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Production and Optimization of Amylase and Glucoamylase from Aspergillus Niger under Solid State Fermentation for Effective Production of Glucose Syrup

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Abstract
The continual demand for sugars and coupled with the chemical hazards associated with chemically produced syrup had led to the development of enzymatic technology which has increased the production of glucose syrups most especially using enzymes from microbial origin. The aim of this work was to isolate, screen and optimized the best cultural condition that can lead to the production of glucose syrups from Nigeria cassava starch using enzymes produced by soil microorganisms. Also to determine proximate and physiochemical properties of the glucose syrup produced from yellow and white maize using microbial enzymes. The results obtained from the preliminary screening showed that most of the isolates obtained were *Aspergillus niger*. It was observed that *Aspergillus niger* had the largest zone of amylase activity of 35.0 mm when compared to *Fusarium pallidorosium* that had the lowest (5.0 mm). The optimal conditions for enzyme production; temperature, incubation period, inoculum concentration, and pH were 30 ^{o}C, 5 days, 1 × 10^{6} CFU/ml, and pH of 5 respectively for the production of glucoamylase and amylase. Generally, strain AMO1 was observed to produced more glucoamylase when compare to amylase production. The crude protein, the colour, viscosity, ash content, reducing sugar content, pH and carbohydrate contents of the syrup from yellow maize were more than that of the syrup from white maize.
Keywords: Glucoamylase; Amylase; Glucose syrups; Cassava; Optimization, *Aspergillus niger*

INTRODUCTION

Microbial-derived enzymes are of greater importance in the production of useful products to mankind and they are produced by different types of micro-organisms including bacteria, fungi, and yeast. Production of enzyme from microbial origin are widely accepted because they are Generally Regarded as Safe (GRAS), easily manipulated so as to obtain enzymes with desired characteristics including predictability, controllable and reliable enzyme contents, and their bulk production which is economically feasible when compare with other sources (Sindhu *et al* 2009).

Production of biotechnologically important enzymes involves screening and identification of filamentous fungi capable of secreting extracellular enzymes with greater activities. (Zambare, 2010). Glucoamylase is produced from different type of fungi but the exclusive production of this enzyme in industry have been obtained mainly by *Aspergillus niger* (Wang *et al.*, 2006), *Aspergillus oryzae* (Biesebeke *et al.*, 2005), *Aspergillus awamori* and *Aspergillus terreus* (Berka *et al.*, 1992) due to their ubiquitous nature and non-fastidious nutritional requirements. Enzymes are majorly produced using solid-state fermentation techniques from microorganisms (Sodhi *et al* 2005).

The wide usage of the enzymes in the industry include high fructose syrups production, cellulose, leather, detergents, liquor, bread, children cereals, ethanol production, as well as in the pharmaceutical and chemical industries such as the synthesis of optically pure drugs and agrochemicals (Pandey and Radhakrishnan, (1993), Gupta *et al.* (2003), Norouzian *et al.* (2006) and Zambare, (2010). Starch syrups are sweet edible products that are widely used in confectionery and other food products. In the United States, these syrups are known as 'corn syrups' as they are produced by acidic or enzymatic hydrolysis of corn starch. However, in Europe, usage of different starches as starting material, with more accurate description 'glucose syrups' is preferred because they contain glucose polymers in addition to glucose (Ipek, 2006). Federal Office of Statistics Annual Report (2000) indicated millions of dollar was spent on the importation of sugar syrups into Nigeria. Glucose syrups are essentially industrial sugars used in the manufacture of food products, and are mainly consumed in the confectionery industry (Akinola, 2004). Glucose syrup can be produced from different starch crops such as potato, cassava, rice, maize.

Corn starch is the major industrial raw material for glucose and fructose syrup production in the US and in many other parts of the world (Cabello, 1999).

Cassava (*Manihot esculenta*), also called manioc or tapioca, is a perennial plant widely grown in many tropical countries, including Nigeria and Brazil on a very large scale. Since cassava roots contain high starch content and low quantity of impurities such as protein and lipid, they are acknowledged as an excellent source of purely suitable starch for a wide range of applications (Tonukari, 2004). Many factories utilize the acid conversion to obtain glucose syrup (especially for the sugar confectionery industry) (Monica and Vionel, 2006). However, the use of enzymes is preferred to acid, because it produces high yields of desired products and less formation of undesired products such as toxic compounds (Silva *et al.,* 2010).

MATERIALS AND METHODS

Cassava (*Manihot utilissima*), white and yellow maize were bought from central market Minna, Niger State, Nigeria. All other chemicals used were obtained commercially and are of analytical grade.

Extraction of Cassava Starch

Cassava was prepared into starch in the laboratory according to standard procedures as described by White *et al*., (1990) modified by Krieger *et al*., (1997). Corn kernels were hand-picked and cleaned to remove foreign material, mold, and broken kernels before analysis. Kernels of whole corn (2, 5, or 10 kernels) were steeped in 5 mL of 1% sodium metabisulfite solution (0.67% SO2) at 45°C for 24, 48, or 72 hr, followed by manual removal of the pericarp and germ with forceps. The separated endosperm was placed in a 50-mL centrifuge tube with 10 mL of distilled water and homogenized using a vortex type tissue homogenizer at 20,500 rpm for 30 sec. The homogenized slurry was filtered by using a 30-µm nylon filter under vacuum with several washes, for a total wash water volume of 500 mL. Coarse and fine fibers and part of the protein were removed during filtration. The starch-protein mixture from the filtrate was further separated by either centrifugation or sedimentation. Each sample was separated three times, with 250 mL of distilled water used for each of the three separations. All treatments were performed in replicates of five, and the results averaged.

Isolation and Characterization of Fungal Organisms

One gram of soil gotten from the boy's hostel premises of Federal University of Technology Minna (FUTMINNA), Niger State was mixed in 9ml of sterile distilled water. Aliquot of 1ml of the mixture was transferred to a tube containing 9ml of sterile distilled water. The procedure was repeated to obtain serial dilution and 0.1 ml from the fourth tube (with the dilution of 4^{-4}) was poured into a petri dish then potatoes dextrose agar was poured into the same Petri dish and gently rocked to mix. The plates were incubated at ambient temperature for 48 hours for the fungal growth. Individual colonies were isolated and subcultured on same medium to obtain pure cultures. The fungal isolates were identified according to the taxonomic key of Alexopoulos *et al*. (1990) on the basis of their macroscopic and microscopic characteristics viewed through a microscope with a magnification of 40 objectives. The organisms were maintained on PDA slants and stored at 4°C. The biochemical and physiological properties of the isolates were confirmed using the protocol of Hewa *et al*., (2014).

