



(RESEARCH ARTICLE)



## Production of amylases by some aspergillus and fusarium species isolated from waste corncobs in Keffi, Nigeria

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### Abstract

Amylases are important industrial enzymes that have wide applications ranging from conversion of starch to sugar syrups, to the production of cyclodextrins for the pharmaceutical industry. This investigation aimed at production of amylases using *Aspergillus* and *Fusarium* species isolated from waste-corncoobs in Keffi Nigeria. Standard microbiological methods were employed for isolation and identification of the fungal isolates. The yields of amylases produced by fungi isolates were determined using Spectrometry. The isolation rate of *Aspergillus* and *Fusarium* species was high in location A, C and D with 60% and location B with 40%. The percentage occurrence of the isolates demonstrated that *Aspergillus carneus* was 40%, *Aspergillus aculeatus* was 60% and *Aspergillus flavus* was 20% while *Fusarium moniliforme* was 80% and *Fusarium redolens* was 40%. The result demonstrated that three species of the fungal isolates *Aspergillus aculeatus*, *Aspergillus carneus* and *Fusarium moniliforme* were found to produce amylases. *Aspergillus aculeatus* isolated from locations C3, D1 and D2 produced 0.018mg/ml, 0.018mg/ml and 0.016mg/ml amylases respectively. Similarly, *Aspergillus carneus* isolated from locations A1 and B2 produced 0.021mg/ml and 0.012mg/ml amylases. *Fusarium moniliforme* isolated from locations A3, C1 and C4 produced 0.010mg/ml, 0.016mg/ml and 0.015mg/ml amylases. Result of effect of (temperature, pH and fermentation time) for production of amylases. Whereas highest amount for amylases produced by *Aspergillus aculeatus* and *F moniliforme* were produced at 28 °C. pH 5.0 was found to be the best optima pH for production of amylases from the fungi studied. *A. carneus* (2.99 mg/ml amylases). The fermentation time showed highest production of amylase by *A. carneus* and *A. aculeatus* after 72 hours while *F. moniliforme* produced at 96hours. The fungi species isolated from soil in keffi can be used for production of amylases.

**Keywords:** Amylases; Spectrometry; Production; Fermentation; Fungi

### 1. Introduction

Amylases are enzyme that convert starch or glycogen. The amylase can be derived from various sources like plant material or products, animal and microbe's products. The major advantage of using microorganism for production of amylase is in large production capacity and microbes are also easy to manipulate to obtain enzymes of desired characteristics Aiyer, 2005[1]. 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 Onoter *et al.* 2011[2]. Although many microorganisms produce this enzyme, the most commonly used for their industrial application are *Bacillus licheniformis*, *Bacillus amyloliquifaciens* and *Aspergillus niger*. Amylases stand out as a class of enzymes, which are of useful applications in the food, brewing, textile, detergent and pharmaceutical industries.

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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[3] and Haq *et al.* 2002[4].

The use of the solid state fermentation (SSF) is advantageous because of the ease of sterilization and process control is easier to engineer in these systems. Depending on the strain and the culture conditions, the enzyme can be constitutive or inducible, showing different production patterns. Submerged fermentation has been defined as fermentation in the presence of excess water. Almost all the large-scale enzyme producing facilities are using the proven technology of SmF due to better monitoring and ease of handling Singhania *et al.* 2010[5]. To meet the growing demands in the industry it is necessary to improve the performance of the system and thus increase the conditions, particularly physical and chemical parameters are important in the development of fermentation processes due to their impact on the economy and practicability of the process Francis and Sabu, 2003[6]. 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.* 2006 [7].

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[8]. 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. This investigation aimed at production of amylases using *Aspergillus* and *Fusarium* species isolated from waste-corn cobs in Keffi Nigeria.

