

## Partial Purification and Characterisation of $\beta$ -Amylase Isolated From Malted Pearl Millet (*Pennisetum glaucum*)

\*KOLAWOLE, A. N. , AYEGBA, D. O. and AJELE, J. O.

Department of Biochemistry, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria.

Copyright resides with the authors in terms of the Creative Commons License 4.0.

(See <http://creativecommons.org/licenses/by/4.0/>).

Condition of use: The user may copy, distribute, transmit and adapt the work, but must recognize the authors and the Nigerian Journal of Biotechnology.

### Abstract

The industrial use of enzymes in the degradation of starch has become predominant over conventional methods of acid starch hydrolysis. As such, cheap sources of starch hydrolysing enzymes are being sought for.  $\beta$ -amylase, an exo-hydrolase which releases maltose by hydrolysing starch from the non-reducing end, is one of such important enzymes found to be common to the seeds of higher plants such as cereal grains. In this research,  $\beta$ -amylase was extracted from malted Pearl Millet grains and partially purified by ammonium sulphate precipitation (0-60%). The activity of  $\beta$ -amylase peaked at 72h of malting (7.40  $\mu\text{mol}/\text{min}/\text{mL}$ ) with specific activity of 1.84  $\mu\text{mol}/\text{min}/\text{mg}$ . The enzyme extract at 72h was purified for characterization assays, giving a 1.33 purification fold and a yield of 9.28% after extensive dialysis against extraction buffer. Enzyme characterisation assays showed pearl millet  $\beta$ -amylase has optimal activity at pH 5.0 and 50 °C while a  $K_m$  value of 10 mg/mL and  $V_{max}$  value of 11.11  $\mu\text{mol}/\text{min}/\text{mL}$  were obtained when corn starch was used as substrate. The enzyme also showed significant activity over a wide temperature and pH range, indicating its potential as an industrial enzyme. The results from this study show that malted Pearl Millet is a cheap source of  $\beta$ -amylase with favourable kinetics and the desired characteristics of activity at low pH and elevated temperatures.

**Keywords:** Partial Purification, Pearl Millet,  $\beta$ -amylase, Starch, Malting, Industrial Potential.

**Correspondence:** [adejokekolawole@futa.edu.ng](mailto:adejokekolawole@futa.edu.ng) Tel: +234 803 3582 082

### Introduction

Starch is a carbohydrate food reserve in plants and known to be one of the most abundant biopolymers on earth (Anto et al., 2006). This polysaccharide, is abundant in the endosperm of several small seeded cereals (millets) and are converted to fermentable sugars during biotechnological and industrial processes of malting (Kolawole and Kolawole, 2015). Several indigenous foods and drinks are made from the flour and malt of these millets across South Asia and Africa (Kolawole and Ebiloma, 2017). Depolymerization of starch may be accomplished by various chemical and physical treatments or by enzymatic treatment.

However, amylases as biocatalysts are now being employed for efficient starch liquefaction and saccharification against the crude acid hydrolysis method (Pervez et al., 2014; Ayodeji et al., 2017).

Pearl Millet (*Pennisetum glaucum*) is an annual, warm-season crop widely grown usually for grazing, hay, cover crop and wildlife. It yields higher than foxtail millet and regrows after harvest if sufficient stubble is left (Chad and Jimmy, 2014). The grass grows 4-8 ft tall, on smooth 1/2–1inch diameter stems, with more upright side shoots (tillers) compared to sorghum (Kajuna and Lewis, 2001). The seeds of Millets are cylindrical, typically white, or yellow,

but there are varieties with colours ranging from brown to purple. Pearl Millet's deep root system grows relatively fast (Hannaway and Larson, 2004), and though, widely distributed across Africa, India, and Asia, Pearl Millet, can be grown throughout the continental United States and Puerto Rico. Despite its wide distribution however, pearl millet is chiefly used as animal fodder material (Chad and Jimmy, 2014) and therefore, discovery of alternative uses for the hardy cereal does not threaten human food production.

Amylases are among the most important enzymes used in several biotechnological applications particularly in starch processing industries to hydrolyse the polysaccharides such as starch into simpler sugars. This is the basis for various industrial processes like preparation of glucose syrups (Prakash et al., 2011).  $\beta$  amylases (EC 3.2.1.2) are exo-hydrolases which release  $\beta$ -maltose from the non-reducing ends of  $\alpha$ -1, 4 linked poly and oligoglucans until the first  $\alpha$ -1, 6 branching point along the substrate molecule is encountered (Saini et al, 2017). It is a starch hydrolyzing enzyme used in foods, pharmaceuticals and brewing industries to convert starch into maltose (Aiyer, 2005). High value is placed on the thermal stability and activity at low pH in industrial processes. However, most enzymes from higher plants and micro-organisms are known to be neither active nor stable at elevated temperatures (Moura et al, 2016). Studies on  $\beta$ - amylase have been highly concentrated on the use of microorganisms, particularly *Bacillus* species such as *Bacillus cereus*, *Bacillus megaterium* and *Bacillus polymyxa* (Femi-Ola and Ibikunle, 2013; Niziolek, 1997; Hensley et al, 1980), as source of the enzyme, with minimal work being done on plant  $\beta$ -amylases.  $\beta$  -amylases from soybean, barley, and wheat have been used industrially (Hesam et al, 2015). However, soybean  $\beta$ -amylase is relatively expensive and barley and wheat  $\beta$ -amylases are lacking in thermostability. The Pearl Millet is thought to be a promising source of  $\beta$ -amylase since  $\beta$ -amylase is one of the major proteins in the cereal, which is responsible for it's usability as a brewer's grain. In addition, the crop is hardy to environmental conditions, pests, and diseases and it's use in enzyme production poses minimal threat to human and animal food production.

