Preliminary Studies on the Digestibility of Maize Starch Using Crude Amylases from *Aspergilus niger*

Suleiman Ali[1], Zainab A. [2], Umar Dan'azumi[1], Mohammed Nur Idris[1], Zakari Abba Ali Garga[3], Shehu Ibrahim Baba Baburo[1], Babagana Umar[1]

[1]*Department of Basic Science Yobe State College of Agriculture, Gujba, Nigeria*
[2]*Department of Biochemistry University of Maiduguri, Nigeria*
[3]*Department of Entrepreneur University of Maiduguri, Nigeria*

Abstract: **- Amylase enzymes are important enzymes employed in starch processing industries for hydrolysis of polysaccharides such as starch and starch based substrates into simple sugar constituents, in this studies, the quantitative production of Maize starch and the Hydrolysis of starch obtained by the crude amylase enzymes from *Aspergillus niger* was investigated. The Results from the various steeping time of the corn starch revealed that at 24 hours yielded 71% of starch, at 48 hours yielded 72.4% of starch and at 72 hours yielded 77.8% of starch. The temperature at which complete granular disruption and the formation of a gelatinized starch solution was found at 64.75^{o}C for initial pasting temperature, 73.00^{o}C for peak temperature and 78.50^{o}C is the temperature at which the starch was completely gelatinized. The digestibility of the starch by crude amylases from *Aspergillus niger* revealed the presence of reducing sugar as 4.70±0.14mg/ml over the period of 10minutes, 5.20±0.28mg/ml over the period of 20minutes, 5.60±0.14mg/ml over the period of 30minutes, 5.30±0.14mg/ml over the period of 40minutes, 5.10±0.14mg/ml over the period of 50minutes and 4.70±0.14mg/ml over the period of 60minutes. At 30 minutes incubation period shows the highest yield of the reducing sugar further incubation result in a decline in the amount of reducing sugar produced. From this studies 30 minutes incubation time, refers to as the optimal hydrolytic reaction time for the starch extracted from our locally produced yellow maize using crude culture filtrate.**

***Keywords*: *Aspergillus niger*, Amylase enzymes, maize**

I. INTRODUCTION

Micro-organism had made significant contribution to the population of foods and beverages in the last three decades. Various industries, such as food, brewing, textile, pharmaceutical and confectionaries depend largely on the various products, especially extra-cellular enzymes produced by these microorganisms (Ibukun and Akindumila, 1998). An extra-cellular amylase, specifically starch digesting amylase has found important application in bioconversion of starch and starch based substrates (Okolo *et al.,* 1995). Industrial conversion of a starch with starch saccharifying amylase has been reported to represent an economically superior alternative to conventional process which uses gelatinizing starch as substrate based on energy utilization and process simplicity (Okolo *et al.,* 1995).

Starch is an abundant source of carbohydrate. It consists of amylose and amylopectin. Amylopectin is formed from linked alpha, 1-4 chain of glucose with linked (α, 1-6) branch points and amylose consists of chain of glucose lined together by α, 1-4 linkage (Parka and Son, 2007). Amylases are starch degrading enzymes that catalyze the hydrolysis of internal alpha 1-4 glycosidic bonds in polysaccharides with the retention of alpha anomeric configuration in the products (Takata *et al.,* 1992).The amylase can be derived from several sources such as plant, animal and microbes (Jiby *et al.,* 2016). Amylases from plants and microbe sources have been employed for centuries in brewing industry. Fungal amylases are widely used for the preparation of oriental foods (Mabel *et al.,* 2006). Amylases of bacteria, fungi and viruses are increasingly studied due to the relative ease of large scale production (low downstream cost as they are extracellular in nature) as compared to amylases from plants and animals and their importance in subsequent application at industry (Ashis *et al.,* 2009). The major advantage of using microorganism for production of amylase is in economical bulk production capacity and microbes are also easy to manipulate to obtain enzymes of desired characteristics (Aiyer *et al.,* 2005**).** The microbial amylases meet industrial demands; a large number of them are available commercially and they have almost completely replaced chemical hydrolysis of starch in starch processing industry (Bernfeld. *et al.,* 1955). Although many microorganisms produce this enzyme, the most commonly used for their industrial application are *Bacillus licheniformis, Bacillus amyloliquifaciens and Aspergillus niger*. (Chengyi et *al.,* 1999, Haq *et al.,* 2002). *Aspergillus niger, Aspergillus awamori and Rhizopus oryzae* have been considered the most important for industrial application (Coutinho, *et al.,* 1997). Amylase of fungal origin was found to be more stable than the bacterial enzymes on a commercial scale, many attempts have been made to optimize culture conditions and suitable strains of fungi (Abu *et al.,* 2005).