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## 2. Material and methods

### 2.1. Study Area

The study area was Keffi local government Area. Keffi is approximately 68km from Abuja, the Federal Capital Territory and 128km from Lafia, the Capital of Nasarawa state. Keffi is located between latitude 8°5'N of the equator and longitude 7°8'E and situated on an altitude of 850m above sea level Akwa *et al.*[9] and Nasarawa is approximately 35km South-west from Keffi.

### 2.2. Sample Collection

Corn cob, also called cob of corn or corn on the cob, is the central core of an ear of corn. It is the part of the ear on which the kernels grow. The ear is also considered a "cob" or "pole" but it is not fully a "pole" until the ear is shucked, or removed from the plant material around the ear. Sample collection was carried out using randomly sampling method was used to collect Twenty (20) corncobs samples, five (5) corncobs sample from each of four selected locations in Keffi Metropolis, Angwan zakara, Angwan Jarne, Nasarawa state university, Pyanko using a clean hand trowel and stored using disposable black polythene bags and transported immediately to the Microbiology Laboratory, Nasarawa State University, Keffi for analysis.

### 2.3. Isolation of fungi

Isolation of fungi was carried out using a method described by Ekeleme *et al.* 2018[10]. Briefly, one (1) gram of the corncob sample was suspended in a test tube containing 9 ml of sterile distilled water to make suspension and ten-fold serial dilution was made by transferring one ml of the corncob suspension to another test tube containing 9 ml of sterile distilled water. These steps were repeated seven times to obtain a dilution of  $10^{-7}$ . From the fourth test tubes, 0.2ml of the aliquot was spread on Potato dextrose agar plates, Malt extract agar and Yeast extract agar and incubated at 28°C for 4 days.

### 2.4. Identification of fungi

Identification of fungi isolated was carried out by the method adopted by Makut *et al.* 2018 [11]. Briefly, the cultural characteristics of fungi were determined by their growth appearance on culture plates and the morphological features were determined microscopically using lactophenol cotton blue staining technique, where lactophenol cotton blue stain was dropped on a clean grease free microscope slide, a small portion of mycelium or colony from the fungi culture plate was dropped on the lactophenol cotton blue with aid of mounted needle, the mycelium was spread well with the two mounted needle and covered with cover slip. The slide was then viewed under the microscope at x40 and x100 lens. The images were identified with reference to fungi standard chart.

## 2.5. Screening for amylolytic activity by fungal isolates

The screening for amylolytic activity was carried using starch agar. The starch agar was inoculated with the fungi isolates and incubated for 4day, Grams iodine was added to the growth on the media incorporated with maize stalk to screen for Amylase production where positive results was indicated by yellow color or clearing, while blue -black areas was indicates areas starch has not been degraded Nimkar *et al.* 2010[12].

## 2.6. Preparation of cultivation substrate for fermentation

Corncoobs were cut into pieces of  $\leq 1$  cm and then dried using an oven at 70°C for 24 hours and grinded into powdered form and sieved using a sieve pore of 0.25 $\mu$  size. Corncoobs powder were pre-treated using autoclave at 121°C for 15 minutes to remove lignin compounds.

## 2.7. Preparation of fermentation substrate

Fermentation media was prepared using a method described by Sumrin *et al.* 2011[13]. Thirty-gram (30g) of corncoobs substrate was mixed with salt nutrients of 0.6g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>; 0.18g KH<sub>2</sub>PO<sub>4</sub>; 0.18 g K<sub>2</sub>HPO<sub>4</sub>; 0.03g MgSO<sub>4</sub>.7H<sub>2</sub>O, maltose and lactose 1g and 60ml H<sub>2</sub>O to form the fermentation media. Then the fermentation media was sterilized for 15 minutes at temperature of 121°C in autoclave.

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## 3. Optimization for amylases production using *Aspergillus* species and *Fusarium* species isolates

### 3.1. Effect of pH on amylases

Effect of pH was carried out following a method described by Makut *et al.* 2018[11]. One hundred (100g) grams of the fermentation substrate was transferred into different conical flasks. The pH ranges were adjusted to, 4.0 4.5, 5.0, 5.5, 6.0 and 6.5 of fermentation media using 1.0 Na and HCl before autoclaving.

