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# Fonio-Derived Amylase: Sustainable Innovations for National Food Security and Employment Creation

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## Abstract

**Background:** Ensuring national food security and reducing unemployment are of high importance, and the innovative and sustainable utilisation of indigenous amylase sources is essential. Fonio (*Digitaria exilis, Digitaria iburua*), an underutilised indigenous cereal, represents a culturally important but economically overlooked source of amylase, an enzyme widely used in various industrial applications. **Objective:** This study explores the production and characterisation of amylases derived from fonio as a sustainable innovation aimed at reducing dependence on imported cereals and, as such, generating employment opportunities **(**state the objective to achieve). **Methodology:** This study explores the potential of fonio as a sustainable and efficient source of amylase, comparing its properties and performance with those of traditional sources. **Results:** The results showed that fonio-induced amylase exhibits high enzymatic activity, stability, and specificity, making it an attractive alternative for industrial uses. An optimal germination condition was achieved by steeping the grains in water or water containing phosphate salts at pH 6.5. However, the second day of germination produced the highest number of amylase. The obtained amount was higher for grains steeped in buffer at pH 6.5 than for those steeped in just water. *Digitaria iburua* produced more amylase than *Digitaria exilis*. *Digitaria exilis* gave 17498 U/mg protein, while Digitaria iburua gave 24332 U/mg protein. Optimal conditions for pH and temperature were investigated, and 30 °C was the best temperature for amylase induction. **Conclusion/ Recommendations:** This research highlights the potential of fonio to contribute to more sustainable and efficient industrial enzyme production, with implications for the food, beverage, and biofuel industries. The use of fonio as a source of amylase offers advantages, including reduced water requirements, low production costs, and sustainability. The research suggests that fonio, a locally grown cereal, could be a total replacement for imported barley for biological applications.

**Keywords:** West African cereal, enzymatic activity, steeping grains, biofuel, sustainability

## Introduction

Amylases are important hydrolase enzymes which have been widely used for many decades. These enzymes randomly cleave internal glycosidic linkages in starch molecules, yielding dextrins and oligosaccharides. Amylases are digestive enzymes predominantly secreted by the pancreas and salivary glands and are present in other tissues at minimal levels. Amylases were first described in the early 1800s and are among the pioneering enzymes to undergo scientific investigation. Amylases are any member or class of enzymes that catalyse the hydrolysis of starch into simpler carbohydrate molecules such as maltose. Amylase is a hydrolytic enzyme from the hydrolase group. The official name of α-amylase is 1,4–α–D–glucan glucanohydrolase; EC 3.2.1.1. α-amylase hydrolyses α -((1-4) glycoside bonding of amylase, which results in

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the formation of maltose (a-glucose disaccharides). In commercial applications, a thermo-labile α-amylase produced from *Bacillus licheniformis* is used. Unlike thermostable α-amylase, it is active and stable at temperatures above 90 °C. In addition, the reactivity of thermo-labile α -amylase is much less dependent on the presence of $Ca^{2+}$ ions and on the pH applied than its thermo-stable counterpart. In the textile industry, substances containing starch or its hydrolyzates are used to glue the wrap. The degumming process is essential to remove the gum that hinders the subsequent technological processes (bleaching, dyeing and printing). α-amylase is used for that purpose (Sojjka-Ledakowicz *et al*., 2000). There are three types of amylases known: alpha, beta and gamma. All three are found in different organisms and catalyse different sites of the starch molecule.

Alpha amylase is widespread among humans and many other mammals. The salivary glands produce a-amylase called ptyalin, whereas the pancreas secretes pancreatic amylase into the small intestine. The optimum pH of alpha amylase is 6.0 – 7.0.