Screening of Isolates for Starch Hydrolysis

The isolates were screened for starch hydrolyzing ability. The fungal isolates were inoculated on 1 % starch PDA plate. The sterilized medium was cooled and aseptically 300 mg of Norfloxacin per 100 ml of medium was added as antibacterial. Each medium plate was point inoculated with the spores of the fungal isolates and incubated at ambient temperature for five days. The growth pattern and starch hydrolysis were evaluated qualitatively only by visual examination after flooding the plates with iodine solution and measuring the clear zones of starch hydrolysis around the growths of the fungal isolates. Hyper-producer of amylase was chosen and employed in subsequent experiments (Akpan *et al*., (1999), Alfred, (2007).

Inoculum Preparation

The best-selected strain was subcultured at 30 ± 2°C under the dark condition and one week-old-PDA slant culture was used for development of inoculum. A spore inoculum was prepared by adding 10-15 ml of sterile Tween 80 (0.8%) to the slant and shaking vigorously using potato dextrose broth. One ml of spore suspension ($1x10^7$spores/ml) was used per flask to carry out solid state fermentation.

Production of Amylase and Glucoamylase Enzyme from Maize Malt

White and yellow maize samples were washed properly with water and soaked in water for 48 hours. The soaked maize was rinsed and the water changed twice a day to prevent it from fermenting and producing odour.

The maize was spread on a perforated tray to allow excess moisture drain and give aeration for germination/sprouting for 5 days with intermittent turning and wetting. The sprouted maize was dried in an oven at 50 °C for 48 hours. The dried maize malt was ground into powder and used as a source of amylase enzyme.

Production of Amylase and Glucoamylase Enzyme through Solid state fermentation

The following procedures were carried out for the production of this enzyme through solid state fermentation using maize as a substrate and inoculated with the isolate that showed the best amylolytic activity in the preliminary screening for hydrolysis of starch.

Preparation and Inoculation of Media for Solid State Fermentation

Maize was reduced to smaller sizes by pounding and used as a substrate for growth of the selected isolate. Sixty grams of ground maize was weighed and mixed with 45ml of basal salt solution consisting of; $(NH_4)_2SO_4$, 4g/l, KH_2PO_4, 2g/l, $MgSO_4$, 1g/l, $FeSO_4.7H_2O$, 6.3µg/l, $MnSO_4$, 0.1µg/l, $ZnSO_4$, 6.2µg/l (Bertolin, *et al.,* 2003). The medium was sterilized by autoclaving at 121°C for 15 mins. The pH was adjusted to 4.5 using acid. About $1x10^7$ spores/ml of the inoculum was used to inoculate the media contained in a conical flask (Patel, *et al.*, 2009). The culture was incubated at 30°C for fermentation to take effect for 7 days.

Extraction of Glucoamylase and Amylase Enzyme Produced Through Solid State Fermentation

After 7 days of fermentation, the whole mixture was treated with 100ml of cold (4°C) distilled water. It was stirred and agitated thoroughly for 30 min then filtered through muslin cloth. The residue was again treated with 100ml distilled water in the same way and filtered. The filtrate was centrifuged at 6000 rpm for 20minutes. The supernatant obtained was again filtered through Whatman filter paper No. 1 so as to obtain a cell-free supernatant which was used as a source of crude enzyme for assaying glucoamylase and amylase(Mahmoud *et al.*, 2007).

Enzyme assay

Two grams of yellow and white maize malt powder used as a source of crude enzyme was dissolved in 20ml of citrate buffer each. The mixture was stirred and agitated for 10mins then filtered. Two millilitres of each filtrate was pipetted into a test tube. Aliquot of 1ml of 1% starch solution was added into the test tube and incubated at 40°C for 30mins. Alpha-amylase and glucoamylase were obtained by incubating 1% of soluble starch and 1% maltose respectively in 0.1 M citrate buffer of pH 5.0 at 45°C for 30 min. Two milliliters of DNS solution was added to each of the test tubes and the tubes were immersed in boiling water bath (100°C) for 5mins to stop the reaction. The tubes were cooled. The final volume was brought to 20ml with distilled water. The absorbance for all the test tubes was measured with a spectrophotometer at 540nm and compared with a standard curve prepared using 0.10 to 1.0 mg of glucose/ml.

Enzyme activities were expressed in International Units (IU). One IU is defined as one µmol of glucose (for amylase and glucoamylase activity) equivalents released per minute per ml under the standard assay conditions by using glucose standard curve (Silva *et al* 2005). Appropriate dilution factors were used during the estimation of enzyme activity. Absorbance was measured at 540 nm using the UV-Visible spectrophotometer. Alpha-amylase and glucoamylase activity were determined according to the method reported by Tang-um *et al.*, (2012).

Hydrolysis of Gelatinized Starch using Amylase and glucoamylase

This was carried out using modified method of Nweke and Abiamere, (2014), 100g of cassava starch was weighed accurately and transferred into a clean 1000ml beaker.

One hundred milliliters of distilled water was measured with a measuring cylinder and poured into the beaker containing the measured cassava starch.

The mixture was stirred thoroughly to produce a white slurry paste of cassava starch and water. Five hundred milliliters of boiling distilled water was added to the starch slurry in the beaker while stirring simultaneously. The slurry was continuously stirred for 10 mins until all the starch was gelatinized forming a thick, translucent paste. Twenty grams of maize malt powder used as a source of amylase was measured and poured into the gelatinized starch paste. The mixture was stirred continuously until liquefaction starts to take place. The mixture was immersed in a water bath to maintain the temperature at 90 °C in order for complete liquefaction to take place. This was allowed for 1hr with intermittent stirring.After liquefaction, the mixture was allowed to cool. The pH was adjusted to 4.5 with acid while monitoring with a pH meter. The temperature was confirmed to be 60 °C with the aid of a thermometer. Twenty milliliters of enzyme filtrate obtained through solid state fermentation by *Aspergillus niger* was introduced into the mixture as the Glucoamylase and amylase sources.

The mixture was stirred and maintained at 60°C for 3hrs in order for saccharification to take place so as to allow the breakdown the entire remaining starch, maltose, and other dextrins into glucose. Thereafter a cheesecloth was used to extract the syrup-like filtrate. The beakers containing syrup-like filtrate were then transferred to a fume cupboard for evaporation and concentration. The pH of the resulting concentrated glucose syrup was determined. The Brix was measured with a refractometer, and the glucose content was determined using Fehling's method of glucose estimation.