### 3.2. Effect of Temperature on amylases

Effect of temperatures was carried out following a method described by Makut *et al.* 2018 [11]. One hundred (100) ml of the fermentation substrate was transferred into different conical flasks and the fermentation media was incubated at 26°C, 28°C, 30°C, and 32°C

### 3.3. Effect of fermentation duration on amylases

Effect of fermentation duration was carried out following a method described by Makut *et al.* 2018 [11]. One hundred (100) ml of the fermentation substrate was transferred into different conical flasks and the fermentation media was incubated at.24hours, 48hours, 72hours, 120hours and 144 hours.

### 3.4. Enzyme extraction from the fermented media

Enzyme extraction was done by adding 0.1 M phosphate buffer to pH 7.0 with the ratio of 100g of the fermented media with 200ml of 0.1 M phosphate buffer. The solution was stirred continually for 30 min using a stirring rod and then filtered using muslin cloth. The extract was centrifuged at 8000 rpm for 20 minutes.

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## 4. Estimation of Enzyme produced

### 4.1. Estimation of Amylases produced

Amylase produced was determined using the method described by Anshikaa *et al.* 2013[14], 0.1ml of phosphate buffer solution of (pH 7) was added to enzyme solution extracted from the fermentation media. Then 1ml of enzyme solution extract was added to a test tube containing 1ml of starch solution (it was considered as the main sample). 1ml of enzyme solution extracted and 1ml of phosphate buffer which is considered as blank or control sample was added to another test tube. The both samples (main and control samples) was incubated at 37°C for 30mins. 2ml of Dinitrosalicylic acid (DNSA) solution was added to each test tube and then transferred to the water bath at boiling temperature for 15mins. The test tubes were kept at room temperature to cool the sample and the solution was transferred to the cuvette. The absorption of the main sample was measured at 540nm in spectrophotometer.

## 5. Result

The isolation rate of fungi species isolated is as given in Table 1. Out of 20 corncobs sample collected the frequency occurrence was 55.0% were the highest occurrence was observed from location A, C and D with 60.0 % and location B had 40.0%.

Cultural and morphological characteristics of fungi isolated from waste corncobs in Keffi are as shown in Table.2. The fungi isolated were *Aspergillus carneus*, *Aspergillus aculeatus*, *Fusarium moniliforme*, *Aspergillus flavus* and *Fusarium redolens*.

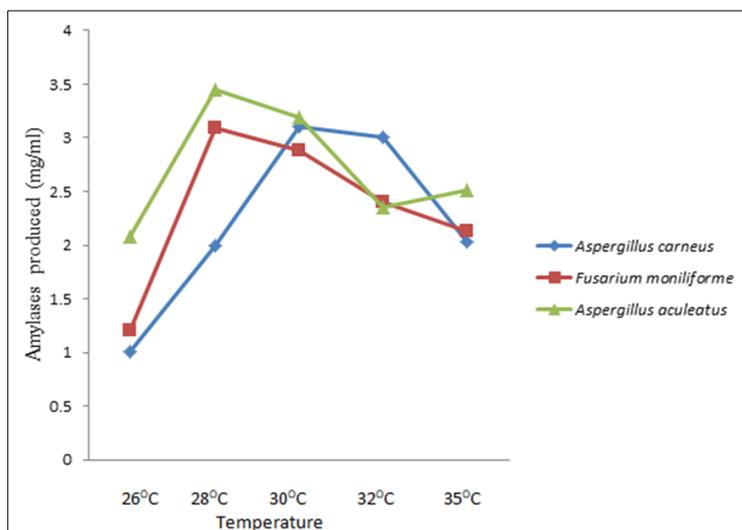
Frequency of fungi species isolated from waste corncobs from selected locations in Keffi is as given in Table 3. Where *Fusarium moniliforme* had the highest occurrence (80.0%) followed by *Aspergillus aculeatus* (60.0%), *Aspergillus carneus* and *Fusarium redolens* had (40.0%) and the least was *Aspergillus flavus* (20.0%)