Ptyalin is mixed with food in the mouth, where it acts upon starches. Although the food remains in the mouth for only a short time, the action of ptyalin continues for several hours in the stomach, where it is mixed with stomach secretions, the high acidity of which inactivates ptyalin. Ptyalin's digestive action depends on how much acid is in the stomach, how rapidly the stomach contents empty, and how thoroughly the food has mixed with the acid. Under optimal conditions, as much as 30-40 per cent of ingested starch can be broken down into maltose by ptyalin during stomach digestion. When food passes to the small intestine, the remainder of the starch molecules are catalysed mainly to maltose by pancreatic amylase. This step in starch digestion occurs in the first section of the small intestine, where pancreatic juice empties. Other enzymes ultimately break down the by-products of amylase hydrolysis into glucose molecules, which are rapidly absorbed through the intestinal wall. The β-amylases are present in yeasts, moulds, bacteria and plants, particularly in the seeds. They are the principal components of a mixture called diastase that is used in the removal of starchy sizing agents from textiles and in the conversion of cereal grains to fermentable sugars. β-amylase has an optimum pH of 4.0-5.0. The γ-amylases are known for their efficiency in cleaving certain types of glycosidic linkages in acidic environments. The optimum pH of γ-amylase is 3.0 (Ehlers & Potter, 2024). The γ-amylases are found in plants and animals. They cleave the last α-1, 4-glycosidic bond and the α-1,6 glycosidic bond in the starch molecule to yield glucose molecules. Their optimum pH is 3. They are members of glycosidase family 15 (Prasad, 2011). The objective of the study is to determine levels of amylase activity in black and white fonio, the effect of days of germination on malted black and white fonio, the effect of induced amylases as a function of pH, and the effect of induced amylases as a function of temperature.

## Materials and Methods

### Materials

White fonio and black fonio grains were purchased from Sabon Gari market in Zaria, Kaduna State, Sabo market at Ilorin, Kwara State and Oja-oba market at Ondo town, Nigeria. These grains were authenticated at the different laboratories.

### Methods

### Experimental Design

This study adopted a laboratory-based experimental design using comparative and analytical approaches.

#### i. *Induction of Amylases*

Amylases were induced by steeping 100 g of screened white fonio and black fonio grains in water and water containing phosphate salts (10 mM sodium phosphate adjusted to pH 6.5)

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separately for 24 h at room temperature, following the method of Adefila *et al*. (2012). Steeped grains of white fonio and black fonio were blotted to remove excess water after 24 h and were spread out in a locally constructed malting chamber at room temperature. The optimal day of germination was determined for each grain by harvesting malt on each day of germination, homogenising to obtain a crude extract, and assaying for amylase activity until a decline in induced amylase activity was observed. The optimum pH and temperature for amylase induction were investigated.

**ii.** ***Extraction of amylase activity***

Induced amylase from white fonio and black fonio malts was extracted by preparing 30% homogenate of the malted grains using water and water containing phosphate sulphate (10 mM sodium phosphate buffer, pH 6.5, containing one mM $CaCl_2$), following the method of Adefila *et al*. (2012). The 30% homogenate was prepared by homogenising 100 g of malted D. exilis and 170 g of malted black fonio in a cold 10 mM sodium phosphate buffer, pH 6.5, containing one mM $CaCl_2$. The crude homogenates were centrifuged at 13,000x*g* for 30 min at 4 ˚C using Hitachi High Speed Refrigerated Centrifuge Himac CR21G H. The pellets were discarded while the supernatants were collected. Amylase activities and protein concentration in each supernatant were determined, and the supernatants were stored at -20 ˚C until further use.

**iii.** ***Standard procedures for amylase activity assay***

The amount of reducing ends released upon starch hydrolysis by amylolytic enzymes was estimated using the modified Bernfeld method (1951). A unit of amylolytic activity was defined as the amount of enzyme which liberated reducing sugar equivalent to 1 µg of D-glucose per minute at 25 ˚C under the standard assay conditions.

An assay mixture of 2 mL in the final concentration contains 10 mM sodium phosphate buffer, pH 6.5, 1 mM CaCl2, 0.2 mL of 1 % soluble starch, and 0.01 mL of the enzyme. The assay mixture was incubated at room temperature for 5 min to allow the enzymatic reaction to occur, after which the reaction was terminated by adding 1 ml of 0.5 mM 3,5-dinitrosalicylic acid. The solution was boiled for 5 min to develop colour; the yellow colour of 3,5-dinitrosalicylic acid turned reddish brown, and it was cooled under a running tap and diluted with distilled water to 10 ml. The optical density was taken at 470 nm using a spectrophotometer. Two blanks were set up for the experiment: the first contained all assay components except the enzyme, while the second contained denatured enzyme (boiled at 100 °C). Glucose was used to prepare the standard curve from which the amounts of reducing sugars liberated were estimated.

