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Exploring the Biotechnological Potential of Amylases from Fonio Grains

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Abstract

Fonio is a fast growing unpopular and underutilized African cereals which could serve as a source of amylases for use in enzymatic saccharification of starch in industrial biotechnological applications. This study investigated optimum conditions for the extraction of amylases from these grains as a replacement to the imported cereals like maize, millet and sorghum. An optimum condition based on days of germination was achieved by steeping grains in water and water containing phosphate salt at pH 6.5 from day 0 – 8. However, second day of germination produced the highest amount of amylases compared to other cereals like maize, millet and sorghum. The obtained amount was higher for grains steeped in buffer pH 6.5 compared to just water which is of traditional practice. Black fonio produced higher amount of amylases 16,540 ± 2160 U/mgprotein when steeped in water compared to white fonio with 15,248 U/ ± 1937 mgprotein. White fonio gave 17,498 ± 1208 U/mgprotein while black fonio gave 24,332 ± 2234 U/mgprotein when steeped in water containing phosphate salt. Optimum condition in terms of pH (4. 0–8.0) and temperature (0-60ºC) were equally investigated and 30ºC was the best temperature for the induction of amylases from white and black fonio. Therefore fonio, a locally grown cereal possesses amylases as a total replacement to imported cereals for biotechnological applications in brewery, alcohol, paper, detergent and beverage industries. This study was limited by the unavailability of fonio grains in the southern part of the country and the researcher had to travel to the north to get the required quantity needed for this study.

Keywords: Amylase; Biotechnology; Fonio; Specific Activity

1.0 Introduction

Fonio grains are small seed grains native to West Africa. It is a tiny, ancient, gluten-free grain originating from West Africa, specifically the savanna regions (Levinson 2018). It is sometimes referred to as Findi or acha which is the term for two cultivated grasses in the genus *Digitaria* that are important crops in parts of West Africa. In West Africa, the species black fonio (*Digitaria iburua*) and white fonio (*Digitaria exilis*) are cultivated; the latter is the economically more important crop. Morales-Payan *et al.,* reported that it is cultivated in the Dominican Republic, apparently brought there from West Africa as a by-product of the slave trade. The early accounts of West African farming testify to the abundance landraces of fonio present in the early twentieth century. Despite its ancient heritage and widespread importance, little is known of fonio evolution, origin distribution and genetic diversity remains scant even within West Africa itself (Adoukonou-Sagbadja *et al*., 2006; Clottey *et al*., 2006). Being a novel food, it has attracted attention from other parts of the world due to the attractive nutritional properties either in whole grain form and being in gluten free (Jour *et al*., 2019). The need for indigenous raw materials for malting industries necessitated the search for local cereals with high diastatic potentials. Germination is a cheap and effective bioprocessing technique used for improvement of the nutritional physicochemical and health promoting properties of seeds. Fonio, a West African cereal with high nutritional value and fast maturity, holds significant biotechnological potential for improving food security, enhancing nutrition and supporting sustainable agriculture through genetic modification and other biotechnological advancements The benefits of germination in fonio grains have not been totally unravelled, hence the need for this study.

Amylases are important hydrolase enzymes which have been widely used since many decades. These enzymes randomly cleave internal glycosidic linkages in starch molecules to yield dextrins and oligosaccharides. Amylases are digestive enzymes predominantly secreted by pancreas and salivary glands and are present in other tissues at minimal levels. Amylases were initially described in the early 1800s and are one of the pioneering enzymes to undergo scientific investigation (Abu, 2023).

Amylases are class of enzymes that catalyze the hydrolysis of starch into simpler carbohydrate molecules such as maltose. Amylase is a hydrolytic enzyme from the hydrolases group. The official name of α-amylase is 1, 4-α - D- Glucan glucanohydrolase; EC 3.2.1.1. α -amylase hydrolyzes α - (1-4) glycoside bonding of amylase, which results in the formation of maltose (α-glucose disaccharides). In commercial applications, a thermo labile α-amylase produced from *Bacillus licheniformis* is used. Unlike the thermostable α -amylase, it is active and stable at temperatures above 90^{o}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 hydrolysate 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 the three are found in different organisms and catalyse different sites of the starch molecule.