Proximate Analysis and physicochemical properties of Glucose Syrup

This was carried out according to the method of A.O.A.C (1990). The proximate analysis and physicochemical properties of Glucose Syrup such as water activity, total soluble solids and pH, colour, moisture, protein, crude fiber, fat, ash and carbohydrate contents were determined.

Effect of incubation temperature on enzyme production

The effect of incubation temperature on enzyme production was determined by incubating the inoculated (with $1x10^7$ density) flasks having 5 g of the substrate from yellow or white maize with 20 ml of mineral medium of pH 5 at different temperatures ranging from 15, 25, 30, 40, 50 and 55 °C for 5 days in a biological oxygen demand (BOD) incubator.

Effect of inoculum concentration on enzyme production

The effect of inoculum size based on the number of spores was studied using the spore concentration of $1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$ and $1x10^8 1x10^9$, $1x10^{10}$spore ml^{-1} of Tween-80 (Kheng and Omar 2005). The flasks having the sterile basal medium were inoculated with the different spore suspensions and were incubated at 30°C on a BOD shaker cum incubator for 96 h and then the partially purified enzymes produced were used for the evaluation of the enzyme activities.

Effect of incubation period on enzyme production

Flasks containing 5 g of substrate from yellow or white maize were inoculated with 1 ml (10^7 spores/ ml) of spore suspension in 20ml of basal medium and incubated at ambient temperature. The enzymes were obtained and assayed after 3, 4, 5, 6,7days interval.

Effect of initial pH on enzyme production

The effect of initial pH on enzymes production was investigated by adjusting the initial pH of Mineral medium to 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0. The flasks containing 5 g of substrate from yellow or white maize and 20 ml of sterile mineral medium were inoculated with 1 ml of spore suspension (10^7 spores) and incubated for 5 days at 28 ± 2 °C in a BOD incubator and enzyme activities determined.

RESULTS

Isolation and Preliminary Screening of Isolates

The soil obtained from FUTMINNA showed the various diversities of fungi present in the ecological niche with many isolates that can be utilized for amylase and glucoamylase production. They were fully characterized using cultural and morphological characteristics (Table 2). The isolate was further screen to select the best isolate that can hydrolyze starch with the aid of their amylolytic enzyme. It was observed that *Aspergillus niger* had the largest zone of clearing of 35.0 mm when compared to *Fusarium pallidorosium* that had the lowest clear zone of 5.0 mm (Table 4). The best-screened isolate with the most amylolytic activity on the starch agar was then coded *Aspergillus niger* AM01 (Fig 1).

Microscopic examination was later used to confirm the features of the most active strain. It was observed that it had a conidial head, radiate, tending to split into loose columns with age. Conidiophore stripes had a smooth-walled, hyaline but often in brown colour (Plate 2).

Biochemical and physiological characterization of isolates

The biochemical and physiological test carried out revealed that *Aspergillus niger* AM01 could utilize dextrose, dextrose, fructose, lactose, maltose, mannitol, sucrose, starch, xylose as a carbon source but it was observed that sorbitol, adonitol, inositol, raffinose were not utilized by the organism.

The best incubation evaluation carried out showed that *Aspergillus niger* AM01 can grow from 20°C to 35°C while the optimum temperature was obtained at 30°C. It can also grow at pH ranges of 5 to 10. Moreover, *Aspergillus niger* AM01 can grow at the salt concentration ranging from 1 % to 4 % (Table 3).

Optimization of Amylase and Glucoamylase Production

The best optimum condition was determined when different substrate was utilized (yellow and white maize). It was observed that the best temperature of 30°C produced the optimum glucoamylase and amylase enzyme of 7.6 IU and 4.3 IU when yellow maize was used as substrate but glucoamylase and amylase enzyme of 5.1 IU and 2.3 IU was obtained when white maize was used.

Also, the best incubation period was on 5 day with optimum glucoamylase and amylase enzyme of 7.5 IU and 4.2 IU obtained when yellow maize was used as substrate but glucoamylase and amylase enzyme of 4.4 IU and 2.3 IU was obtained when white maize was used. The best inoculum concentration that favours the best enzymes activities was 1×10^6 CFU/ml. It was discovered that the optimum glucoamylase and amylase enzyme of 6.3 IU and 4.1 IU were obtained when yellow maize was used as substrate but glucoamylase and amylase enzyme of 3.7 IU and 2.5IU was obtained when white maize was used. Moreover, the optimum pH was pH of 5. It was observed that the optimum glucoamylase and amylase enzyme of 6.5 IU and 4.2 IU were obtained when yellow maize was used as substrate but glucoamylase and amylase enzyme of 5.1 IU and 3.2 IU was obtained when white maize was used (Table 3 and 4).

Production of Glucose Syrup

The most active screened strain *Aspergillus niger* AM01 was then utilized for the conversion of gelatinized cassava flour to glucose syrup using sprouted white and yellow maize Plate 3.

Proximate Analysis and Physicochemical Properties of Glucose Syrup

The Crude protein and Carbohydrate values of the syrup from yellow maize enzymes was more than the syrup from white maize (Table 4). 0.50 ± 0.2^b, 0.75 ± 0.4^a crude protein for white maize amylase and glucoamylase values and 0.50 ± 0.1^b, 0.76 ± 0.3^a values for yellow maize respectively. The carbohydrate values for both maize types for amylase and glucoamylase are 13.10 ± 3.1^c, 13.25 ± 2.5^c and 14.25 ± 3.3^b and 15.20 ± 2.6^a respectively.

Table 2: Preliminary screening of various strains of fungi isolated for amylase production

S/N	Fungus	Amylase Activity (Clear zone mm)
1	*Rhizopus stolonifer*	10.0 ± 1.1^b
2	*Saccharomyces cerevisiae*	3.0 ± 0.5^d
3	*Aspergillus niger*	35.0 ± 2.8^a
4	*Aspergillus flavus*	12.0 ± 1.0^b
5	*Fusarium oxysporium*	8.0 ± 0.5^{bc}
6	*Fusarium pallidorosium*	5.0 ± 0.3^{cd}

Means with different superscripts within the same column are significantly (P=0.05) different

Fig1: A: *Aspergillus niger* AM 01 growing on Starch agar

B. Colour change after treatment with lugol's iodine

Table 2: Characterization of the isolated fungi.