**iv.** ***Protein concentration determination***

The protein concentration in the crude supernatants was determined using the Coomassie dye-binding assay, following the Bradford (1976) method, with bovine serum albumin as the standard protein. The method measures the increase in absorbance at 595 nm of Coomassie Brilliant Blue G-250 upon binding to protein.

**Sample Size**

Fonio grain samples: 3 locations and 5 independent batches per location.

Enzyme production experiments: Each experimental condition was conducted in triplicate.

Comparative assays: Fonio-derived amylases was tested against commercial amylase across multiple substrates and conditions with three replicates per treatment.

Total experimental replicates: A minimum of 60-90 experimental observations, which was sufficient for a robust statistical analysis, was employed

All analyses were conducted using standard statistical software of GraphPad Prism

**Statistical Analysis**


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Data obtained from enzyme activity assays, optimization studies and comparative evaluations will be analysed using statistical tools such as descriptive statistics: Mean ±standard deviation (SD) was used to summarize enzyme activity, and level of significance: statistical significance was set at $p < 0.05$.

**Results**

***Levels of amylase activity in white fonio and black fonio grains***

Figure 1 is the summary of the levels of amylase activities in the crude homogenates of malted white and black fonio grains steeped in water and water containing phosphate salts. A higher amount of amylase was found in black fonio grains steeped in water containing phosphate salts compared with grains steeped in water for both grains.

***Effect of days of germination on malted white fonio and black fonio grains***

Grains of white fonio and black fonio were steeped in water and water containing phosphate salts and were germinated for a couple of days in a locally constructed malting chamber. Harvested malts were subjected to homogenization, and a specific activity of 17,948 U/mg protein and 24,337 U/mg protein for white fonio and black fonio, respectively, at day two (2) of germination produced the highest amount of amylolytic enzymes for both grains, as summarised in Figure 2.