### 5.1. Production of amylases using different fermentation condition

The highest amylases was produced at 30°C by *Aspergillus Carneus* with 3.10 mg/ml followed by 32 °C, 35 °C with 2.03 mg/ml 28°C with 2.00 mg/ml and the least was at 26 °C with 1.01 mg/ml. *Fusarium moniliforme* produced highest At 28 °C, 3.09 mg/ml followed by 30 °C with 2.88 mg/ml, 32 °C with 2.40 mg/ml 35 °C with 2.01 mg/ml and at 26 °C with 1.20 mg/ml. *Aspergillus aculeatus* produced highest amylases at 28 °C with 3.45 mg/ml followed by 30 °C with 3.19 mg/ml, 35 °C with 2.51 mg/ml, 32 °C with 2.35 mg/ml and the least was at 26 °C with 2.08 mg/ml as given in Figure1.

### 5.2. Effect of different fermentation duration on amylases production

The highest amylases produced by *A. carneus* was observed after 72 hours with 3.10 mg/ml followed by 96 hours with 3.01 mg/ml, 120 hours with 2.03 mg/ml, 144 hours with 1.97 mg/ml and 24 hours with 0.61 mg/l.



**Figure 1** Effect of temperature on amylases production by *Aspergillus* species and *Fusarium* species

**Table 1** Isolation rate of fungi species from waste corncobs in Keffi

Locations	No sample	No (%) isolated
A	5	3 (60.0)
B	5	2(40.0)
C	5	3(60.0)
D	5	3(60.0)
Total	20	11 (55.0%)

**KEY:** A = Angwan zakara; B = Angwan Jarne; C = Nasarawa state university; D = Pyanku

**Table 2** Cultural and Morphological Characteristics of Fungi Isolated from waste corncobs in Keffi

Fungal Isolate	Cultural	Morphological
<i>Aspergillus carneus</i> ;	Colonies are typically black in colour with white cleistothecia developing within and upon the conidial layer. Reverse may be olive to drab-grey or brown	Branched conidiophores with chains of conidia like a brush
<i>Aspergillus aculeatus</i>	Yellow or yellowish green colonies with distinct margin, the colony reverse is brownish to dark in colour.	Conidiophores arise from a foot cell. Club shaped vesicles at top of conidiophores. Conidia are found in chains and surface is irregularly rough.
<i>Fusarium moniliforme</i>  <i>Aspergillus flavus</i>	Colonies are usually fast growing, pale or bright colored (depending on the species) with or without a cottony aerial mycelium. The colour of the thallus are red or purple shades  Colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with	Produce both macro- and microconidia from slender phialides.  Macroconidia are hyaline several-celled, fusiform to sickle-shaped Conidiophore stipes are hyaline and coarsely roughened, often more noticeable near the vesicle. Conidia are globose to subglobose
<i>Fusarium redolens</i>	Conidial heads are typically radiate, later splitting to form loose columns biserial but having some heads with phialides borne directly on the vesicle cleistothecia  Colonies growing slowly; surface usually orange to deep apricot due to confluent conidial slime; aerial mycelium sometimes floccose and whitish.	Conidiophores loosely branched, with short, often swollen phialides. Macroconidia strongly curved and pointed at the apex, mostly one septate, Microconidia absent

