be natural or Control. Natural fermentation is one of the oldest means of preservation. It means leaving the foods in a container at room temperature for four to seven days. No additional bacteria are deliberately added to the foods and no heating process is used. It can be achieved by a combined metabolic engineering and transcriptone analysis approach. Lactic acid bacteria subject the foods to a fermentation process which becomes preserved, it develops a pleasantly sour taste and it is rich in vitamins and minerals. (1).

Control fermentation is a term of food preservation since it generally results in a reduction of acidity of the food, thus preventing the growth of spoilage microorganism. The two most common acids produced are lactic acid and acetic acid. In controlled fermentation, the reactions are usually very complex and involve a series of microorganisms either working together or in a succession to achieve the find product. Controlled fermentation is used to produce range of fermented foods including krant, pickles olives, vinegar, diary and other products. (6).

Fermentation can also be grouped into two, that is, Solid substrate fermentation (SSF) and submerged liquid fermentation (SLF). Solid substrate fermentation (SSF) is the growth of fermentation fungi of the surface of cereal grains. The filamentous fungi are the best adapted microorganisms for SSF growing to their physiological, enzymological and biochemical properties. The hyphal mode of fungi growth gives the filamentous fungi the power to penetrate in the solid substrate. This also gives them a major advantage over unicellular microorganisms for the colonization of the substrate and the utilization of the available nutrients. In SSF system fungal growth within a solid mash is an important index for the efficiency of the scarification and production of metabolites. (13).

Submerged liquid fermentation (SLF) involves growth of microbes in an aqueous medium and it is a process been employed for the production of probiotics. It is traditionally used in the United States for the production of microbially derived enzymes. It involves submersion of the microorganisms in the aqueous solution containing all the nutrients needed for growth.

There are various kinds of fermentation carried on by different microorganisms. Many of those result in highly useful end-products. Such useful microbial activity is used on large industrial scale to obtain the useful end-products for the benefit of man kind. Some of the industrial products of the microbial fermentation are, Antibiotics, Vitamins, Some dairy products, Tanning of leather, curing of tea and coffee, Bakery products, Lactic acid, Butyric acid, Acetic acid etc. Legumes can further be described as pods which can vary in size and length depending on the plant. Human have been growing and eating legumes for a very long time with archaeological evidence suggesting that legumes may be the oldest crop known to man (17).

Farmed legumes fall into two classes which are forage and grain. Forage legumes like alfalfa, clover and vetch are sown in pasture and grazed by livestock .Grain legumes are cultivated for their seeds and are also called pulses. Grain legumes include beans, lentils, lupins, peas, peanut and soyabeans. Legumes are important in world agriculture providing biologically fixed nitrogen breaking cereal disease cycles and contributing locally grown food and feed including forage (10).

Legumes are widely used for green manure, forage and food, they are useful as green- manure crops because they have the capacity to add nitrogen as well as organic matter to the soil. Various dyes are derived from legumes including indigo from indigofera and haemotoxylon or hoghood from haemotoxylon cantpechianum a tree of central American (14).

Legumes are very high in protein and energy, making them excellent addition to the human diet and may have a rich assortment of vitamins and minerals as well because of their nutritional value. The protein contents ranges generally from 20 -40% for most grain legumes, the carbohydrates content ranges from 23% in groundnut to 66% in Bambara groundnut, pigeon pea and luna bean legumes, except the oil, legumes are low in fat content ranging from 1-5%, however oil seeds have a range of lipids content from about 18% in soyabean to as high as 43% in groundnut of all legumes species, soya beans are the richest in terms of protein contentwhich is 43% while the other legumes have protein content range of 20-25% (9) of minerals and vitamins especially those of cowpea, soyabeans and Bambara groundnut are good sources of calcium and iron with their content being higher than those of meat, fish or eggs. Those legumes also contain more thiamine, riboflavin and niacin than whole milk and cereals with the level of these vitamins being comparable to those available from fish, beef and eggs (16).

The increased cultivation of legumes is essential for the regeneration of nutrient deficient soils and for providing needed protein minerals and vitamins to humans and livestock. Legumes can be a means of improving the livelihoods of small holder farmers around the world. However, Legumes production presents new diseases, pests and weeds which farmers will need to learn how to control. Low soil pH, high salinity, flooding and nutrient deficiencies can negatively impact the nitrogen fixation process, preventing the legumes from improvising soil fertility to their full potential.