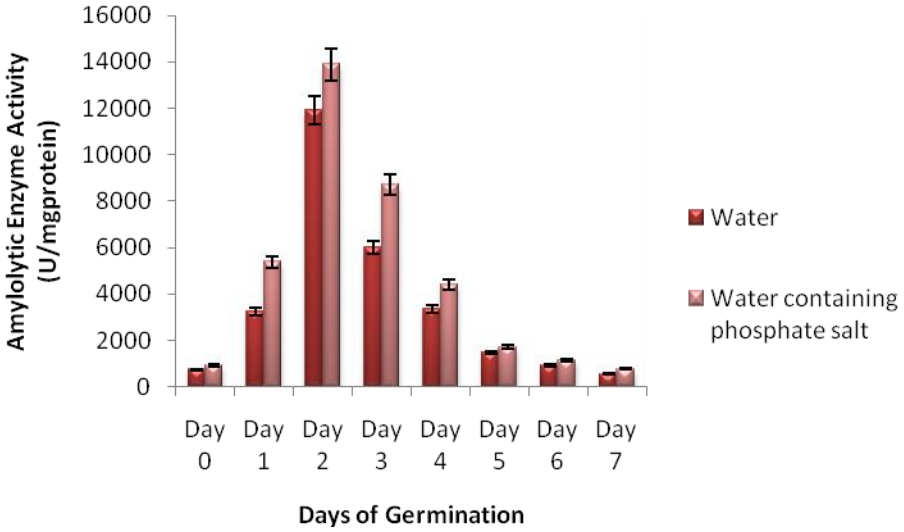


Figure 1 **a:** Level of induced amylases in white fonio malt


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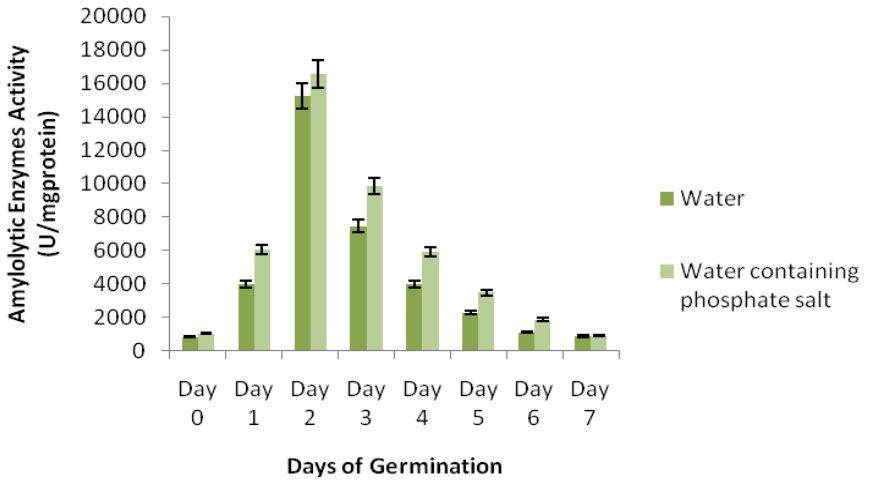


Figure 2: Level of induced amylases in black fonio malt

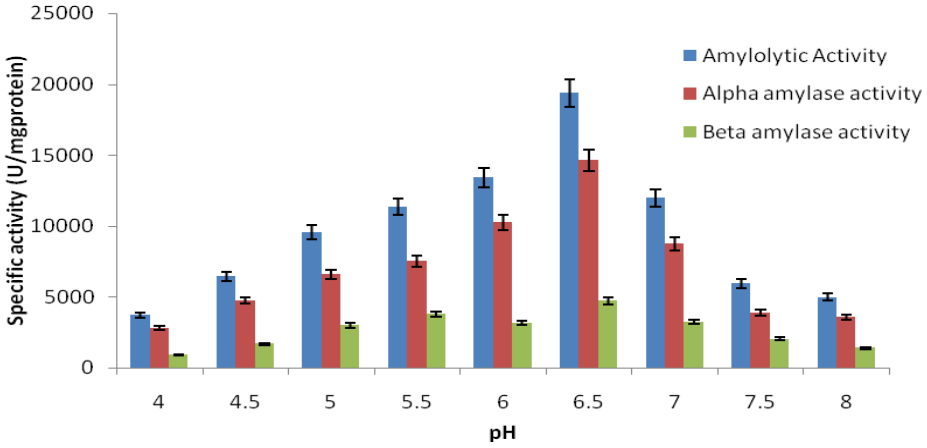


Figure 3: Profile of induced amylases as a function of pH from malts of white fonio

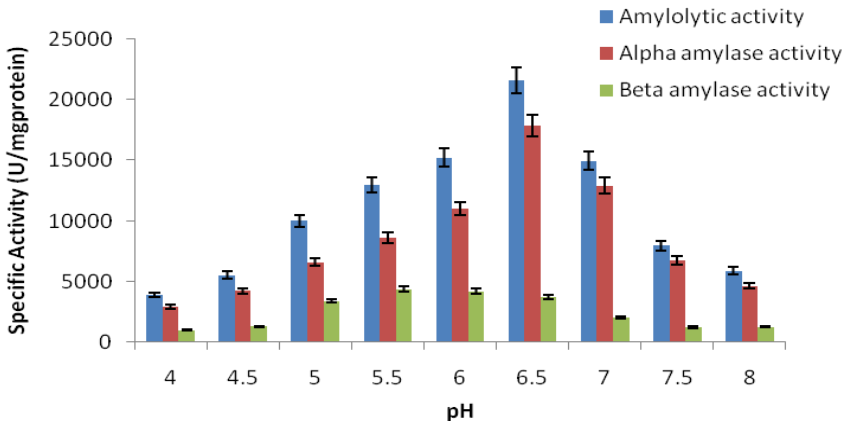


Figure 4: Profile of induced amylases as a function of pH from malts of black fonio