Isolate code No	Characteristics	Probable fungus
1	Colony whitish becoming grayish-brownish sporangiophores and brown-black sporangia, often over 20mm high. Sporangiophores are colourless to dark brown, smooth or slightly rough-walled stolons opposite the branched rhizoids. Sporangia globose to subglobose, ovoid, blackish-brown at maturity.	*Rhizopus stolonifer*
2	Colonies of *Saccharomyces* grow rapidly and mature in three days. They are flat, smooth, moist, glistening or dull, and cream to tannish cream in color. Blastoconidia (cell buds) are observed. They are unicellular, globose, and ellipsoid to elongate in shape. Multilateral (multipolar) budding is typical.	*Saccharomyces cerevisiae*
3	Colonies at 25^{o}C attaining a diameter of 4-5cm within 7 days, usually consisting of a compact white or yellow basal felt with a dense layer of dark brown to black conidiophres. Conidial heads, radiate, tending to split into loose columns with age. Conidiophore stipes smooth-walled, hyaline but often in brown colours. Vesicles globose to subglobose.	*Aspergillus niger*
4	Colonies at 25 ^{o}C attaining a diameter of 3-5cm within 7 days, usually consisting of a dense felt of yellow-green conidiophores. Conidia heads, radiate tending to split into loose columns with age. Conidiophores stipes smooth-walled, hyaline but often in brown colours.	*Aspergillus flavus*
5	Colony at 25^{o}C attaining a diam. of 4.5 cm in 4 days. Aerial mycelium sparse or floccose, becoming felting, whitish or peach, usually with the purple tinge more intense near the medium surface. Variable in shape and size, ovoid-ellipsoidal to cylindrical, straight or slightly curved.Conidiophores are usually short branched on phialides.	*Fusarium exosporium*
6	Colonies 25 ^{o}C attaining a diameter of 6cm in 4 days, white often with a peach tinge. Aerial mycelium floccose, whitish or peach often changes to brownish. Sporodochia absent. Conidia 3-5(7) septate fusiform straight or somewhat curved, wedged shaped, apical cell beaked, chlamydospore often sparse, hyaline, smooth-walled globose.	*Fusarium pallidorosium*

Table 3: Biochemical and physiological characteristics of *Aspergillus niger* AM 01

Sugars	Acid production from sugars	temperature (^{o}C)	Growth at different temperatures	pH	Growth at different pH	NaCl (%)	Growth at different NaCl conc.
Sorbitol	-	5	-	4	-	1	+
Adonitol	-	10	-	5	+	2	+++
Dextrose	+	20	+	6	+	3	++
Fructose	+	25	++	7	+	4	++
Inositol	-	30	+++	8	+	5	-
Lactose	+	35	++	9	+	6	-
Maltose	+	40	-	10	+	7	-
Mannitol	+	45	-			8	
Raffinose	-	50	-			9	-
Sucrose	+					10	-
Xylose	+						
Starch	+						

+++ = Utilized strongly, ++ = Utilized moderately, + = Utilized slightly,- = No activity

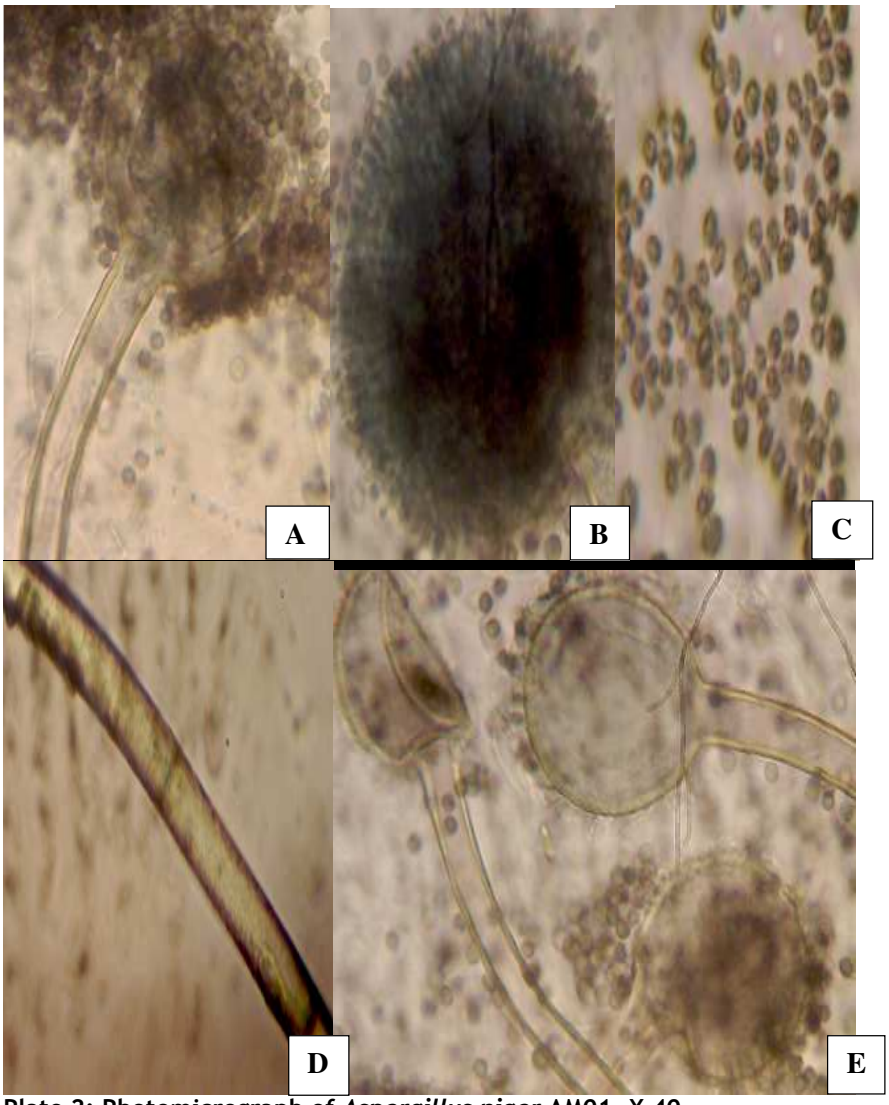
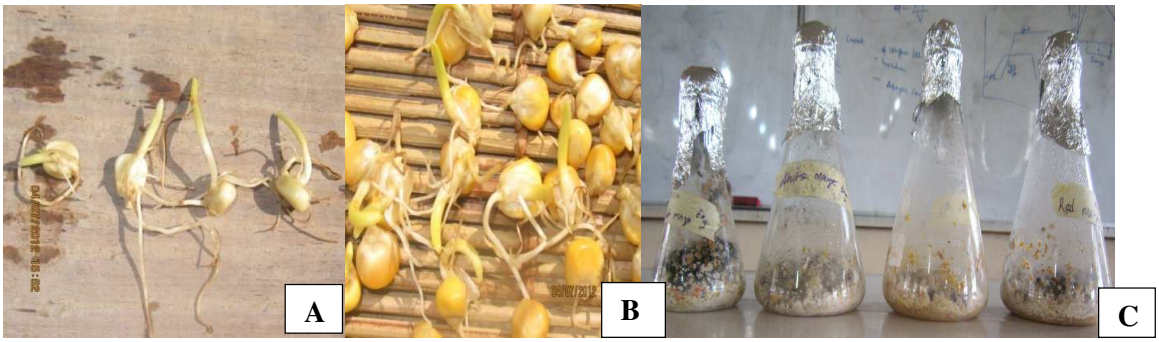