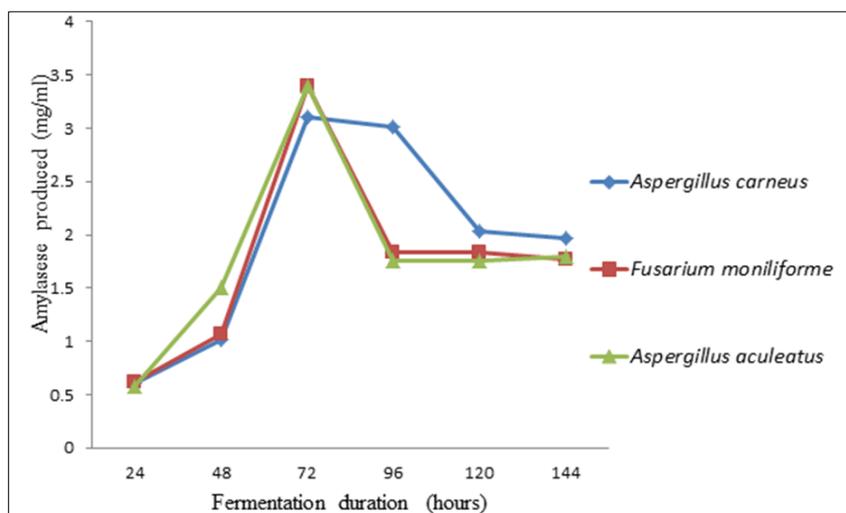
**Table 3** Percentage occurrence of fungi species isolated from waste corncobs from selected locations in Keffi

Location						
Fungi isolates	No sample	A	B	C	D	Occurrence (%)
<i>Aspergillus carneus</i>	5	+	+	-	-	2(40.0)
<i>Aspergillus aculeatus</i>	5	-	-	+	++	3(60.0)
<i>Aspergillus flavus</i>	5	+	-	-	-	1(20.0)
<i>Fusarium moniliforme</i>	5	+	-	++	+	4(80.0)
<i>Fusarium redolens</i>	5	-	+	-	+	2(40.0)

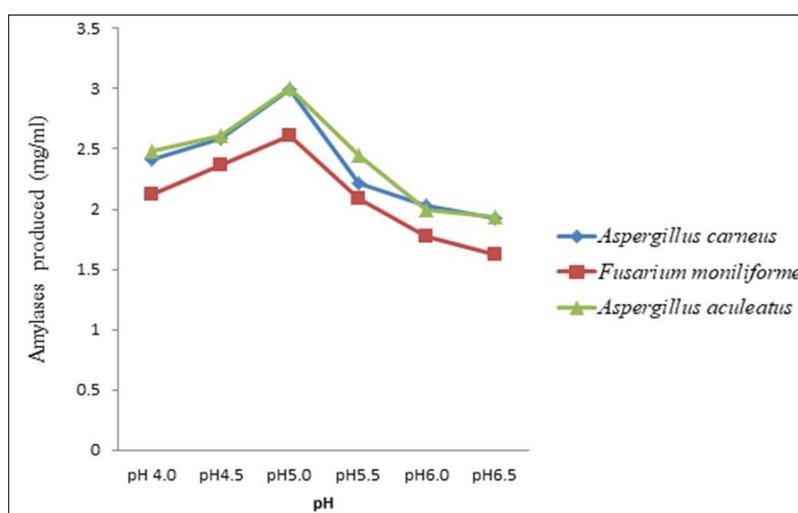
The highest amylases produced by *F. moniliforme* was at 96 hours with 3.40 mg/ml followed by 72 hours with 2.81 mg/ml, 120 hours with 1.83 mg/ml, 144 hours with 1.77 mg/ml, 48 hours with 1.07 mg/ml and 24 hours with 0.52 mg/ml. *Aspergillus aculeatus* produced highest after 72 hours with 3.39 mg/ml followed by 96 hours with 3.15 mg/ml, 144 hours with 1.79 mg/ml, 120 hour with 1.75 mg/ml, 48 hours with 1.51 mg/ml and at 24 hours with 0.58 mg/ml as shown in Figure 2.