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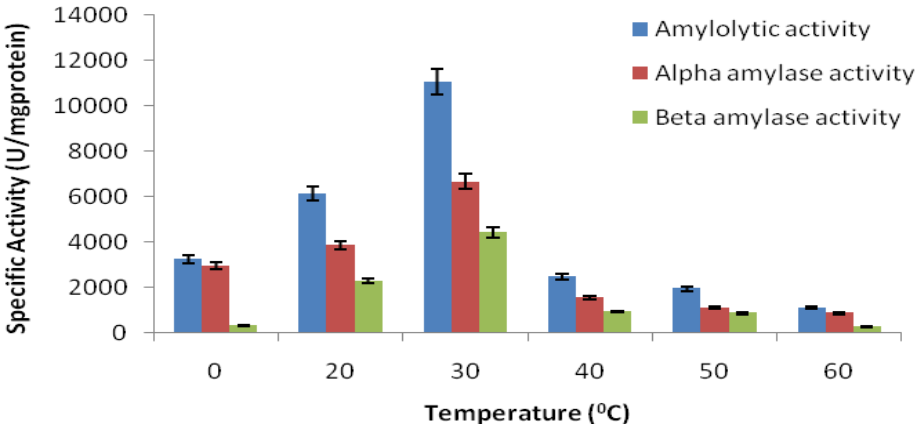


Figure 5: Profile of induced amylases from white fonio as a function of temperature steeping conditions.

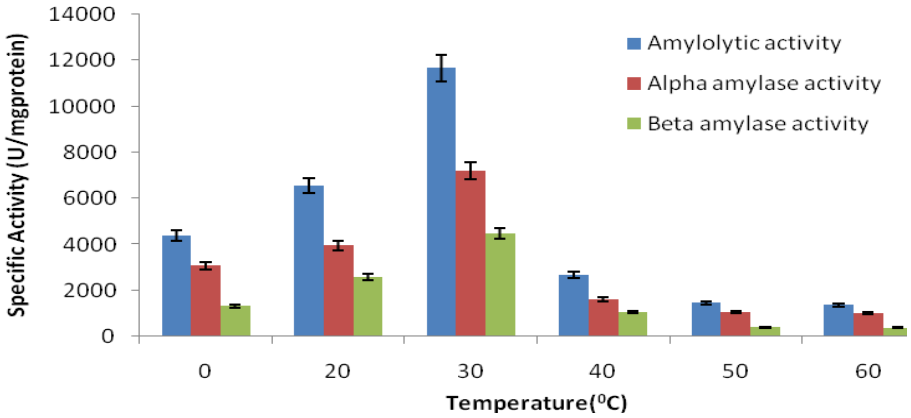


Figure 6: Profile of induced amylases from black fonio as a function of temperature steeping conditions.


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**Sample Results Interpretation:**
**Comparative Analysis of Fonio-Derived Amylase and Commercial Amylases**

The comparative evaluation of fonio-derived amylase against fungal utilised amylases revealed notable differences in enzymatic performance, stability, and substrate preference. Fonio-derived amylases demonstrated competitive and catalytic efficiency when assessed under standardised assay conditions.

**Enzyme Activity and Optimal Conditions**
Fonio-derived amylases exhibited a high specific activity with an optimum temperature within the range commonly employed in food processing operations. While fungal amylases showed comparable maximum activities, the fonio enzyme maintained appreciable activity over a broader temperature range. This suggests enhanced adaptability of the fonio-derived amylase to variable processing conditions.

**Thermal and pH Stability**
Stability studies revealed that fonio-derived amylase retained a higher percentage of residual activity after prolonged exposure to elevated temperatures compared to fungal amylase. This improved thermal stability implies reduced enzyme denaturation during processing, potentially lowering enzyme dosage requirements and operational costs.