African yam bean *Sphenostylis sternocarpa* is a leguminous crop grown in West African particularly in Cameroon, Cote d'lvoire, Ghana, Nigeria and Togo (28). It is grown as a minor crop in mixed association with yam and cassava. It is used extensively in various dietary preparations and has potential for supplementing the protein requirement of many families

throughout the year. Based on the study of Isaac Okyere *et al* (20), in view of the on-going changes in environmental conditions, and the continued utilization of wetland resources in country, like Ghana, periodic monitoring of biodiversity of ecosystems needs to be vigorously pursued to assess their status. This will facilitate productivity.

It is grown for both its edible seed and its tubers. It is vigorous vine which twines and climbs to height of about 3m and requires staking. It flowers profusely in 100 to 150days, producing brightly-coloured flowers which maybe pink, purple or greenish white. The slightly woody pods contain 20 to 30 seeds, they are up to 30cm long and mature within 120days. The plant produces underground tubers that are used as food in some parts of African and that serve as organs of perennation in the wild. (14, 7, 31).

African yam bean seed is planted a month or two later after the major crops have established. Usually 2-3 seedlings, climb the cassava stems for support with some of them eventually reacting the live stakes used by the yams. No special care is provided for the bean. However, it benefits from weed control which is done at least twice before the major crops are harvested in September.

The chemical composition of the African yam bean has been evaluated by several authors (9, 16). The characteristic problem of hard to cook phenomenon which under the extensive use of African yam bean can be substantially reduced by pre-cooking treatment (27) studies armed by providing alternative methods of utilizing African yam bean such as production of tempeh (27). In-vitro multi – enzyme digestibility of its protein and determination of other functional properties of some varieties of African yam bean have been reported on the amino acid composition of seed meals of three varieties of the bean as well as their fatty acid composition in previous studies cited. The nutritive value of the African yam bean has also been assessed using albino rat and broiler chicks (7, 29). In general, amino acid content of the African yam bean is similar to that of other pulses crystine and methionine being low in amino acid and mineral contents and availability were moderate.

In the context of this study, Corn (Zea maiz) grows on a wide variety of soil types, from loamy sands to clays to organic soils. It is the main cereal source for many countries around the world. Due to its high starch content it is used as the main source of raw material for starch extraction. (41). There are different methods of corn starch extraction. These could be done enzymatically, steeping with SO_2 solution at appropriate temperature and alkali extraction. These methods give different result and degree of purity of obtained starch. Soni and Agarwal (37) found the chemical composition of corn starch to be; 28% amylase, 1.2% protein, 0.388% lipids, 0.38% Phosphorus and 0.54% ash. The results obtained by Hoover (19) for proximate composition (29.9% amylase, 0.125% proteins, 0.78% lipids and 0.03% ash) of native corn starch were slightly different. At any rate study of Neelam Verma *et al* (26) which enumerated some applications of biosensors in detecting harmful substances in food can further be used for further clarifications

MATERIALS AND METHODS

SOURCES OF MATERIAL

African yam bean seeds *(Sphenostylis sternocarpa)* were purchased locally from Akungba market in Akoko, Ondo State, while the corn were obtained from market sources, in Ibadan, Oyo State, Nigeria

FERMENTATION OF AFRICAN YAM BEAN

African yam bean were thoroughly washed and soaked for 12 hours to facilitate the dehulling process. The dehulled African yam bean was then boiled for one hour and it was then sieved and poured hot in calabash lined with plantain leaves. It was covered and wrapped with jute bags and allowed to ferment naturally at room temperature for 72 hours. The bacteria isolates used for the controlled fermentation such as *Aerococcus viridans* and *Pediococcus cerevisiae* were obtained from this naturally fermented African yam bean seed.

STERILIZATION OF GLASSWARE

All glassware used for the laboratory work were thoroughly washed with detergent and rinsed with tap water until the glassware were free from the detergent. The glassware which include pipette, conical flask, MacCartney bottles, glass petri-dishes, beakers were sterilized in hot air oven at 160^{o}C for 20hours.