Amylases stand out as a class of enzymes, which are of useful applications in the food, brewing, textile, detergent and pharmaceutical industries. They are mainly employed for starch liquefaction to reduce their viscosity, production of maltose, oligosaccharide mixtures, high fructose syrup and maltotetraose syrup. In detergents production, they are applied to improve cleaning effect and are also used for starch de-sizing in textile industry (Chengyi, *et al.,* 1999, Haq, *et al.,* 2004). Due to the increasing demand for these enzymes in

various industries, there is enormous interest in developing enzymes with better properties such as raw starch degrading amylases suitable for industrial applications and their cost effective production techniques (Sivaramakrishnan, *et al.*, 2006). Selection of appropriate carbon and nitrogen sources or other nutrients is one of the most critical stages in the development of an efficient and economic process (Konsula *et al.*, 2004). The growth and enzyme production of the organism are strongly influenced by medium composition thus optimization of media components and cultural parameters is the primary task in a biological process (Djekrif-Dakhmouche, *et al.*, 2004).

Starch, the raw material required for the production of low molecular weight products (glucose/dextrose, maltose, maltotriose and dextrin is widely applied in sugar, spirits, textile as well as brewing (Selmi *et al.*, 2000). 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). Starch is found in the endosperm of cereal grains (Stare and McWilliam, 1977), roots and tubers of crops (Fox and Cameron 1982; Omemu *et al.*, 2004). The conversion of starch to various sweeteners is achieved through a chemical (acid) or an enzymatic process. The use of enzymes however has more advantages to the former (Yankov *et al.*, 1986) due to the formation of undesirably coloured and flavoured breakdown products, and the process appears to be totally random which is not influenced by the presence of α-1, 6-glucosodic linkages and its difficulty to control (Chaplin, 2004) 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). The industrial processing of starch to glucose, maltose and dextrin involves gelatinization, liquefaction and saccharification processes (Hall, 2001).

Raw starch granules are relatively insoluble, nondispersible and resistant amylolysis. Before the starch granule can be readily hydrolyzed by the amylases, the crystalline structure must be disrupted. This can be achieved by gelatinization with chemicals or heat or by extensive mechanical treatment (Leach and Schoch 1961).Starch gelatinization is associated with the disruption of granular structure causing starch molecules to dissolve in water and, as such, is one of the starch's most important and unique properties (Lineback, et al., 1986).As the crystalline organization of the starch granuleis destroyed, the granule swell in the presence of water become more susceptible for the enzymatic breakdown by amylases.

Cereal grains such as maize, millet and sorghum are important staple foods found in the diet of the people within the Northeastern Nigeria. These cereals are widely cultivated within the sub region, and to a larger extent, the country with an aggregate annual production of 23.9 million tones in year 2003 (FAO Reviews, 2006). However, despite their importance, a large proportion of these cereals are lost yearly due to non-availability of appropriate technology and industry to harness these into various useful products such as glucose syrup, maltose syrup, high fructose corn syrup and maltodextrins (Zainab *et al.*, 2011). the objectives of this work was to study the production crude Amylases by using *Aspergillus niger*a nd to analysed its ability to hydrolyses corn starch from our local yellow Maize.

II. MATERIALS AND METHODS

Sample collection/Substrate collection

Yellow Maize (local brought tolerance variety (local comp 3DT)) used as starch substrate was obtained from Borno state Agricultural development program office, Maiduguri. Borno state, Nigeria.