Plate 2: Photomicrograph of *Aspergillus niger* AM01. X 40
A: Mature sporangium with hyphae, B: Conidial heads C: Spores D: Hypae E: Conidophore stripes with smooth-walled



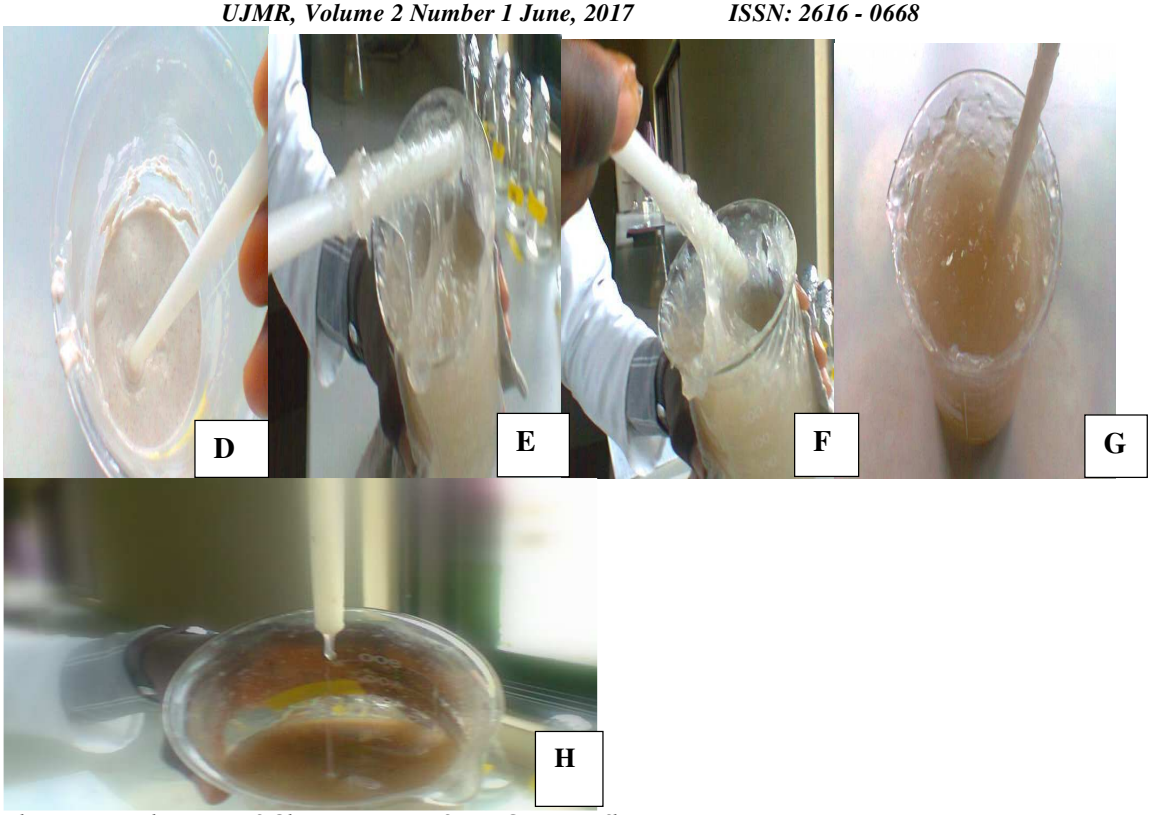
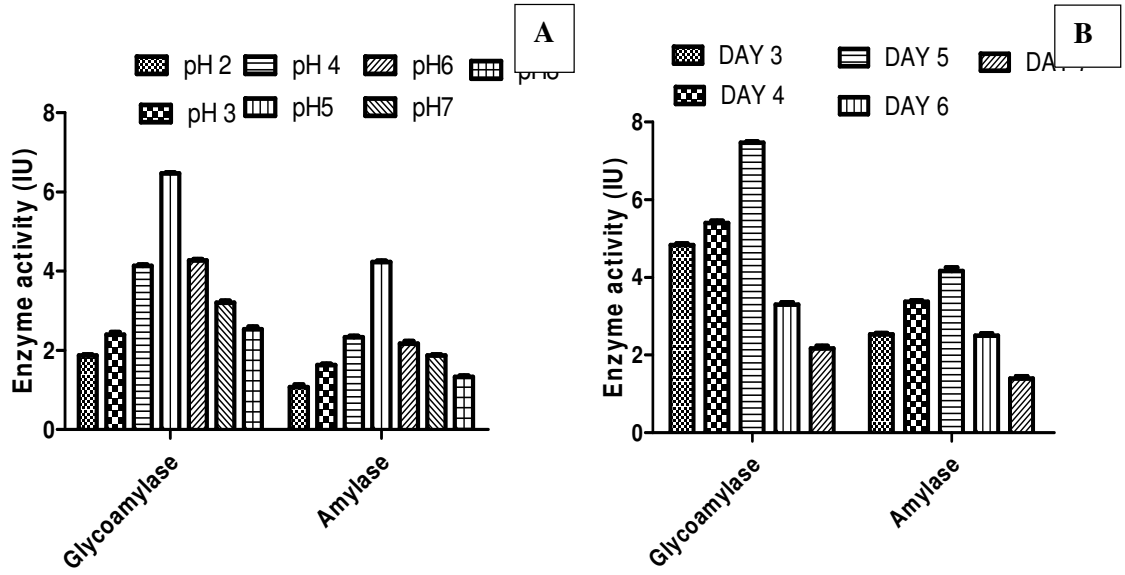


Plate 3: Production of Glucose syrup from Cassava flour
A: Sprouted white maize B: Sprouted yellow maize C: Solid state fermentation D:intermittent stirring E: Complete liquefaction F: Syrup from white maize G: Syrup from yellow maize; H: Process of pH adjustment.