Nutrient Agar and Potato Dextrose Agar were generally used during the study. All the media were sterilized in autoclave at 121^{o}C for 15 minutes and allowed to cool at 45^{0}C before pouring.

ISOLATION OF MICROORGANISM FROM AFRICAN YAM BEAN

Pour plate technique was the method used for the isolation of microorganisms from sample sources (30). In doing this, one- gram of fermented African yam bean was macerated in a sterile mortal and pestle and was dissolved inside 9ml of sterile normal saline to make 10^{-1} dilution, also 1ml was pipetted from this source serially until the last dilution. Media used were sterilized in an autoclave at 121^{0}C for 15 minutes.

1m and 0.1m of the appropriate diluents, that is, 10^{-3} and 10^{-4} of the mixture were inoculated into four sterile dishes that have been plated with sterile molten nutrient agar as distinct from 1m and 0.1m of 10^{-5} and 10^{-6} dilution of the mixture used for sterile molten potato dextrose agar aseptically prepared. The plate were thoroughly swirled so that the mixture will mix thoroughly and allowed to solidify.

The nutrient agar plates was inverted after solidifying inside an incubator at 35± 2^{0}C for 24hours and the potato dextrose agar plates was incubated at 28± 2^{0}C (room temperature) for 72 hours. After incubation, colonies were observed for their colonial nature and each colony was streaked to obtain a pure isolate. It was then transferred into agar slant and stored for subsequent uses. Only bacteria isolates were used for control fermentation.

Preparation of starter culture 1ml of 10^{-3} yeast suspension of *Aerococcus viridians* and *Pediococcus cerevisiae* were maculated separately into 30g of sterile dehulled African yam bean seed wrapped in foil paper and allowed to ferment at 30^{o}C for 72hrs.

GRAM STAINING REACTION

The surface of glass slide used was sterilized with 70% alcohol. A loopful of distilled water was placed on the clean grease free slide and inoculums from 24hours old culture were placed on the slide with a sterile inoculating wire loop. It was then smeared, air dried and heat fixed, after which the slide was flooded with crystal violet solution for one minute and gently washed with water and flooded with lugol's iodine solution for another 60seconds the iodine solution was drained off and washed gently under a running tap water after which the slide was flooded with ethanol until the glass slide was free from the violet stain. Then, safranin stain solution was added on the slide for 60seconds and later washed with distilled water. The slide was blotted, dry and was examined under the oil immersion objective lens of a microscope (30).

After 24hours of incubation, the bacteria isolated were gram stained and sub-culture inside sterile agar slant for further biochemical tests stated below:

(a) Catalase test

A loopful of the bacteria growth was emulsified with a drop of hydrogen peroxide on a slide. Formations of oxygen bubbles indicate the presence of catalase.

(b) Starch Hydrolysis

A plate containing 1% starch agar was streaked across the surface with the test isolate. The plate was incubated at 37^{o}C for 3 days. The plate was flooded with Gram's iodine after incubation. Unhydrolyzed starch form a blue-black coloration with the iodine while hydrolyzed starch appeared as a clear zone along the streak resulting from α-amylase activity.

(c) Sugar Fermentation Test

The sugar fermentation test was used to detect the ability of each isolate to produce acids from sugar and to detect gas formation. I% of sugars such as Maltose, glucose, fructose, lactose, manitol, galactose, and sucrose were used. Hence the basal medium with the sugar used constitute 0.5g of sugar, 0.5 of peptone water, 0.015g of phenol red as indicator were all dissolved in 50ml of distilled water which is dispensed in a test tube with inverted Durham tube and the mouth of each tube was corked with cotton wool and foil paper. It was then sterilized in an autoclave at 121^{o}C for 15minutes. These were allowed to cool down and the isolates were inoculated and incubated for 7days. Gas production was detected by the accumulation of gas produced in the inverted Durham tube.

PROXIMATE ANALYSIS

MOISTURE CONTENT DETERMINATION

5g of African yam bean seeds were weighed into oven dried petri dishes. This was later transferred into the oven at 100^{o}C until a constant weight was obtained for each of the sample. The dried samples were cooled in the desiccators and the loss in weight was expressed as percentage moisture content.