This paper therefore presents a  $\beta$ -amylase from malted pearl millet and describes some of its properties with a view to exploiting the enzyme for industrial purposes.

## Materials and Methods

### *Collection of Sample*

Newly cultivated and harvested grains of pearl millet were obtained from the Oja Oba market, Akure, Ondo State, South-western Nigeria and were authenticated at the Department of Crop, Soil and Pest Management, the Federal University of Technology, Akure, Nigeria.

### *Malting of Pearl Millet Grains*

The malting of the pearl millet grains was done as described by Kharkrang and Ambasht (2012) with some modifications. Dried grains Pearl Millet (300 g) was weighed into a plastic vessel. The grains were carefully rinsed and then soaked with distilled water for 6 h. The soaked grains were sieved and spread in an incubator set at 25 °C where they were left to grow and germinate. An aliquot sample from the soaked grains was obtained to represent the grain at 0 h. The germinating grains were sprinkled with little quantity of distilled water every day until the 5th day and also sampled daily for analysis (Macnicol et al., 1993)

### *Extraction of Crude Enzyme from the Malted Pearl Millet Grains*

The extraction of crude  $\beta$ -amylase from the germinated grains was done as described by Kharkrang and Ambasht (2012) with some modifications. The vegetative parts of the samples were removed by rubbing them between the palms, followed by suspension in distilled water and decantation. 30 g of each sample was weighed and homogenized using an electric blender with 7.5 mL of 50 mM sodium phosphate buffer pH 6.9. The homogenized samples were then centrifuged at 6000 rpm x g at 4 °C for 25 min. The obtained supernatant was used as the crude enzyme solution.

### *Beta Amylase Activity Assay and Determination of Total Protein*

Amylase activity assay was carried out according to the method of Bernfeld (1955).

Briefly, 0.5 mL of enzyme solution was added into a test tube containing 0.5 mL 1% starch solution in 50 mM Sodium acetate buffer pH 4.8. The reaction was allowed to proceed for 5 min at 40°C before termination. The amount of reducing sugar released was estimated using dinitro salicylic acid (DNSA) reagent as described by Miller (1959). One unit of enzyme is defined as the amount of enzyme that is required to liberate 1  $\mu$ mol of maltose per min under the described assay condition.

The total protein content in the sample was determined using the method of Bradford (1976) using Bovine Serum Albumin (BSA) as the standard protein.

#### *Partial Purification of $\beta$ -amylase from Crude Extract*

Precipitation of  $\beta$ -amylase from the crude enzyme was done stepwise until it attained 60% ammonium sulphate saturation and the solution was incubated overnight in a cold laboratory system. The resulting suspension was centrifuged at 10,000 rpm x g for 15 min at 4 °C. The pellet was obtained as the precipitated  $\beta$ -amylase and was suspended in 2 mL of 50 mM sodium phosphate buffer pH 6.9. The precipitate was dialysed extensively, as described by Sattayasai (2012), with some modifications, against 50 mM sodium phosphate buffer pH 6.9

### **Characterization of Partially Purified $\beta$ -amylase**

#### *Effect of Temperature on the Activity of $\beta$ -amylase from Pearl Millet*

The effect of temperature on  $\beta$ -amylase activity was determined at different temperatures between 25°C and 80°C in 50 mM sodium acetate buffer solution, pH 4.8 under standard assay conditions previously described.

#### *Effect of pH on the Activity of $\beta$ -amylase from Pearl Millet*

The effect of pH on the activity of the partially purified  $\beta$ -amylase was determined by performing the enzyme activity assay earlier described at different buffer solution pH using the following buffers (50 mM): Sodium acetate buffer (pH 4.0 – 5.0), potassium phosphate buffer (pH 6.0 - 7.0) and Tris-HCl buffer (pH 8.0 - 9.0) at 25°C.

#### *Kinetic Parameters of $\beta$ -amylase from Pearl Millet*

Kinetic parameters ( $K_m$  and  $V_{max}$ ) were determined at best conditions by measuring the initial reaction rates with soluble starch at various concentrations (0.5 – 4 %) for  $\beta$ -amylase. The accurate values of the apparent kinetic parameters were obtained from double reciprocal plot by Lineweaver and Burk (1934).

### **Results and Discussion**

#### *Total Crude Protein Content*

A protein standard curve was obtained using the method of Bradford (1976) using Bovine Serum Albumin (BSA) as the standard protein (Figure 1). At 0h, the total protein content was 1.36 mg/mL which increased slightly to 1.46 mg/mL after 24 h and 2.27 mg/mL at 48<sup>th</sup>h. A gradual increase in the protein concentration was noticed from the beginning of the malting process up till 72<sup>nd</sup> h with a value of 4.02 mg/mL before a sharp drop to 2.54 mg/mL at 96<sup>th</sup> h of malting (Figure 2). The protein concentration reaching maximum at 72<sup>nd</sup> h in this study agrees with the reports of Egwin and Oloyede (2006) and El Nour et al. (2013), with both studies placing the maximum protein concentration of malted millet between 72<sup>nd</sup> h and 96<sup>th</sup> h of germination. This increase in protein content can as well be correlated with the activity of the enzyme which was observed to peak at the 72<sup>nd</sup> h of malting.