Source of Fungi

Pure isolated (purified and characterized) *Aspergillus niger* fungi was collected from plant pathology laboratory of crop science Department Faculty of Agricultural Science, University of Maiduguri, Borno state - Nigeria.

Corn Starch Extraction

Extraction and quantitative determination of yellow maize starch in the laboratory was done according to standard procedures as described by White *et al.*, (1990) modified by Krieger *et al.*, (1997). In this experiment the Corn kernels was handpicked and cleaned to remove foreign material, mold, and broken kernels before analysis. About Five grams (5g) of the cleaned yellow maize were steeped in 30 ml of 1% sodium metabisulfite solution at room temperature for 24, 48, and 72 hour, (four replicate of five grams starch at each of the steeping hours), followed by manual removal of the pericarp and germ with forceps and crushed to softening using pestle and mortar. Then placed in a 50-ml centrifuge tube with 10 ml of distilled water and homogenized using a vortex type tissue homogenizer at (Ultra Turrax, 170W, 20000 rpm) at 5000 x g for 2 minutes. The homogenized slurry was filtered by using a muslin cloth filter with several washes until the wash water became clear with a total volume of 100ml.The starch slurry was allowed to sediment for 20 hours at room temperature after which the supernatant decanted away and crude starch was washed three times again with 100ml distilled water and allowed to re-sediment again for another 20 hour and re-decanted again. The starch and residues obtained was sundried. All treatments were performed in replicates of four, and the results were averaged.

Starch Yield

In this study, the dried material obtained was referred to as starch, even though the material is not completely pure. The various starch yields were determined as described by Ji *et al.*, (2004). Adopted by Zainab *et al.*, (2011).

% yield = Dry weight of Starch Recovered from Extraction / Dry weight of whole grains x 100.

The weight of starch and that of corn kernels were measured by the same balance to enhance accuracy (Mettalr Toledo weight balance).

Gelatinization of starch

A suspension of extracted starch was gelatinized to make it susceptible for enzymatic breakdown as described by Ji 2004 *et al*., with little modification.

About 3.5g of starch granules was mixed with 10ml of distilled water, the insoluble mixtures in a beaker was immersed in a water bath of constant temperature of 96 ^{o}C with constant stirring. The starch was cooked for 5-7 minutes until the granules swelled up and completely absorbed all the water. The temperature at which the starch starts gelatinizing was noted as initial temperature, temperature at which all the water absorbed was noted as peak temperature where the temperature at which the gelatinization was completed was noted as final temperature and recorded. This procedure was repeated in four replicate the mean was recorded.

Production of Crude Amylase Enzymes

Production of amylases was carried out according to the method described by Omemu *et al.,* 2004 adopted by sheriff *et al.,* (2012).

Preparation of Mineral Salt Media

Mineral salt medium consisting (MSM) of KH_2PO_4 (0.1%) NH_4NO_3 (0.1%) $NH_4(S0_4)_2$ (0.1%) $MgS0_4.7H_2O$ (0.1%) $CaCl_2$ (0.1%) $FeSO_2.7H_2O$ (0.05%) and starch (2%), was prepared and sterilized at 121°C for 15 minutes and cooled to room temperature before inoculation with the fungi.

Inoculation

10ml of sterilized distilled water under aseptic conditions was introduced into petri dish containing fungal spores. It was then scraped with sterilized wire loop to loosen the spores. 0.5ml spore suspension of fungi was inoculated to the mineral salt medium. It was then incubated with intermittent shaking for about 72 hours at room temperature. After which the MSM was filtered to remove the fugal mycelium from the medium through clean sterilized muslin cloth filter. The filtrate contains the crude amylase was kept at 4^{o}C in refrigerator for further use.

Crude Enzyme Extraction

The medium was centrifuged at 8000 rpm for 10 min. The supernatant was filtered through Whatmann No1 filter paper and the filtrate was used as crude enzyme suspension, as described by Murugan *et al.,* (2016).