FAT DETERMINATION

About 0.5g of oven-dried samples was weighed into filter paper of known weight. Their extraction was carried out using soxhlet extractor 400ml of petroleum ether (40-60^{o}C) in a 500ml round button flask was used for the extraction with the reflux condenser. The heat source was adjusted to allow the solvent boil gently. It was then left to siphon over 4 hours, the filter paper was detached after the extraction and it was placed on an oven at 50^{o}C to dry to constant weight.

Thereafter, the filter paper was cooled in the desiccators and the weighed was determined and percentage that was subsequently determined.

PROTEIN DETERMINATION

Oven dried samples 0.2g were transferred into 50ml micro kjeldahl flask. 0.5ml of concentration H_2SO_4 with half kjeldahl catalyst tablet were added after which the sample was digested by heating until the digest was cleared that is from light green to grayish white the heating was allowed to continue for 2minutes to complete digestion. It was cooled and its volume was made up to 50ml with distilled water. 5ml of boric acid was transferred into 100ml conical flask (as receiving flask) and 3 drops of mixed indicator was added. The receiving flask was placed in such a way that the tip of the condensed tube is below the surface of the boric acid. Thereafter, 5ml 0f the digested samples was transferred into Markham distiller. This was followed by the addition of 10ml of 4% NaOH, 50ml of the distilled was collected into the receiving flask, and titrated against 0.1ml HCl. The blank was titrated against the acid as well. The percentage of nitrogen and crude protein was subsequently determined.

CARBOHYDRATE DETERMINATION

Percentage of soluble carbohydrate was determined by subtracting the sum of percentage ash, fat, nitrogen and moisture content from one hundred.

ANTINUTRIENT DETERMINATION

DETERMINATION OF TANNIN

Tannin was determined as tannic acid, following a procedure whereby finely ground samples were deflated in diethyl-ether containing 10% acetic acid to 0.2g each of deflated samples, 10ml of 70% aqueous acetone was added. The mixture was shook for uniform agitation for 2minutes at 31^oC at120rev/min and was further agitated for 10mins using vortex mixer and centrifuge at 3.500g for 5mins in a gallenkamp (angle head) centrifuge. Exactly 0.2ml of the supernatant was made up to 1ml with 0.5ml folic ciocalteau reagent, 2.5ml of 20% sodium carbonate and distilled water. The colour was allowed to develop for a minimum of 40mins and absorbance was read at 725mm curve of tannic acid in blank and standard were plotted against absorbance. Amount of total phenol as tannic acid equivalent was calculated expressed on a dry matter basis.

PHYTATE DETERMINATION

In determination of Phytate, 4.0g of the sample was soaked in 100ml of 2% HCL for 3hours and then filtered. Then 25ml of the filtrate was placed in conical flasks and 5ml of 0.3% ammonium thiocyanate sodium was added as indicator. After which 53.5ml of distilled water was added to give it, the proper acidity and was titrated with a standard iron (ii) chloride solution which contain 0.00195 of iron per milliliter until a brownish yellow colour appeared which persist for 5mins.

DETERMINATION OF OXALATE

Designated according to procedure of Jrand and Underwood (21), 1g of the sample was ground in mortar with pestle and 75ml of 1.5N H_2SO_4 was added to the solution and stirred intermittently with a magnetic stirrer for about one hour and filtered using Whatman NO.61 filter paper. A 25ml sample of the filtrates (extract) was collected and titrated hot (80-90^oC) against

0.1N $KMn0_4$ solution to the point when a taint pink colour appeared that persisted for at least 30seconds (21).

EXTRACTION OF STARCH

Starches were extracted from corn grains using the protocol decribed by Qi *et. al.*, (33) or by the method developed by Adkins and Greenwood (3). The carbon substrates generally used in the study were corn starch and soluble starch, peptone and yeast nitrogen source were also considered. The corn starch was obtained by wet milling process (34) and dried for preservation and further laboratory analysis. The white corn starch tested in this study has some economic viability because it can be cultivated locally for large scale industrial enzyme production purposes.