### 5.3 Effect of pH on Amylases and cellulases production by different fungi species

The highest amylases was produced by *A. carneus* at pH 5.0 with 2.99 mg/ml followed by pH 4.5 with 2.5 mg/ml, pH 4.0 with 2.4 mg/ml, pH 5.5 with 2.21 mg/ml, pH 6.0 with 2.03 mg/ml and pH 6.5 with 1.92 mg/ml. *Fusarium moniliforme* produced highest at pH 5.0 with 2.6 mg/ml followed by pH 4.5 with 2.37 mg/ml, pH 4.0 with 2.12 mg/ml, pH 5.5 with 2.09 mg/ml, pH 6.0 with 1.77 mg/ml and at pH 6.5 with 1.62 mg/ml respectively. *A. aculeatus* produced highest amylases at pH 5.0 with 3.01 mg/ml followed by pH 4.5 with 2.61 mg/ml, pH 4.0 with 2.48 mg/ml, pH 5.5 with 2.45 mg/ml, pH 6.0 with 1.99 mg/ml and pH 6.5 with 1.94 mg/ml as shown in Figure 3.



**Figure 2** Effect of fermentation duration on amylases production by *Aspergillus* species and *Fusarium* species



**Figure 3** Effect of pH on amylase production by *Aspergillus* species and *Fusarium* species

## 6. Discussion

Amylases are one of the most important industrial enzymes that have a wide variety of applications ranging from conversion of starch to sugar syrups, to the production of cyclodextrins for the pharmaceutical industry. Amylases have been widely used for the industrial production due to advantages such as cost effectiveness, consistency, less time and space required for production and ease of process modification and optimization Burhan *et al.* 2003[15]. This study is focused on the production of amylases by *Aspergillus* and *Fusarium* species isolated from waste corncobs in Keffi, Nigeria.

In this study the occurrence of fungi was higher in Angwan jarme and pyanku than in Angwan Zakara and Tudu wada and fungi isolated from corncobs was not surprising because fungi play an important role in breaking down most of

agriculture waste in the environment. The effect of temperature on amylase production as observed in this study was in agreement with studies reported earlier by different authors such as Haq *et al.* 2002 [4] and Khan *et al.* 2011[16] that temperature of 30°C and 28°C has been the optimum temperature for amylase production. In the study it was observed that *A. carneus* produced highest amylase at 30°C, *F. moniliforme* and *A. aculeatus* produce highest amylase and cellulose at 30°C this showed that temperature is an important parameter in fermentation process which helps in maximum production of this important organic enzymes.

Fermentation duration is an important fermentation parameter that can not be over emphasizes the production amylase using fungi required long incubation time which defers from one species to another. In the study it was observed that highest amylase was produced after 72hours by *F. moniliforme* and *A. aculeatus* and *A. carneus* which is similar to study reported by Uguru *et al.* 2011 [17] and Singh *et al.* 2009[18] who reported highest amylase production after 5days of fermentation.

The effect of pH on the enzyme activity is depending on the number of factors such as microorganism use in the production, time and temperature. In general enzymes are less stable at high temperature over time at pH value near the limit of the optimum. The optimum pH should be determined to be under certain conditions. In such case it is important to choose an enzyme with a pH range from 4 to 11 Gimbi *et al.* 2002[19] and Akpan and Adelaja, 2004[20]. The *Aspergillus* species is about the pH range between 3-5 which is in agreement in this study because it was observed that highest amylase was produced at pH 5.0 from this study fermentation parameter is important in the production of this important enzyme that is used in day-to-day production of different industrial products.

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## 7. Conclusion

In this study, it was observed that fungi such as *A. cerneus*, *fusarium moniliforme* and *A. aculeatus* were isolated from waste corncobs from different locations in Keffi, had the ability to produces amylases. It was also observed that the *A. aculeatus* produced highest amylase at different fermentation parameters such as temperature, pH and fermentation duration followed by *A. cerneus* with the least been *F. moniliforme*.

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## Compliance with ethical standards

### Acknowledgments

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### Disclosure of conflict of interest

Authors have declared that no competing interests exist.

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