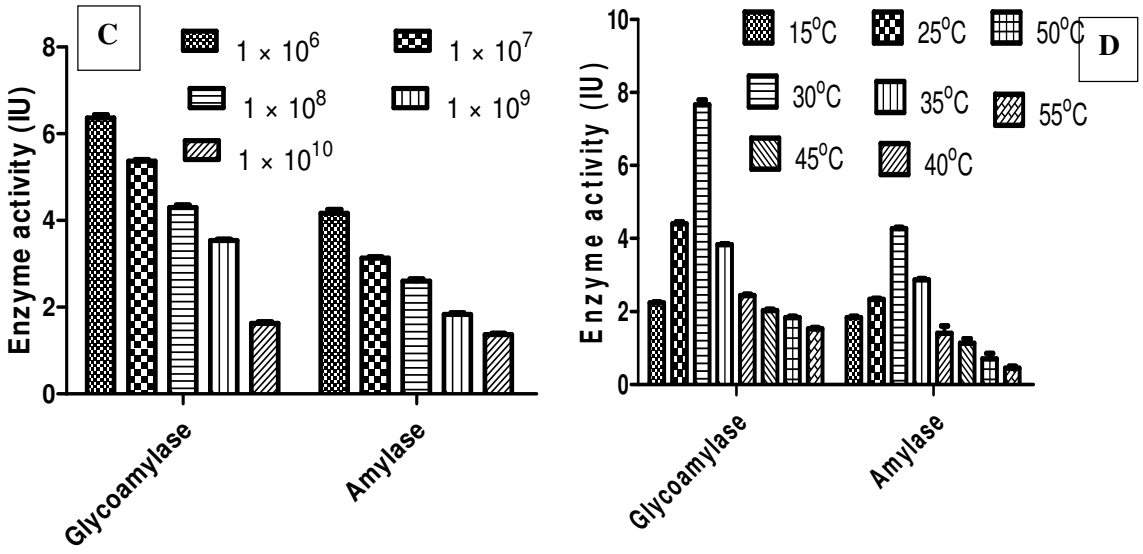
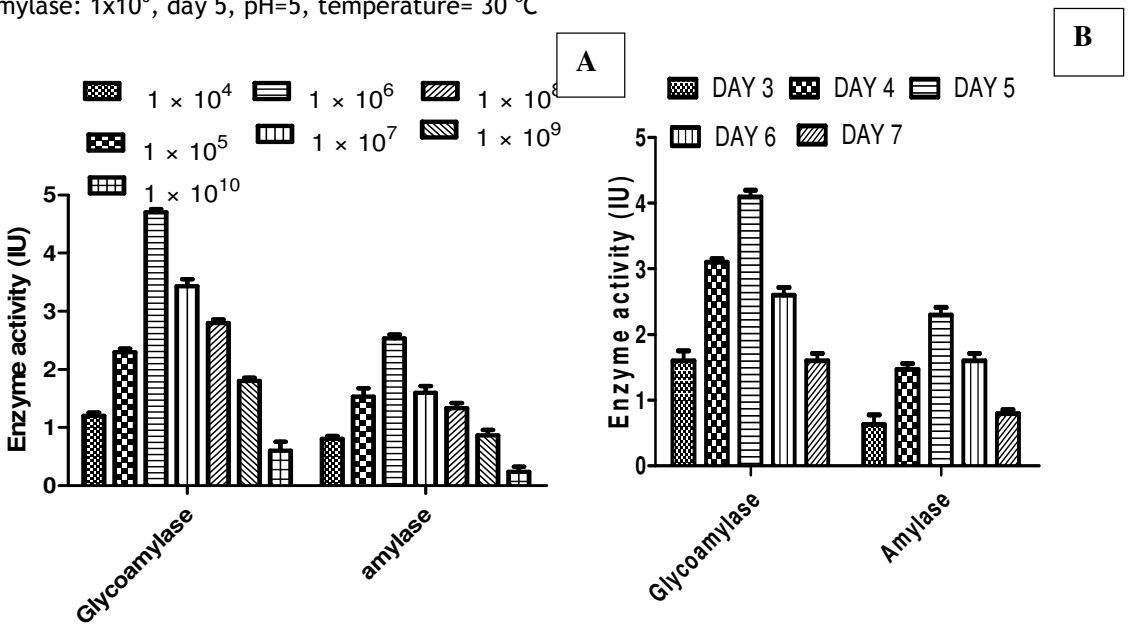


Fig 1: Influence of different factors on enzyme activities when yellow maize was used as a substrate;
A = pH, B=Incubation days, C=Inoculum development, D = Temperature.

Summary:

Glucoamylase: 1x10^6, day 5, pH=5, temperature= 30 ^{o}C;
Amylase: 1x10^6, day 5, pH=5, temperature= 30 ^{o}C



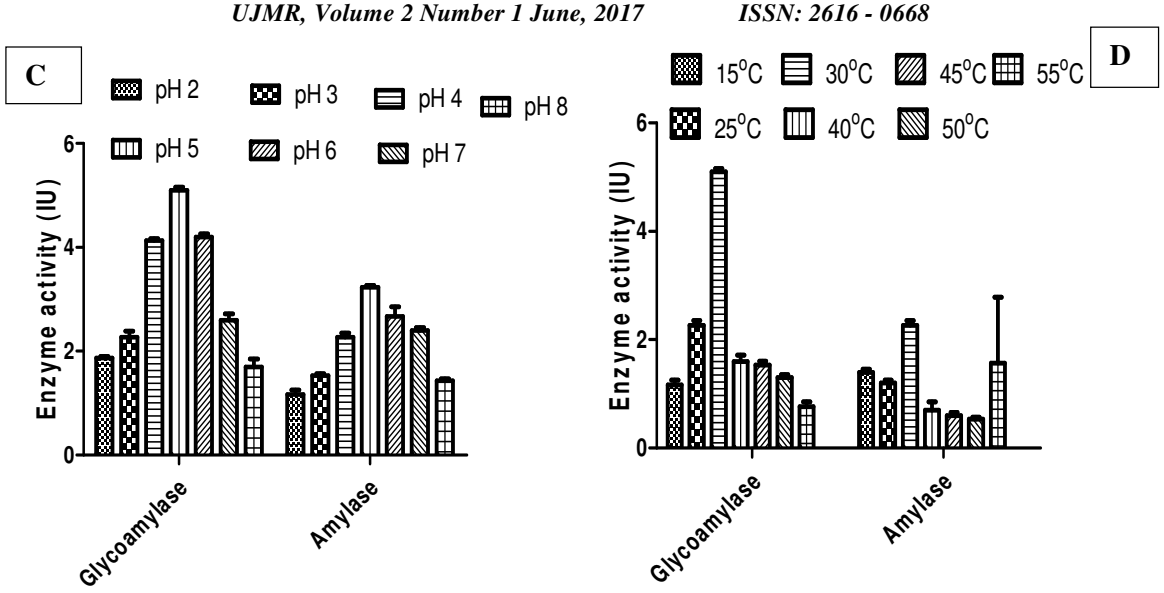


Fig 2: Influence of different factors on enzyme activities when White maize was used as a substrate;
A=Inoculum development, B=Incubation days, C = pH, D = Temperature.