#### *Activity of $\beta$ -amylase in Crude Extract*

Cereal amylases play a very important role in the metabolism of germinating cereals (Agbo et al., 2017). These enzymes are synthesized under the influence of plant growth hormones such as gibberellic acid upon activation by water imbibition and they exist in multiple forms (Muralikrishna and Mirmala, 2005). Germinated pearl millet grains were investigated as a possible source for industrially viable  $\beta$ -amylase. The results of the study showed promising amylase activity from the germinated grain extract. A  $\beta$ -amylase activity standard curve was obtained using the method of Bernfeld (1955) (Figure 3). The activity of  $\beta$ -amylase at 0h of malting was 5.23  $\mu$ mol/min/mL while the specific activity was 3.86  $\mu$ mol/min/mg protein. After 24 h of malting,  $\beta$ -amylase activity as well as the specific activity showed a slight increase, being

5.65  $\mu\text{mol}/\text{min}/\text{mL}$  and 3.89  $\mu\text{mol}/\text{min}/\text{mg}$  protein respectively. At 48h, a more marked increase was noted with  $\beta$ -amylase activity being 6.25  $\mu\text{mol}/\text{min}/\text{mL}$  and specific activity being 4.61  $\mu\text{mol}/\text{min}/\text{mg}$ .  $\beta$ -amylase activity peaked at 72 h being 7.65  $\mu\text{mol}/\text{min}/\text{mL}$  in agreement with reports on maximum  $\beta$ -amylase activity of malted millet between 72h and 96h (Egwin and Oloyede, 2006; El Nour et al., 2010; El Nour et al., 2013) but the specific activity plummeted significantly to 1.85  $\mu\text{mol}/\text{min}/\text{mg}$ , a phenomenon which can be correlated with the noted increase in protein content of the Pearl Millet grains at this time.  $\beta$ -amylase activity at 96h showed a marked decrease from the peak down to 6.13  $\mu\text{mol}/\text{min}/\text{mL}$  while the specific activity increased slightly to 2.41  $\mu\text{mol}/\text{min}/\text{mg}$  (Figure 4). Degradation of the starch endosperm of the malted pearl millet grains is thought to be responsible for this. This is explained by the

phenomenon in which the starch content of the grains, as well as other metabolic resources begin to run out, therefore impeding the germinating grain's ability to produce more enzymes and other proteins (Kolawale and Ebiloma, 2017).

#### *$\beta$ -amylase Partial Purification Process*

The 72<sup>nd</sup> h extract which showed maximum  $\beta$ -amylase activity was chosen for partial purification. Purification was carried out by 60% ammonium sulphate precipitation and dialysis. The clear solution remaining after the ammonium sulphate precipitation showed no  $\beta$ -amylase activity. The summary of purification procedure is shown in Table 1. The two step purification process revealed a specific activity of 2.44  $\mu\text{mol}/\text{min}/\text{mg}$  yield of 9.28% and purification fold of 1.33.

**Table 1.** Summary of partial purification of  $\beta$ -amylase from Pearl Millet (*P. glaucum*) using 50mM Sodium phosphate buffer pH 6.9.

Fraction	Vol (mL)	Protein Content (mg/mL)	$\beta$ -amylase Activity ( $\mu\text{mol}/\text{min}/\text{mL}$ )	Total activity ( $\mu\text{mol}$ )	Specific activity ( $\mu\text{mol}/\text{min}/\text{mg}$ of protein)	Yield (%)	Purification fold
<b>Crude Enzyme</b>	30.00	4.02	7.40	221.85	1.84	100	1.00
<b>Ammonium Sulphate Precipitation (at 60%)</b>	2.00	3.71	8.10	16.134	2.17	7.28	1.18
<b>Dialysis against Buffer</b>	2.50	3.38	8.24	20.588	2.44	9.28	1.33

#### *Characterisation assays of Pearl Millet $\beta$ -amylase*

The partially purified 72<sup>nd</sup> h extract was subjected to characterisation assays to determine the usefulness of the enzyme in the industry.

#### *Kinetic analysis of $\beta$ -amylase from Pearl Millet*

The reaction between the partially purified  $\beta$ -amylase and corn starch obeyed the Michelis-Menten rule (Figure 5), reaching

saturation at 10 mg/mL of substrate concentration. The  $K_m$  value obtained from the Lineweaver-Burk plot for the Pearl Millet  $\beta$ -amylase is rather high, 10.00 mg/mL (Figure 6), indicating a low affinity of the enzyme for the corn starch substrate while the  $V_{max}$  value (11.11  $\mu\text{mol}/\text{min}/\text{mL}$ ) is comparable to the results reported (12.50  $\mu\text{mol}/\text{min}/\text{mL}$ ) by Tapan et al. (2006) for *Heliodiaptomus viduus* (Gurney)  $\beta$ -amylase.