Crude Enzyme Assay

Enzyme assay was carried out by modified method Omemu *et al.,* (2004). About 0.5ml of gelatinized corn starch was placed into six different test tubes labeled A-F at room temperature 1ml of crude phosphate buffered (pH 4.6) culture extract was added to each test tube. After 10 minutes interval of incubation time 2 to 3 drops of NaOH solution was added to stop the reaction by making the test reaction medium Alkaline. This was followed by addition 1ml dinitrosalicylic acid reagent (DNS) to each test tube and boiled for five minutes then cooled under running tap water. After the addition of 20ml distilled water, the optical density of the solution containing the brown reduction product was determined photo metrically at 540nm by means of Corning colorimeter (253) and a blank was prepared in the same manner without enzyme. A calibration curve established with glucose was used to convert the colorimeter reading into milligram of glucose or maltose.

III. RESULTS AND DISCUSSION

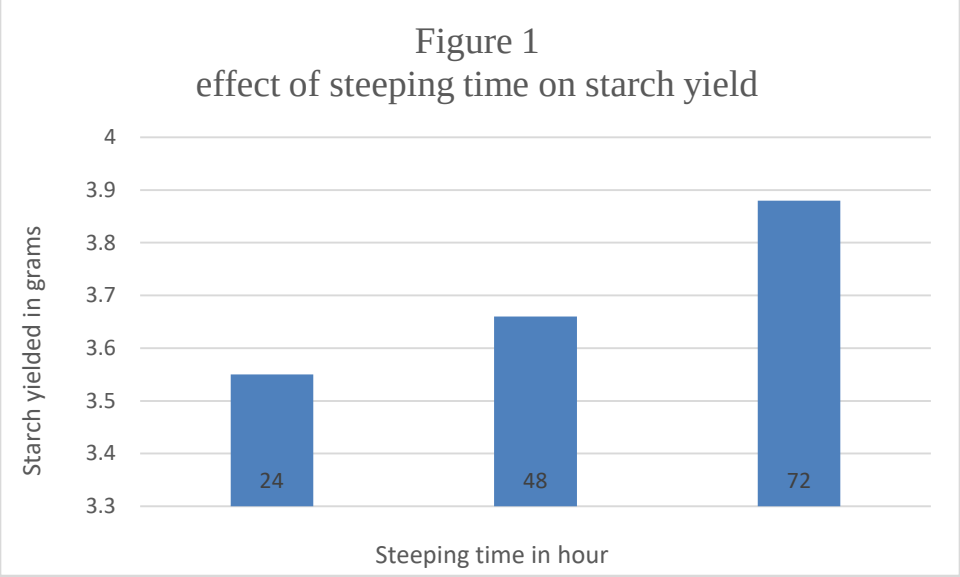

Table 1: Gelatinization Temperature in Degree Celsius

Initial Pasting Temperature in °C	Peak Temperaturein °C	Final Temperature in °C
64.75±1.42	73.00±0.10	78.50±0.50