Summary:
Glucoamylase: $1x10^6$, day 5, pH=5, temperature= 30 ^oC;
Amylase: $1x10^6$, day 5, pH=5, temperature= 30 ^oC

Table 4: Proximate, Physicochemical Properties and Enzyme Activities of Syrup Produced by *Aspergillusniger*AM01 using yellow and white maize

Proximate and Physicochemical paprameters	Amylase activity (mm)			Glucoamylase activity (mm)		
	White maize	Yellow maize	Control	White maize	Yellow maize	Control
Moisture	84.0±3.2^{bc}	82.0±4.1^{c}	98.0±2.9^{a}	86.0±3.2^{bc}	88.0±2.6^{b}	95.0±3.1^{a}
Ash	0.23±0.1^{c}	0.22±0.1^{c}	0.85±0.3^{a}	0.25±0.1^{c}	0.26±0.1^{c}	0.75±0.2^{b}
Protein	0.50±0.2^{b}	0.50±0.1^{b}	0.41±0.1^{bc}	0.75±0.4^{a}	0.76±0.3^{a}	0.31±0.1^{c}
Carbohydrate	13.10±3.1^{c}	13.25±2.5^{c}	6.4±1.1^{e}	14.25±3.3^{b}	15.20±2.6^{a}	8.4±0.6^{d}
Fat	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
Fiber	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
Sulphate	1.36±0.2^{bc}	1.28±0.9^{c}	1.62±0.3^{a}	1.42±0.2^{b}	1.45±0.1^{b}	1.60±0.3^{a}
Viscosity (%)	56.4±3.5^{d}	62.1±3.2^{c}	38.6±2.8^{f}	69.2±3.7^{b}	73.4±5.1^{a}	43.8±3.9^{e}
pH	5.68±0.4^{a}	5.53±0.3^{a}	4.2±0.4^{b}	6.0±0.2^{a}	6.3±0.2^{a}	5.7±0.3^{a}
Brix	8.7±0.6^{c}	9.4±0.5^{b}	6.8±0.4^{d}	10.9±0.8^{a}	11.3±0.4^{a}	6.3±0.2^{e}
Reducing sugar(mg/ml)	20.0±1.0^{bc}	22.0±0.9^{ab}	24.0±2.1^{a}	16.0±2.0^{d}	18.0±1.3^{cd}	22.0±0.8^{ab}
Enzyme activities	5.0±0.2^{b}	5.4±0.1^{b}	1.4±0.2^{d}	7.5±0.8^{a}	7.9±0.4^{a}	2.8±0.6^{c}

Means with different superscripts within the same row are significantly (P=0.05) different

DISCUSSION

The strain used during this study showed that it has the ability to utilized starch and other sugars as a source of carbon and energy. The result obtained during this study by Mchunu *et al.*, (2013) observed that monosaccharides showed good assimilation by *Trichoderma reesei* and *Aspergillus niger*. *Aspergillus niger* AM 01 was observed to possess amylopectin enzyme which enhances it starch degrading ability. These findings are in line with the research conducted by the various researcher (Omemu *et al.*, 2005; Pandey *et al.*, 2006 and Sasi *et al.*, 2010) where the collection of potent species was made by plate method. However, zones of clearing was associated with the amount of enzyme formed (amylase) during analysis of enzyme activity using starch as a sole source of carbohydrate.

The amylolictic property of *Aspergillus niger* AM 01 isolated from soil of FUTMINNA was check by blue-black coloration obtained after flooding the plate with starch iodine. The occurrence of amylolytic organisms from the soil agrees with earlier reports of Adebiyi and Akinyanju (1998) that the soil is known to be a repository of amylase producers. *Aspergillus niger* AM 01was selected among all the isolated strains on starch agar. This is because it gave a direct visual indication of starch hydrolysis (Akpan *et al.*, 1999a).

This study showed that the utilization of raw starch to by the crude enzymes of *A. niger* AM 01 is significantly dependent on the starch source and time of incubation. This agrees with earlier reports of Okolo *et al.*, (1995) that the susceptibility of starch granules to digestion by amylase is dependent on starch source and length of amylase or glucoamylase treatment. The high digestibility of raw cassava starch observed in this study is similar to the findings of Okolo *et al.*, (1995). The ability of the crude amylase of *A. niger* AM01 to hydrolyze the root starches especially cassava starch presents a remarkable property since these root starches are abundantly available in the tropics. According to Anthony *et al.*, (1996) and Oluwole *et al.*, (1999), over 30 million tons of cassava is lost yearly since cassava is perishable after harvesting. Conversion of raw cassava by this enzyme means that some of the cassava could be used as raw materials by the starch industry for value-added products. This will reduce wastage and improve economic gains. The result obtained from the proximate analysis is in line with Oino Food Ltd. (2004).

CONCLUSION

The fungal enzyme from *Aspergillus niger* using white maize as the substrate for solid state fermentation has proven to be a good tool for the enzyme hydrolysis of cassava starch for the production of glucose syrup. This can be harnessed by industries for the commercial production of glucose syrup.

It was concluded from this study that the enzymes, glucoamylase, and amylase, produced by *A. niger* both isolated from FUTMINNA soil showed optimal characteristics, such as optimum pH and temperature and as well good thermal stability, that can be used in the industrial process such as starch processing. The amylolytic enzymes produced by *A. niger AM01* are suitable for biotechnological applications, considering the high productivity of these enzymes, the good capacity to degrade starch and its derivates.

Furthermore, the technology used for glucose and fructose syrup production using Nigeria cassava starch and enzymes produced by soil microorganisms was successful and can be carried out on an industrial scale.

REFERENCES

Akinola, S. O., Ayanley, T. A. (2004). The use fungal glucoamylase enzyme for the production of Glucose syrup from cassava starch. Journal of Life and Physical Sciences.1(2): 138-141

Akpan I. Bankole MO, Adesemowo AM (1999a). A rapid plate culture method for screening of amylase producing microorganisms. Biotechnol. Tech.13: 411-413.