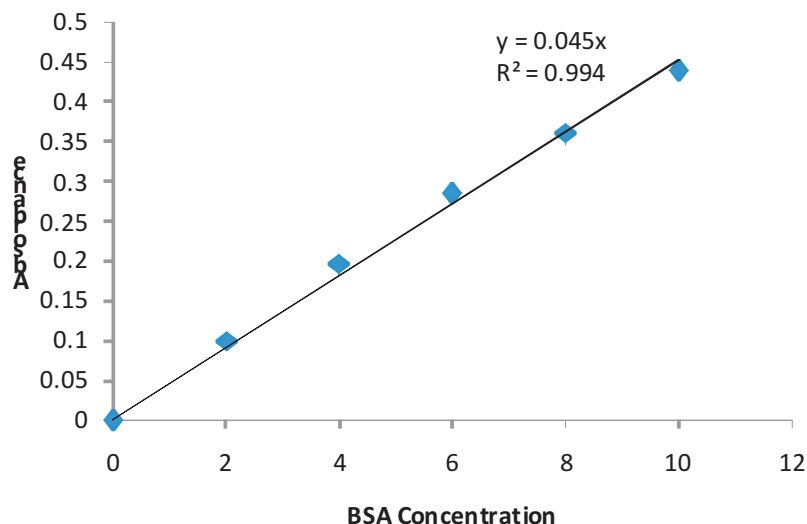
### Effect of pH on $\beta$ -amylase Activity

At pH 4, the relative activity was 55.66% while at pH 5.0 the relative activity peaked at 100% after which a successive decline was recorded at pH 6 (74.83%), pH 7 (52.12%), pH 8 (35.66%) and pH 9 (29.07%) (Figure 7). The partially purified Pearl Millet  $\beta$ -amylase revealed optimum activity at pH 5.0, showing significant preference for acidic pH than most bacterial  $\beta$ -amylases which have their pH optimum at around pH 6.0 (Femi-Ola et al, 2013; Madi et al, 1987) while exhibiting similarity to the optimum pH 5.5 reported by Oyefuga et al. (2011) for  $\beta$ -amylase isolated from *Saccharium officinacium* nodes. Most commonly described  $\beta$ -amylase lack significant activity at extreme pH (2-3) (Eke and Oguntimehin, 1992; Hyun and Zeikus, 1985). Extreme pH can initiate chemical reactions that can destroy amino acid residues of the protein molecules of the enzyme, thus resulting in irreversible deactivation of the enzyme (Creighton, 1990). Thus, Pearl Millet  $\beta$ -amylase shows potential for industrial processes such as starch liquefaction which require low pH environments (Richardson, 2002)

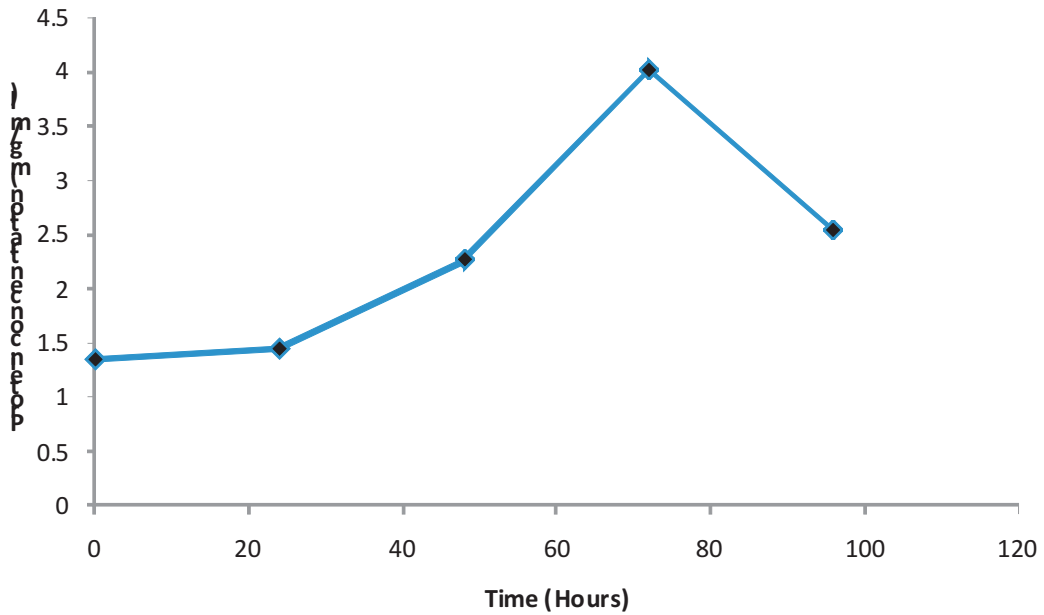
### Effect of Temperature on $\beta$ -Amylase Activity

The thermal studies showed that there was a gradual increase in the activity of the partially purified  $\beta$ -amylase as the assay temperature