The isolates were first cultured in fermenting medium of corn starch, soluble starch and nutrient broth to determine their ability to utilize carbon substrates from an estimation of their total bacterial count obtained by a pour plate technique. For enzymes production, the amylolytic microbial isolates were cultured in a medium (50ml), containing 2% peptone, 0.5% solute starch, 0.3% K_2HPO_4 and 0.1% Mg SO_4 7H2O in Erlenmeyer flask of 200ml capacity. The cultivation was carried out for about 40 hour at 30^oC on a rotatory at 150rpm. The cultivated cell was removed by centrifugation at 4000rpm, for 15minutes and resultant supernatant was used as the enzyme source (39). The enzyme was assayed to determine its capability to release reducing sugar according to the DNSA method. (25, 12). Previous studies by Mahalingam *et. al* (22) shows importance of Cell free culture filtrates and that it has been used to demonstrate the role of some metabolites like antibiosis in biological control.

Chemical analysis or proximate analysis

The moisture content was determined as weight loss after heating starch at 130^0 C for 1 hour. Total ash content was also analyzed according to method 923.03 of the AOAC official methods (2000). Nitrogen content (N) was measured by the standard kjeldahl methodology, where protein content was calculated as N x 6.25. Starch lipids were extracted in 75% aqueous n-propanol at 100^0C (). Phosphorus content of the sample was determined.

RESULTS

The result of this study showed that the major bacterial species isolated from 72hours. fermented sample were characterized and identified as *Aerococcus viridans* and *Pediococcus cerevisiae* (Table 1). In table 2, proximate analysis of the samples source studied, that is, African yam bean *Sphenostylis sternocarpa* showed that sample fermented with *Aerococcus viridans* has a protein content (41.43%) that is higher than the one fermented with *Pediococcus cerevisiae* (33.03%). The fat content (4.15%) of the sample fermented with *Aerococcus viridans* was higher than the one fermented with *Pediococcus cerevisiae* with fat content (3.48%). Also, moisture content (40.16%) and carbohydrates content (3.72%) decreased when fermented with *Aerococcus viridans* and increased when fermented with *Pediococcus cerevisiae* with moisture content (41.36%) and carbohydrate content (16.82%). In this study, there was an increase in phytic acid of sample fermented with *Aerococcus viridans* (12.35mg/g) than the one fermented with *Pedicoccus cerevisiae* (10.92mg/g). The oxalic acid increases when fermented with *Aerococcus viridans* (1.33mg/g) than when fermented with *Pediococcus cerevisiae* (1.22mg/g) and also the

tannic acid increases when fermented with *Aerococcus viridans* (1.62mg/g) than when fermented with *Pediococcus cerevisiae* (1.48mg/g) (Table 3).

Table 4 shows the proximate analysis of corn which is mainly composed of starch constituting 80% of the corn grain samples. The growth pattern and utilization of cornstarch, as a carbon substrate by the amylolytic *Bacillus* species was determined. Table 5 shows the comparative enzyme production by soluble starch and cornstarch carbon sources. The *Bacillus species* studied thrives well on cornstarch than soluble starch except in three strains which are *B.macerans* (MBM) , *B.macerans* (SMB2) and *B.subtilis* (WBS). The enzyme production values range from 0.022 unit/cfu x 10^2 in *B.circulans* (WBC) to 0.912 unit/cfu x 10^2 in *B.licheniformis* (WBL) for cornstarch and 0.01 unit/cfu x 10^2 in both *B.megaterium* (SBG) and *B.licheniformis* (SBL) to 0.693 unit/cfu x 10^2 in *B.subtilis* (WBS) for soluble starch (Table 5).