Alexopoulos, C. J., Charles, W. M. and Blackwell, M. (1990) Introductory mycology, 4th edition. Blakiston company, Philadelphia. 15-630.

Anthony OE, Yusuf C, Murray M (1996). Culture of Saccharomyces cerevisiae on hydrolysed waste cassava starch for production ofbaking quality yeast. Enzyme Microbial Technol. 18(7): 519 – 525.

Berka, R.M., N. Dunn-coleman, M. Ward, 1992. Industrial enzymes from Aspergillus species. In Aspergillus: Biology and Applications. Bennets, J.W., & Klich, M.A. (Edn.). Oxford, Heinemann, pp., 155-202.

Biesebeke, R., E. Record, N. van Biezen, M. Heerikhuisen, A. Franken, P. J. Punt, C. A. van den Hondel, 2005. Branching mutants of Aspergillus oryzae with improved amylase and protease production on solid substrates. Appl. Microbiol. Biotechnol., 69: 44-50.

Cabello, C. (1999). Amylases using in glucose syrup production. Seminario Brazileiro De Tecnologia Enzimatica - Eezitec, Rio de Janeiro. Anais 1: V1-V3.

Federal Office of Statistics, Nigeria.(2000). Annual Report 2000. pp616.

Gupta, R., P. Gigras, H. Mohapatra, V.K. Goswami, B. Chauhan, (2003). Microbial α-amylases: biotechnological perspective. Process Biochem., 38: 1599-1616.

Hewa O. H, Hakki A, Hero M. I, Ali G. (2014). The Effect of Some Sugars on the Growth of Aspergillus niger. KSU J. Nat. Sci., 17(4),

Ipek, C. (2006). Colour formation in wheat starch-based glucose syrup and use of Activated carbons for sugar decolourization. A Thesis Submitted to the Graduate School of Natural and Applied Sciences of Middle East Technical University. 7-18

Krieger, K. M., Duvick, S. A., Pollak, L. M., and White, P. J. (1997). Thermal Properties of Corn Starch Extracted with different blending methods: Microblender and homogenizer. Cereal Chem. 74: 553 – 555.

Mahmoud, M. A., Al-Agamy, M. H. M., El-Loboudy, S. S. and Ashour, M. S. (2007).Purification and characterization of neutral protease from soil strain of Bacillus subtilis. Journal of Microbiology, 26:212-317.

Mchunu, N. P., Permau, K., Alam, M., Singh, S., 2013. Carbon utilization profile of a thermophilic fungus, Thermomyces lanuginosus using phenotypic microarray. Advances in Bioscience and Biotechnology, 4: 24-32.

Monica, M., Vionel, M. (2006).Hygienic aspects of the production of glucose syrups through Acid hydrolysis. Food Technology.Vol. x No. 1

Norouzian, D., A. Akbarzadeh, J.M. Scharer, M.M. Young, 2006. Fungal glucoamylases. Res. Rev. Paper, 24: 80-85.

Nweke F.N, Abiamere O. C (2014). Glucose syrup production from cassava peels and cassava pulp. Int.J.Curr.Microbiol.App.Sci (2014) 3(12) 781-787

Oino Food Ltd. (2004). Glucose syrup, www.oinofood.com/glucosesyrup.htm, China. Accessed January 2017.

Okolo BN, Ezeogu LI, Mba CN (1995). Production of raw starch digesting Amylase by Aspergillus niger and Bacillus alvei grown on Native starch Sources J. Sci. Food Agric. 69:109-115.

Oluwole OB, Joaquim AA, Olatunji AU, Ozumba AU, Odunfa SA (1999).Assessment of the pasting and organoleptic qualities of cocoyam flour from selected Xanmthomonas sp. Proceedings of 23 rdannual conference of NIFST.

Omemu, A. M., Akpan, I., Bankole, M. O., Teniola, O. D., 2005. Hydrolysis of raw tuber starches by amylase of Aspergillus niger AM07 isolated from the soil. Afr. J. Biotechnol., 4 (1): 19-25.

Pandey, A., Nigamp, V. T., Socco, L., Singh, D., Mohan, R., 2006. Advances in microbial amylases. Biochem, 31: 35-152

Pandey, A., S. Radhakrishnan, (1993). The production of glucoamylase by Aspergillus niger NCIM 1245, Process Biochem., 28: 305–309.

Sasi, A., Kani, M., Panneerselvam, A., Jegadeesh, G., Muthu, K., Kumar, M.R. 2010. Optimizing the conditions of amylase by an Esturian strain of Aspergillus sp. Afr. J. Microbiol. Res., 4 (8): 581-586.

Silva E D, Gomes E, Souza S R, Grandi R P (2005). Production of thermostable glucoamylase by newly isolated Aspergillus flavus A.1.1 and Thermomyces lanuginosus A 13.37. Braz. J. Microbiol. 36: 75-82.

Silva, R. N., Asquieri, E. R., Quintino, F. P., Monteiro, V. N.(2010). Production of glucose and fructose syrup from cassava (Manioc esculentaCrantz) starch using enzymes produced by microorganism isolated from Brazilian cerrado soil. Ciencia e Tecnologia de Alimentos.30(1): 213-217

Sindhu R, Suprabha G N, Shashidhar S (2009). Optimization of process parameters for the production of alpha-amylase from Penicillium janthinellum (NCIM 4960) under solid state fermentation. Afr. J. Microb. Res. 3(9): 498-503.

Sodhi H K, Sharma K, Gupta J K, Soni S K (2005). Production of a thermostable alpha-amylase from Bacillus sp. PS-7 by solid state fermentation and its synergistic use in the hydrolysis of malt starch for alcohol production. Process. Biochem. 40: 525-534.

Tang-um, J., and Niamsup H. Chitinase production and antifungal potential of endophytic Streptomycesstrain P4, Maejo Int. J. Sci. Technol. 2012; 6: 95-104.

Tonukari N. J., Osumah, L. I. (2010). Production of yeast using Acid hydrolyzed cassava and poultry manure Extracts. African Journal of Biochemistry Research. 4(5): 119-125

Wang, X.J., J.G. Bai, Y.X. Liang, 2006. Optimization of multienzyme production by two mixed strains in solid-state fermentation. Appl. Microbiol. Biotechnol., 73: 533-540.

White, P. J., Abbas, I., Pollak, L. M., and Johnson, L. (1990). Intra-and Inter-population Variability of thermal properties of maize starch. Cereal Chem. 67: 70 -73.

Zambare, V., 2010. Solid state fermentation of Aspergillus oryzae for glucoamylase production on agro-residues. Int. J. Life Sci., 4: 16-25.