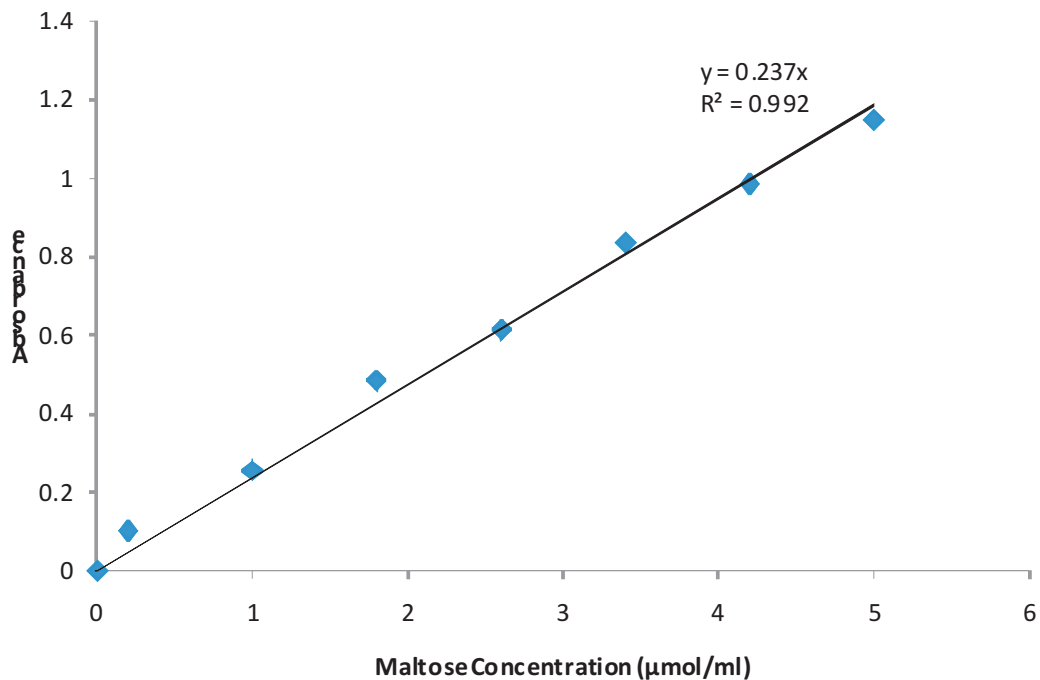
condition increased between 25°C, 30 and 40°C with relative activities of 42.94%, 46.86% and 71.166%, respectively. Maximum activity was recorded at 50 °C while temperature upwards of 60°C showed a decline in the relative activity of the amylase (Figure 8). The optimal activity temperature at 50 °C is comparable with reported values 50°C to 60°C for  $\beta$ -amylase from microbial sources including *Actinomycetes* and *Bacillus subtilis* (El Nouret al., 2013; Femi-Ola and Ibikunle, 2013; Obi and Odibo, 1984). At 60°C is the enzyme still shows appreciable activity, comparable with the enzyme's relative activity at room temperature, 25°C (41.72% to 42.94% respectively). Relative activity at 70 and 80 was recorded at 16.02% and 7.33% respectively. The decline in activity below or above the optimum temperature results from the changes in the protein structure due to the changes in the temperature of the environment (Agbo et al, 2017). Loss of activity at temperatures far exceeding the optimum temperature is usually irreversible (Prescott et al, 2008). An optimum temperature of 50°C places this amylase as a fairly thermostable enzyme, sharing it's optimum temperature (and pH) with acid amylase, an industrially used amylase isolated from *Aspergillus niger* (Vengadaramana, 2013).



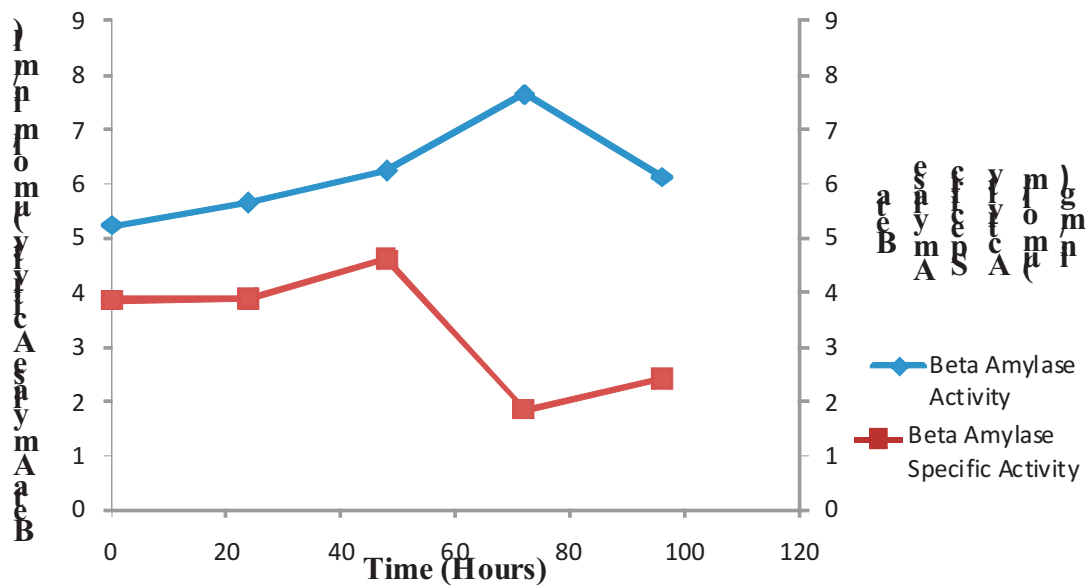
**Figure 1:** Bovine Serum Albumin (BSA) protein concentration standard curve. The BSA concentration range was between 2µg/mol-10µg/mol. Each point represents the mean  $\pm$  SD values of independent experiments carried out in triplicate.