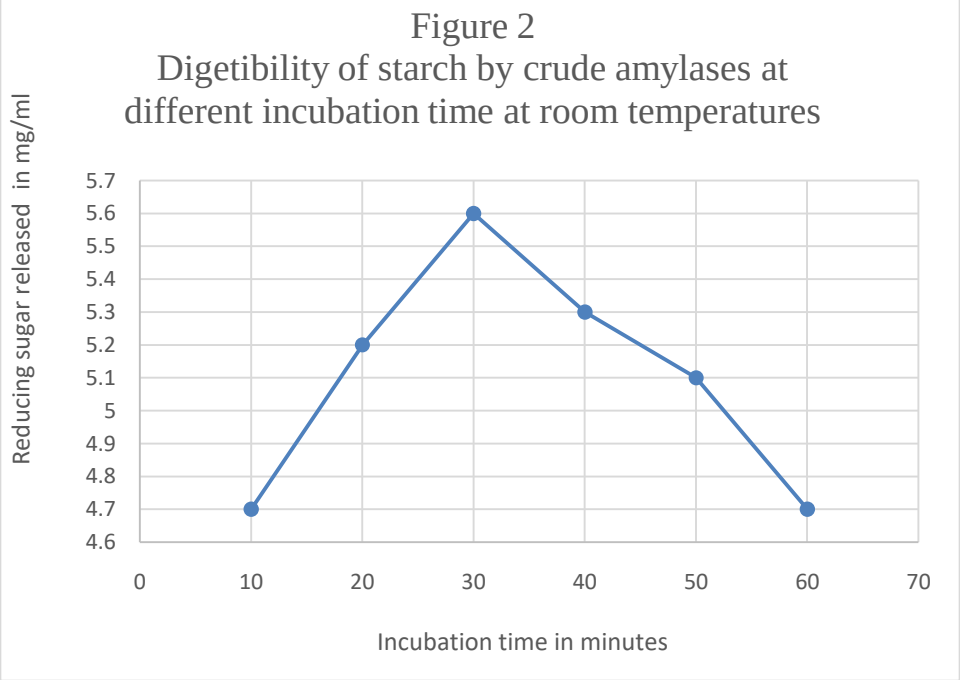

Figure 1 shows the amount (in grams) of starch yielded at various steeping time,3.55g, 3.66g and 3.89g of dried starch yielded at 24, 48 and72hours steeping time respectively. This result shows that, the starch yield increased with an increased in time from24 hoursto72hours this is in agreement with the findings of Wang and Johnson, (1992) who reported that the protein was probably more completely separated from starch when steeping time was greater than 48hrs resulting in high starch yield. A similar pattern of results was reported by Zainab *et al.*, (2011) and Sheriff *et al.*, (2012).

The starch granule's initial pasting, peak and gelatinization temperatures shown in table 1(Mean of four replicate).From the result the64.75°C initial pasting temperature, is in agreement with observation of Jing-ming and Sen-lin (1990) reported that as, the granules lost their integrity and formed a molecular network at 64 °C. Sheriff *et al.*, (2012) reported similar result as the lowest on set gelatinization temperatures for the yellow maize starch. Also, the observation by light microscopy of Wajira *et al.*, (2006) reported that, the large starch granules swelled first and began to break apart at around 55 to 60 °C; small granules did not start to disintegrate until 65 °C. But from our result, the peak and gelatinization temperature is slightly differ from that of previous authors. The differences might be due to plant variety or cultivation method of the used maize plant (Yamin, *et al.*, 1997), corn steeping time (Ji *et al.*, 2004, Sheriff *et al.*, 2012) or the concentration difference (3.5g of starch in 10ml of water) (Tester, *et al.*, 1990, Donald, *et al.*, 2001).

Figure 2 shows the digestibility of maize starch using crude Amylases enzyme from *Aspergilus niger* as 4.70±0.14mg/ml over the period of 10minutes, 5.20±0.28mg/ml over the period of 20minutes, 5.60±0.14mg/ml over the period of 30minutes, 5.30±0.14mg/ml over the period of 40minutes, 5.10±0.14mg/ml over the period of 50minutes and 4.70±0.14mg/ml over the period of 60minutes which showed an increase in reducing sugar concentration as the reaction time increased from 10 minutes through to 60 minutes. At 30 minutes incubation period shows the highest yield of the reducing sugar, further incubation resulted in a declined in the amount of reducing sugar produced. From this studies 30 minutes incubation time, refers to as the optimal hydrolytic reaction time for the starch extracted from our locally produced yellow maize using crude culture filtrate (crude enzymes) from *Aspergillus niger* a fungi species isolated from soil of our local environment.

IV. CONCLUSION

In conclusion, the fungal crude enzyme from *Aspergillus niger* produces amylases that have capabilities of converting Maize starch to products of commercial interest. And also, the

locally produced Yellow Maize (local brought tolerance variety) starch used, has great potential to use as substrate for amylase enzymes produced by locally isolated strain of *Aspergillus niger* fungi.

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