Alpha amylase is widespread among living organisms of humans any many other mammals, an a-amylase called ptyalin is produced by the salivary glands whereas pancreatic amylase is secreted by the pancreas 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 up to several hours in the stomach until the food is mixed with the stomach secretions, the high acidity of which inactivates ptyalin. Ptyalins digestive action depends upon 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 to 40% of ingested starches can be broken down to maltose by ptyalin during digestion in the stomach. When food passes to the small intestine the remainder of the starch molecules are catalyzed mainly to maltose by pancreatic amylase. This step in starch digestion occurs in the first section of the small intestine, the region into which the pancreatic juices empty. These by-products of amylase hydrolysis are ultimately broken down by other enzymes into molecules of glucose which are rapidly absorbed through the intestinal wall.

β-amylases are present in yeasts, molds, 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

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)

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 a member of the glycosidic hydrolase family 15(Prasad, 2011)

2.0 Materials and methods

2.1 Materials

White fonio and black fonio grains were purchased from Sabon Gari market in Zaria, Kaduna State, Nigeria. These grains were authenticated at the Herbarium of Department of Biological Science, Kaduna State University, Kaduna, Nigeria. The Voucher number is 6/6.

2.2 Methods

2.2.1 Induction of amylases

Amylases were induced by steeping 100 g of screened white and black fonio grains in water and water containing phosphate salts (10 mM sodium phosphate adjusted to pH 6.5) 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. Grains were sprinkled with respective medium of steeping (water and water containing phosphate salt) at 12 h intervals (twice a day) for optimization of the germination condition. Optimum day of germination was determined for each grain by harvesting malts on each day of germination, homogenizing to obtain a crude extract and assaying for amylases until a decline in induced amylase activity was obtained. Optimum pH and temperature for amylase induction was investigated.

2.2.2 Extraction of amylase activity

Induced amylase from white and black fonio malts were 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 1 mM $CaCl_2$), following the method of Adefila *et al*. (2012). The 30% homogenate was prepared by homogenizing 100 g of malted white fonio and 170 g of malted black fonio in cold 10 mM sodium phosphate buffer pH 6.5 containing 1 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.

2.2.3 Standard procedures for amylase activity assay

The amount of reducing ends released upon starch hydrolysis by amylase was estimated using the modified method of Bernfeld (1951). A unit of amylase 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 condition.

An assay mixture of 2 mL in the final concentration contains 10 mM sodium phosphate buffer, pH 6.5 containing 1 mM $CaCl_2$, 0.2 mL of 1 % soluble starch and 0.01 mL of the enzyme. The assay mixture was incubated for 5 min at room temperature for enzymatic reaction to occur, after which the reaction was terminated with 1 ml of 0.5 mM 3,5-dinitrosalicylic acid. The solution was boiled for 5 min for colour development, the yellow colour of 3,5-dinitrosalicylic acid turned into reddish brown and 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 blank consisted of all assay components except the enzyme while the second contained denatured (boiled at 100ºC) enzyme. Glucose was used to prepare the standard curve, from which the amounts of reducing sugars liberated at 470 nm were estimated.

2.2.4 Protein concentration determination

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

3.0 Results

3.1 Levels of amylase activities in white and black fonio grains

Figures 1 and 2 indicate 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. Higher amount of amylase was found high in black fonio grains steeped with water containing phosphate salts compared with grains steeped in water for both grains.