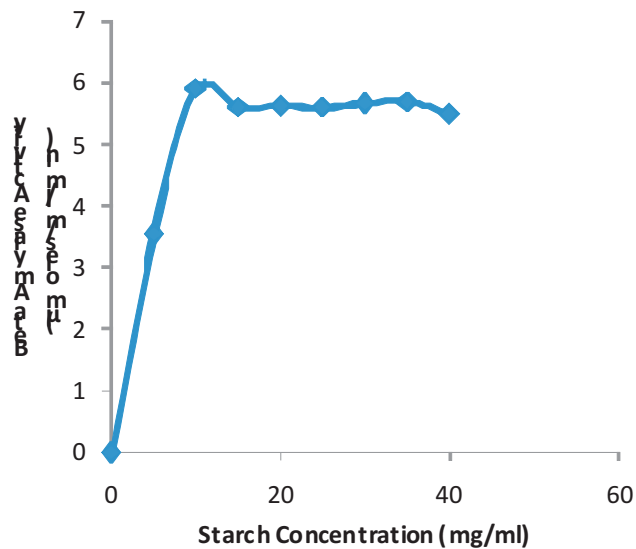
**Figure 2:** Protein concentration curve showing the dynamics of protein concentration from 0-96 h at 24 h intervals at 25°C at near neutral pH (50mM Sodium phosphate buffer pH 6.9). A steady rise in total protein content is observed from 0h till 48h. Maximum protein concentration is indicated by the peak at 72h of germination, followed by a decline in total protein content at 96h. Each point represents the mean  $\pm$  SD values of independent experiments carried out in triplicate.



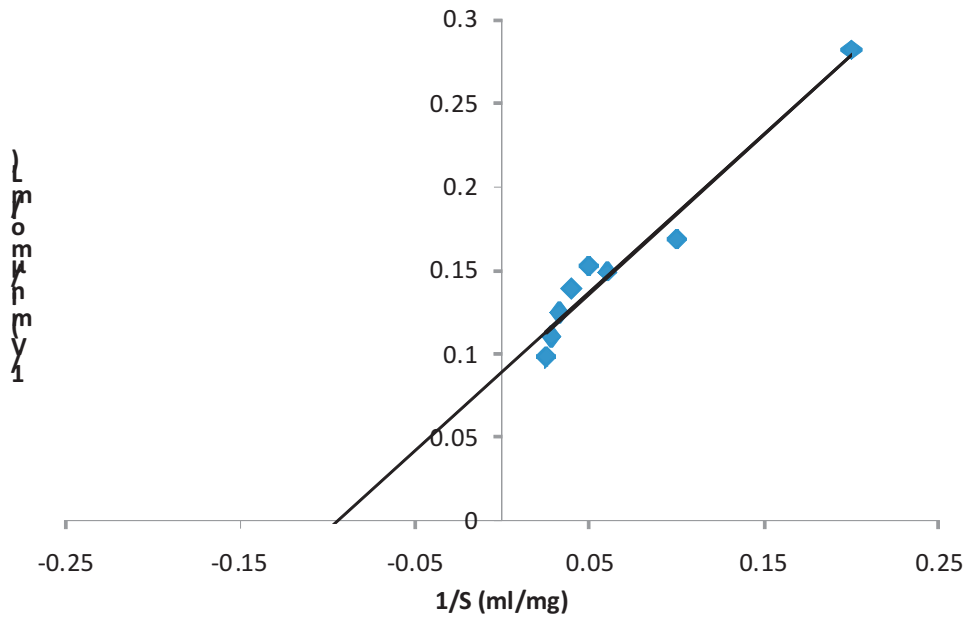
**Figure 3:** Maltose standard curve. The Maltose solution concentration range was between 0.2 $\mu\text{mol/ml}$  - 5.0 $\mu\text{mol/ml}$ . Each point represents the mean  $\pm$  SD values of independent experiments carried out in triplicate.