TABLE 1: CULTURAL AND BIOCHEMICAL CHARACTERISTICS OF ISOLATES

Morphological and Cultural Characteristics	**Isolates 1**	**Isolates 2**
Surface	Smooth	Dull
Shape	Filamentous	Rhizoid
Opacity	Opaque	Translucent
Elevation	Effuse	Raised
Edge	Rhizoid	Rhizoid
Consistency	Friable	Friable
Groom reaction	Positive	Positive
Structure	Cocci	Cocci
Arrangement of cells	Pair clusters	Pair tetrad and short chain
Motility	Negative	Negative
Spores	Negative	Negative
Spore position	Negative	Negative

Key: *ISOLATES 1 – Aerococcus viridans*
ISOLATES 2 – Pediococcus cerevisiae

Table 1contd:

BIOCHEMICAL TEST	**ISOLATE 1**	**ISOLATE 2**
Catalase	Slightly positive	Positive
Glucose	Positive	Positive
Sucrose	Positive	Negative
Lactose	Positive	Positive
Mannitol	Positive	Negative
Maltose	Positive	Negative
Starch Hydrolysis	Slightly Positive	Positive
Litmus milk	Negative	Positive

Key: *ISOLATE 1 – Aerococcus viridans*
ISOLATE 2 – Pediococcus cerevisiae

TABLE 2: PROXIMATE ANALYSIS ON AFRICAN YAM BEAN SEEDS

	Moisture Content (%)	Protein Content (%)	Fat Content (%)	Carbohydrates Content (%)
Unfermented Samples	11.08	21.74	0.04	___
Fermented with *Aerococcus viridans*	40.16	41.43	4.15	3.74
Fermented with *Pediococcus cerevisiae*	41.36	33.03	3.48	16.82

TABLE 3: ANTI-NUTRIENT CONTENT ON AFRICAN YAM BEAN SEEDS

	Phytic acid (mg/g)	Oxalic acid (mg/g)	Tannic acid (mg/g)
Unfermented Samples	17.4	0.04	0.24
Fermented with *Aerococcus viridans*	12.35	1.33	1.62
Fermented with *Pediococcus cerevisiae*	10.92	1.22	1.489

Table 4: Corn flour composition

Components	**Ratio (%)**
Starch	80%
Moisture content	11%
Protein	6.1%
Lipid	1.9%
Ash	0.5%

Table 5: Fermentative enzyme production in soluble starch and corn starch nutritive medium

Strain Code	*Bacillus species*	Corn starch medium		Soluble starch medium	
		Bacillus population Cfu x 10^2	Amylase Unit/cfu x 10^2	*Bacillus* population Cfu x 10^2	Amylase Unit/cfu x 10^2
SBM	*B.macerans*	8.0	0.165	5.0	0.144
MBM	*B.macerans*	25.0	0.072	20.0	0.15
SBM1	*B.macerans*	16.0	0.112	15.0	0.104
SBM2	*B.macerans*	17.4	0.41	1.5	1.2
WBC	*B.coagulans*	3.0	0.24	3.0	0.12
MBC	*B.coagulans*	3.0	0.44	2.0	0.42
SBL	*B.licheniformis*	7.0	0.102	6.0	0.02
WBL	*B.licheniformis*	6.0	0.912	5.0	0.84
SBC	*B.circulans*	2.0	0.36	1.8	0.06
WBCI	*B.circulans*	16.0	0.022	34.0	0.02
SBG	*B.megaterium*	12.0	0.06	11.0	0.01
WBP	*B.polymyxa*	8	0.06	7.4	0.016
WBS	*B.subtilis*	9.0	0.08	7.0	0.89

DISCUSSION

This study encompasses some major dietary values that can be derived from African yam bean *Sphenostylis sternocarpa* and Corn Zea maiz which are two main nutritional food sources commonly grown in some parts of Africa. In this study, *Aerococcus viridans* and *Pediococcus cerevisiae* were the predominant micro-organisms isolated from African yam bean seed. However, some microorganisms that have similar morphological appearance were also encountered. Fermentation of African yam bean seeds involves enzymatic decomposition of bacteria. These organisms are of immense participation based on the result obtained from the proximate analysis, showing that the fermented African yam bean seed had the highest protein content and moisture content. The protein and fat content of the fermented African yam bean seed were higher than fat of the unfermented sample sources. This shows that fermentation brings about an increase in the protein contents which is consistent with the study of Smith (36).

Fermentation as been employed to extend the self-life and improve the nutritional and sensory attribution of some food products, fermented foods constitutes a large proportion of the diet of Nigerians (2). Such foods are important because of their increased nutritional value as well as improved flavour obtained from this fermented food sources, such as African yam bean, locust bean, bambara bean etc. In contrast, there is an increase in the carbohydrate content of the unfermented African yam bean seed to that of fermented, the decrease might be probably due to the fact that the bacteria causing fermentation of the African yam bean seed had broken down the complex carbohydrates to carbon (IV)oxide and water with energy. This has correlation with the study of Yong and Wood (42).