**Discussion**
This study optimised germination conditions to induce amylases in white and black fonio. Grains became swollen after steeping in water and water containing phosphate salts, which could be a result of the higher water absorption capacity of these grains, probably because of their thin seed coat. High malting loss has previously been reported with these grains, as observed in the preliminary experiments in this work. High malting loss in white fonio and black fonio grains was prevented by steeping grains in a large volume of water (1:20 (w/v) of water). After 24 h, white fonio and black fonio were blotted out and germinated in a locally constructed malting chamber. To ensure uniform germination, grains were sprinkled with respective steeping mixture at 12 h intervals, contrary to the six h usually employed for other grains such as sorghum, maize and millet. However, studies have shown that the rate of water diffusion in grains depends on factors such as steeping duration, water temperature, grain dimensions, and protein content, and possibly on the quantity of available oxygen (Francis, 2003).
Maximal enzyme activity was obtained after 2 days, which is fewer days than for other grains (Adewale *et al*., 2006; Adefila *et al*., 2012). The enzyme was extracted, and the resulting supernatants were assayed for amylase activity. The amylase induced is a function of the days of germination, as the highest amylase activity was obtained on the second day (48 h) of germination with 17498 U/mg protein and 24337 U/mg protein for white fonio and black fonio, respectively (Figure 4.1a and b) for grains steeped in water containing phosphate salt. About 5000 U/mg protein was the difference in amylase activity induced under the same conditions, but with only water as the steeping medium. Water is traditionally the medium for steeping in most industries. The observed reduction in amylase activity after 48 h indicates that induced amylases may have been degraded to produce other biomolecules required by the growing plant.
This study, therefore, established that more amylases were induced in D. exilis and D. iburua grains within a very short germination (2 days) when compared with other grains, such as sorghum and millet, which require 3-5 days for maximal amylolytic activity (Egwim & Oloyede, 2006). This implies that white fonio and black fonio grains would generate a far higher amount (quantity) of amylolytic enzymes. This will invariably increase the economic value of these underutilised African grains.
According to Osman's (2002) method, amylases from malts of white fonio and black fonio were split into respective amylases (α and β) because a good mixture of α and β-amylases is required for complete

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saccharification of starch in all starch-based industries. Malts of *D. exilis* and *D. iburua* contain good amounts of α and β-amylases, although with a very good quantity of β-amylase. Generally, industrial enzymes, such as amylases, require only minimal downstream processing and are therefore relatively crude preparations. Commercial utilisation of amylases does not require purification, except for applications in the pharmaceutical and clinical sectors, which require high-purity amylases (El Nour *et al*., 2013). The crude amylolytic enzyme supernatants obtained from malts of *D. exilis* and *D. iburua* were experimented with as a function of pH.

**Conclusion:**
The study demonstrated that amylase derived from black and white fonio exhibits competitive enzymatic activity, favourable stability profiles, and strong substrate affinity when compared with fungal amylases. These further confirm the potential of fonio-derived amylase as a locally sourced, cost-effective alternative for biotechnological applications.

**Recommendations:** Further studies are needed to assess the feasibility of large-scale production of fonio-derived amylase. Molecular characterisation and genetic studies are recommended to improve enzyme yield and stability. Government and private-sector stakeholders should support enzyme biotechnology using indigenous crops to enhance the development of local content.

**Scientific implications of the study**: The study contributes to enzymology by providing new data on the catalytic and stability properties of fonio-derived amylase. This study expands the knowledge base on underutilized cereals as viable sources of industrial enzymes. It supports the concept of enzyme–substrate compatibility between indigenous crops and locally derived enzymes. And also provides a scientific framework for developing climate-adapted enzymes suitable for tropical processing conditions.

**Limitations to the study**: The study was conducted at laboratory scale. Only fungal amylase was used for comparison due to availability and cost constraints.

**Ethical consideration**: Fonio grains used in the study were obtained through legal and ethical sourcing from local markets. Laboratory experiments were conducted following institutional biosafety and chemical safety guidelines. Data were honestly collected, analyzed, and reported without fabrication or manipulation.

**Conflict of interest:** The authors declare no conflict of interest regarding the conduct, analysis, and publication of this research. The study was carried out independently without influence from commercial enzyme manufacturers or funding bodies.

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