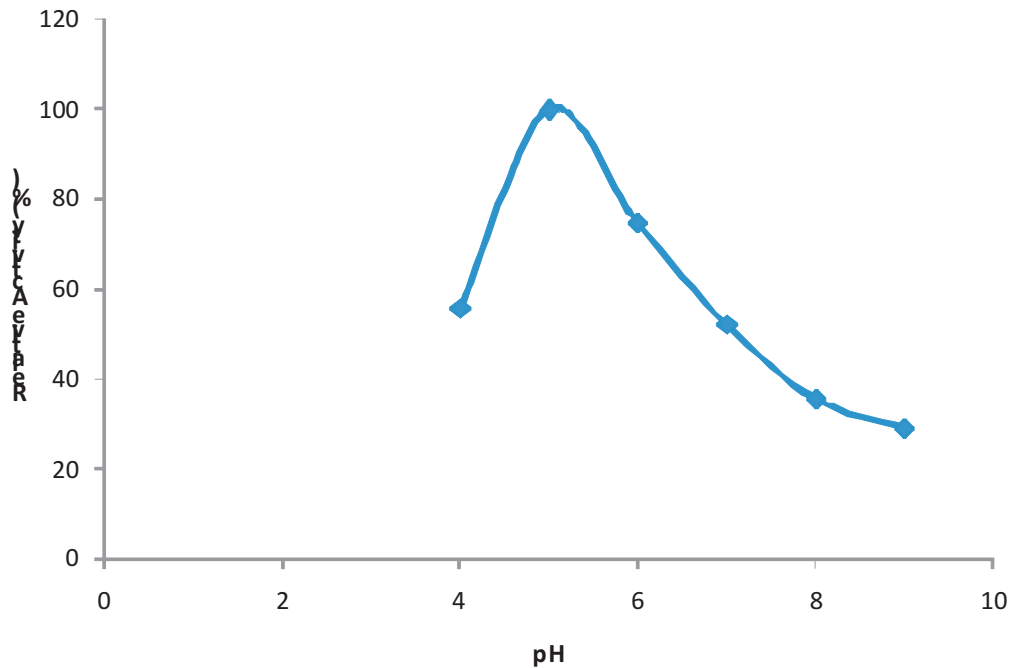
**Figure 4:**  $\beta$ -amylase activity curve showing the dynamics of activity and specific activity from 0-96 h at 24 h intervals at 25°C at acidic pH (16mM Sodium acetate buffer pH 4.8). Activity peaks at 72h while specific activity drops, showing that while amylase activity is maximum at 72h, protein content also exhibits a massive increase at that point. This is then followed immediately by a drop in  $\beta$ -amylase activity and a slight rise in specific activity due to a reduction in the total protein content of the germinating grain. Each point represents the mean  $\pm$  SD values of independent experiments carried out in triplicate.



**Figure 5:** The Michellis Menten curve of the effect of substrate concentration (1% - 4% Starch) on the activity of partially purified pearl millet  $\beta$ -amylase at 25°C in acidic pH (16mM Sodium acetate buffer pH

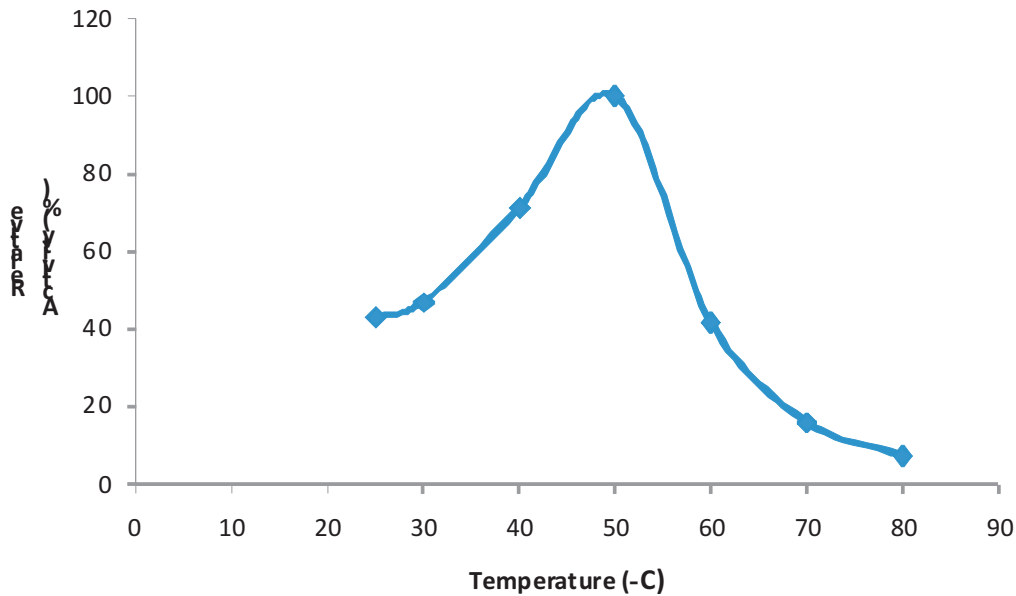


**Figure 6:** The Lineweaver-Burk plot of the effect of substrate concentration (1% - 4% Starch) on the activity of partially purified pearl millet  $\beta$ -amylase at 25°C in acidic pH (16mM Sodium acetate buffer pH 4.8).  $V_{max}$  was determined to be 11.11  $\mu\text{mol}/\text{mL}/\text{min}$  while  $K_m$  was 10 mg/mL. Each point represents mean  $\pm$  SD values of independent experiments carried out in triplicate.



**Figure 7:** The effect of pH on partially purified pearl millet  $\beta$ -amylase at 25°C within the range of pH 4-9, using 16mM Sodium acetate buffer from pH 4-5, 16mM Sodium phosphate buffer from pH 6-7 and 16mM Tris-HCl buffer from pH 8-9. Each point represents the mean  $\pm$  SD values of independent experiments carried out in triplicate.





**Figure 8:** The effect of temperature on partially purified Pearl Millet  $\beta$ -amylase at pH 4.8 (16mM Sodium acetate buffer) within the range of 25°C to 80°C. Each point represents the mean  $\pm$  SD values of independent experiments carried out in triplicate.

### Conclusion

Pearl millet (*Pennisetum glaucum*) is a cheap source of  $\beta$ -amylase. Analysis of the partially purified enzyme from the cereal shows desirable properties of an industrial enzyme. Its wide pH and temperature range of activity, as well as its optimum activity at elevated temperatures and acidic pH is good indication of its potential for industrial application.