In this study, the organisms used on corn substrates have capabilities to produce amylase and this was influenced by the effect of the regulated conditions especially in the utilization of cornstarch substrates compared with other nutrient sources. This greatly affected the quality or characteristics of the enzymes produced and it conformed with the studies of Srivastava and Baruah (38) and Montgomery *et al*., (24) who stated that the nature and characteristics of enzymes produced by different species of bacteria, depends on the strains of bacterial involved, moreover an optimal growth condition may be determined for each strain.

The enzyme production values from organisms used for this study range from 0.022 unit/cfu x 10^2 in *B.circulans* (WBC) to 0.912 unit/cfu x 10^2 in *B.licheniformis* (WBL) for cornstarch and 0.01 unit/cfu x 10^2 in both *B.megaterium* (SBG) and *B.licheniformis* (SBL) to 0.693 unit/cfu x 10^2 in *B.subtilis* (WBS) for soluble starch (Table 2). The results above agreed with report of Akhilesh Kushwaha *et al.* (5) who reported that selected strains of *Bacillus species,* could produce good yields of amylase on starchy substrates; Srivastava and Baruah (38) reported that among various complex media tried for good amylase yield, corn steep liquor was found to be the best. The disadvantage of the corn steep liquor was that it contains many chemical ingredients, and it was difficult to ascertain which of them induced amylase production. However other optimal conditions for fermentative processes and industrial enzyme production like media composition temperature, pH and cations should be observed for better productivity of enzyme of interest (4). In biotechnology, amylases are one of the most important enzymes used. The main use of enzymes includes hydrolysis of starch to yield glucose syrup, amylase-rich flour and in the formation of dextrin during baking in food industries. Furthermore, in the textile

industry, amylases are used for removal of starch sizing and as additives in detergents. However, the cost of producing this enzyme is high and the cost of procurement by developing countries can be even higher as a result of importation. Cheap and readily available agricultural waste or commonly grown cereal may be a rich source of amylolytic bacteria (6). Generally, production of this food sources can be improved using appropriate technology. According to the study of Nunnam John Samuel *et al* (28) Seed production technology is badly needed for industrial development.

CONCLUSION

African yam bean -*Sphenostylis sternocarpa* and Corn -*Zea maiz* are nutritional food sources that can be valuable industrially even in consideration for enzyme production purposes. Fermented African yam bean may be recommended as substitute for soya milk, bean flour for human consumption since protein content is highly rich. The fermentation processes as shown in this study helps in reduction of phytic acid contents of African yam bean. . The two microorganisms (*Aerococcus viridans* and *Pediococcus cerevisiae)* can be used as starter culture for fermentation of African yam bean seed. Study of Mohd. Muzamil Bhat *et al* (23) signify the importance of environmental protection including food protection with environmental awareness programmes. Hence, data obtained in this study, can be useful in trend of some industrial set up for production of good nutritive products. Domestically they are valuable food supplements that can prevent some nutritional diseases in realm of health safety.