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

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

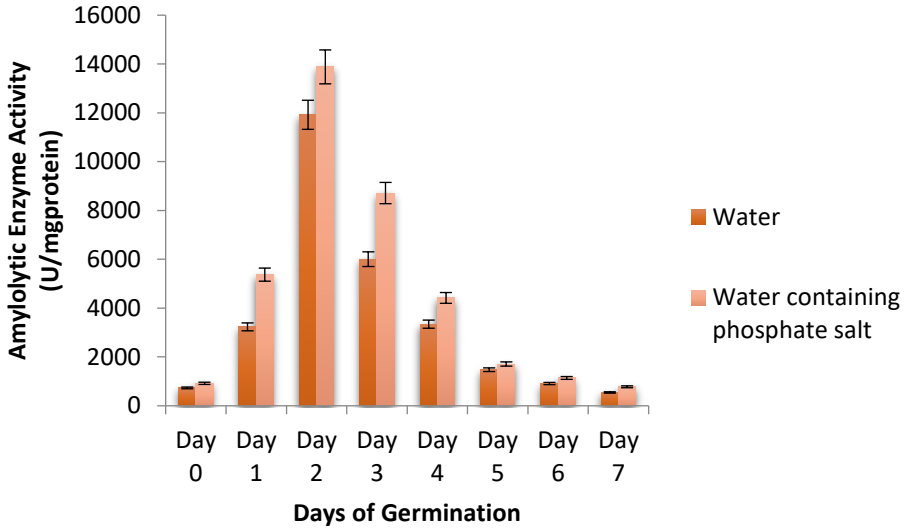

Figure 1: Level of induced amylases in white fonio malt

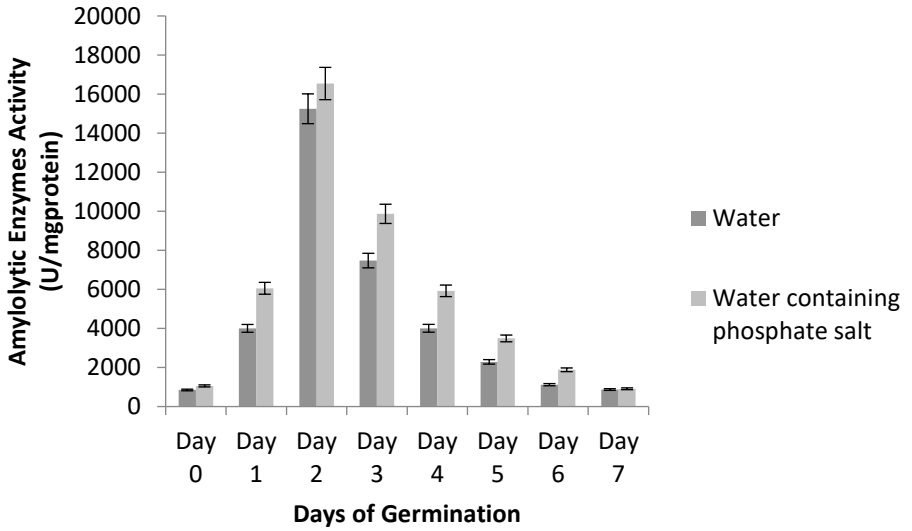

Figure 2: Level of induced amylases in black fonio malt

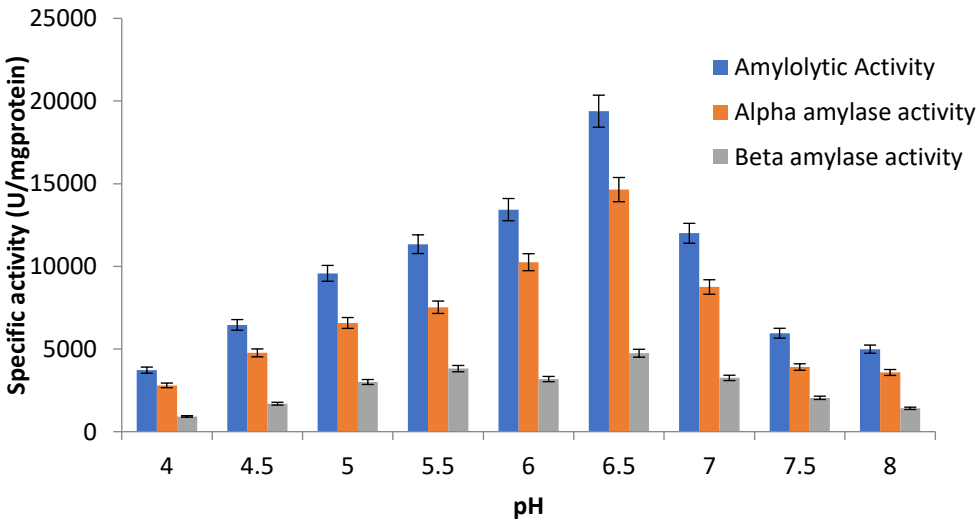

Figure 3: Profile of induced amylases induced as a function of pH from malts of white fonio