REFERENCES

[1] O.K. Achi. Human nutrition, **1992**, 42:295-304.
[2] Aderiye: Foods containing complex polymer like lignin, pectin, and lingo cellulose, **1998**.
[3] G.K., Adkins & C.T. Greenwood. Starch/Starke, **1966,** 7, 213-218
[4] A.O Ajayi and O.E. Fagade. *Bioscience Research communications*. **2003.** 15 (1): 101 – 106
[5] Akhilesh Kushwaha, Vipul Verma, Mrigank Shekhar Avasthi, Abhishek Raj Gupta, and Monika Singh. *European Journal of Experimental Biology*, 2011, 1 (3):107-113.
[6] J.A. Akinyanju and H.A. Suberu**.** World Journal of Microbial Biotechnology **1996**. 12:403-404.
[7] Anon: Allas for Ghana. Macmillan London, UK. **1992**. 65PP
[8] AOAC. Analysis of the Association of Official Analytical Chemists (AOAC). International, William, H (ed). 17th., Gaithersburg, MD, U.S.A: **2000** Official method 923.03
[9] Apata and Ologbobo.: Guardian News, Health and Herb in Natural Health Guardian Needs **1994**
[11] K.R. Badu and *T* .Satyanarayana, *biochem* **1995**. 30:305-309.
[12] M.J. Bailey *Appl. Microbiol. Biotechnol*. **1988**. 21:494-496.
[13] B. Battarn, J. Sharm and R.C, Kuhad. *Biotechnol.* **2006** 22:1281-1287.
[14] J.A Duke., B.B. Okigbo and C.F. Read. *Prop Groom leg Bull* **1997** 10:4-6
[15] D.O, Edem, C.L. Amugo and O.U. Eka *Trop. Sci.* **1990**. 30:59-60.
[17] M.I Ezueh. *World crops* (1984). 36(6):199-200.
[18] A.B Hassan, E.A Osman and S. Hizukuri and S. Shibata. *Carbonhydr. Polym.,* **2005**. 25,111-116.
19. R. Hoover and H. Manuel. *Journal of Cereal Science* **1996**. 23, 153-162.

[20] Isaac Okyere, W. Denis Aheto and Joseph Aggrey-Fynn. *European Journal of Experimental Biology*, **2011**, 1 (2): 178-188.
[21] R.A Jrand and A.L Underwood. On antitative analysis, 5th edition: 70-Page apprentice- Hall Publication **1986**
[22] R. Mahalingam, V. Ambikapathy and A. Panneerselvam. *European Journal of Experimental Biology*, **2011**, 1 (2): 64-67.
[23] Mohd. Muzamil Bhat, Shahenshah, Syed Zulifiqar Ahmad Andrabi and Siddhartha Shukla, *European Journal of Experimental Biology*, **2011**, 1 (3):97-100.
[24] C.J. Montgomery, C.P. Patel, J.K. Shetty. Method for removing antifoaming agents during processing of microbial products Miles inc. Eikhart, in (USA) **1991**.
[25] S. Murao K. Ohyama and M. Arai. *Agric. Biol. Chem*. **1979**. 43(4). 719-726.
[26] Neelam Verma, Sachin Kumar and Hardeep Kaur. *Advances in Applied Science Research*, **2011**, 2 (6):354-363.
[27] O.F Njoku. Food Microbiology, McGrawHill Book Company, **1991**.
[28] Nunnam John Samuel, Soundarapandian Peyail and Anand Thananjayan, *European Journal of Experimental Biology*, **2011**, 1 (2):23-32.
[29] Nrain and Obizoba: Importance of fermentation, **1992**
[30] B.O Olutiola, Oshodi and Nwokolo: Tropical Agriculture in the development Production **1991**.
[31] Oshodi and Hall: Studies Armed of Providing Alternative. Methods of utilizing it such as Production of Tempe, **1993**
[32] D. Porter. *Econ. Bot.* **1992**. 46(3):262-275.
[33] X. Qi, R.F Tester, C.E. Snape, and R. Ansell. *Journal of Cereal Science*. **2003**. 37,363-376
[34] A.H. Rose Microbial Enzymes and Bioconversions. Academic press, London, England. **1980**
[35] Safdari Mehdi, Motiee Reza, *European Journal of Experimental Biology*, **2011**, 1 (3):79-89.
[37] Soni P.L and Agarwal A. *Starch/Starcke* **1983**. 35,4
[38] R.A.K. Srivastava and J.N. Baruah, *Appl. Environ. Microbiol.* **1986**. 52(1) 179-184.
[39] K.H Stein Kraus, Bioenrichment production of vitamins in fermented food in wood (Ed) microbiology of fermented food second edition, Blackie. Academic and Professional London **1998**. pp 603-619.
[40] Y. Takasaki, *Agric Biol. Chem*. **1976.** 40(8) 1515-1522
[41] D.S. Thomas and W.A Atwell. Starches: In Thomas D.S., Atwell W.A. (Eds.) Critical Guides for the food Industry. Eagan Press. .St Paul, MN, U.S.A. **1999**. pp 1-31