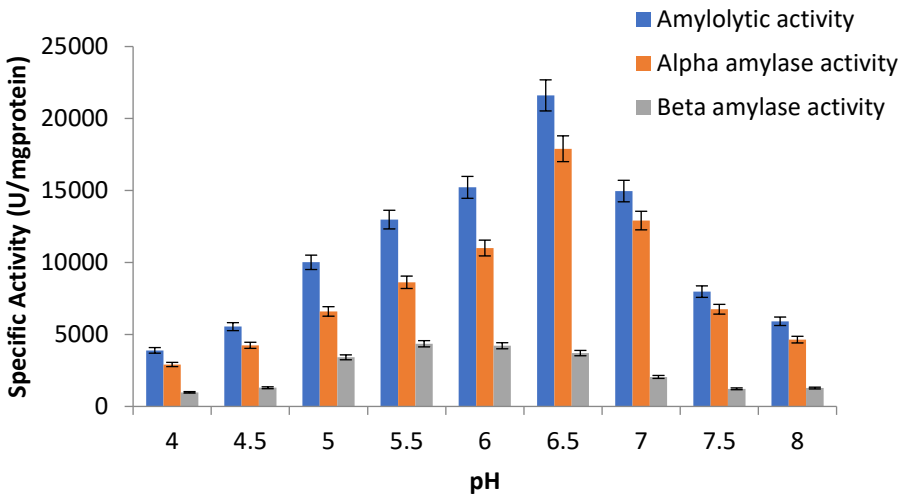

Figure 4: Profile of induced amylases induced as a function of pH from malts of black fonio

Table 1: Amylase composition of different grains

Grain	Amylase composition (U/mg protein)	Days of Germination	References
Millet	1,001	3-5	Usha *et al*., 2011
Maize	2667	3-5	Awoyinka and Adebawo,2014
Sorghum	16,000	3	Adefila *et al*., 2012
Acha	17,948	2	-
Iburu	24,337	2	-

4.0 Discussion

This study optimized germinating conditions for the induction of amylases in white and black fonio. Grains became swollen after steeping in water and water containing phosphate salts as this could be as a result of higher water absorption capacity of these grains, probably because of their thin seed coat. High malting loss has previously been reported with these grains, which was experienced in the preliminary experiments in this work. High malting loss in white and black fonio grains was prevented by steeping grains in large volume of water (1:20 (w/v) of water). After 24 h white 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 interval contrary to the 6 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 some factors like steeping duration, water temperature, grain dimension, and protein content of grains and possibly the quantity of available oxygen (Francis, 2003).

Maximal enzyme activity was obtained in 2 days which is lower than the number of days obtainable with other grains which are between 3-5 days (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 second day (48 h) of germination with 17498 U/mg protein and 24337 U/mg protein for white and black fonio respectively for grains steeped in water containing phosphate salt. About 5000 U/mg protein was the difference in the amylase activity induced under the same condition but with only water as the medium of steeping. Water is traditionally the steeping medium in most

industries. The observed reduction in the amylase activity after 48 h is an indication that induced amylases have possibly been degraded to produce other biomolecules required by the growing plant.

This study therefore established that more amylases were induced in white and black fonio grains within a very short germination (2 days) when compared with other grains such as sorghum and millet which are 16,000 U/mg protein and 2,667 U/mg protein respectively (Egwim and Oloyede, 2006; Adefila *et al*., 2012). This implies that white and black fonio grains would generate far higher amount (quantity) of amylases. This will invariably increase the economic value of these underutilized African grains.

According to Osman, 2002 method, amylases from malts of white and black fonio were splitted into respective amylases (α and β) because a good mixture of α and β-amylases are required for complete saccharification of starch in all starch-based industries. Malts of fonio equally contain good amounts of α and β-amylases which further establishes its application for biotechnological uses.

Generally, industrial enzymes among which are amylases require a little downstream processing and hence are relatively crude preparations. Commercial utilization of amylases does not require purification except for their applications in pharmaceutical and clinical sectors which require amylases of high purity (El Nour *et al*., 2013). Therefore, amylase extracts from fonio-derived grains can be used without purification in industries like detergents, paper, brewery which does not require enzyme purity but for wider usage of these amylases, further studies is required on its purification techniques, thermal stability and effects of various metal ion so, the induced amylases in order to increase its application varieties

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