### References

- Agbo, K. U., Eze, S. O., Okwuenu, P. C., Ezike, T. C., Ezugwu, A. L. and Chilaka, F. C. (2017). Extraction, Purification and Characterization of Sprouting Pearl Alpha-Amylase for Biotechnological Applications. *J. Plant. Biochem. Physiol.*, 5(1) 1 - 6.
- Aiyer, P. V. (2005). Amylases and their applications. *Afr. J. Biotech.*, 4: 1525 - 1529.
- Anto, H., Trividi, U. B., and Patel, K. C. (2006). Glucoamylase production by solid-state fermentation using rice flake manufacturing waste products as substrate. *Bioresour Technol.*, 97(10): 1161 - 1166.
- Ayodeji, A. O., Bamidele, O. S., Kolawole, A. O. and Ajele, J. O. (2017). Physicochemical and kinetic properties of a high salt tolerant *Aspergillus flavus* glucoamylase. *Biocatal. Agric. Biotech.* 9: 35 - 40.
- Bernfeld, P. (1955), Amylase: Alpha and Beta. *Meth. of Enzymol.*, 1: 149 - 158.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing: the principle of protein-dye binding. *Anal. Biochem.*, 72: 248 - 254.
- Chad, L. and Jimmy, H. (2014). Foxtail Millet, Pearl Millet, USDA Agricultural Research Service, Pp. 972 - 990.
- Creighton, T. E. (1990). Protein Function: A practical approach. Oxford University Press, Oxford.
- Egwin, E. C. and Oloyede, O. B. (2006), Comparison of  $\alpha$ -amylase activity in some sprouting Nig. Cer. *Biokem.*, 18(1): 15 - 20.

- Eke, O.S. and Oguntimehin, G.B. (1992). Partial purification and characterisation of Alpha-amylase from *Bacillus cereus* BC 19q. J. Agric. Sci. Technol., 2 (2): 152 – 157.
- El Nour, M.E.M., Yagoub, S. O. (2010). Partial purification and characterization of  $\alpha$  and  $\beta$ -amylases isolated from *Sorghum bicolor* cv. (Feterita) malt. J. Appl. Sci., 10(13): 1 - 6.
- El Nour, M. E. M., Yagoub, S. O. and Al Jarbough, A. (2013). Purification and characterization of  $\alpha$  and  $\beta$ -Amylases isolated from millet (*Pennisetum Glaucum*) malt. Amer. J. Sci. Ind. Res. 4(2): 183 - 189.
- Femi-Ola, T, O. and Ibikunle, I. A. (2013). Purification and Characterization of Beta-Amylase of *Bacillus subtilis* Isolated from Kolanut Weevil. J. Biol. Life Sci., 4(1): 68 - 78
- Femi-Ola, T .O., Oshokoya, A. H. and Bamidele, O. S. (2013). Kinetic properties of beta-amylase from *Bacillus subtilis*. IOSR-JESTFT, 2(4):19 - 23
- Hannaway, D. B. and Larson, C. (2004). Forage fact sheet: Pearl Millet (*Pennisetum americanum*). Oregon State University, Corvallis, OR. Pp. 456 - 460.
- Hensley, D. E., Smiley, K. L., Boundy, J. A., and Lagoda, A. A. (1980). Beta-amylase production by *Bacillus polymyxa* o a corn-steep-starch-salts medium. Appl. Environ. Microbiol., 39(3): 678 - 680.
- Hesam, F., Tehrani, T., Balali. G. R. (2015). Evaluation of  $\beta$ -amylase activity of sweet potato (*Ipomoea batatas*) cultivated in Iran. J. Food. Biosci. and Tech., 5(2): 41 - 48
- Horváthová, V., Janeček, Š. and Šturdík, E. (2000). Amyolytic enzymes: their specificities, origin and properties. Biol. Bratis., 56(6): 605 - 615.
- Hyun, H. H. and Zeikus, J. G. (1985). General Biochemical Characterisation of Thermostable extracellular  $\beta$ -amylase from *Clostridium thermosulfurogenes*, Appl. Environ. Microbiol., 49: 1162 – 1167.
- Kajuna, S. D. M. and Lewis, B. (2001). Millet: Post-Harvest Operations, Sokoine Univ. of Ag., Morogoro, Tanzania, Pp. 676 - 688.
- Kharkrang, K. and Ambasht, P.K (2012). Purification and characterization of alpha amylase from seeds of pearl millet (*Pennisetum typhoides*). J. Prot, Proteom., 3(1):47 - 60
- Kolawole, A. N. and Ebiloma, I. B. (2017). Modulation of steeping conditions influence the diastatic enzymes and protein profile in pearl millet malt. Biokemistri., 29(1): 1 - 11.
- Kolawole, A. N. and Kolawole, A. O. (2015). Diastatic enzymes malting process optimisation of African finger millet for biotechnological uses. Afri. J. Biochem. Res., 9(6): 81 - 88.
- Lineweaver, H. and Burk, D. (1934). The determination of enzyme dissociation constants. J. Amer. Chem. Soc., 56(3):658 - 666.
- Macnicol, P. K., Jacobsen, J. V., Keys. M. M. and Stuart, I. M. (1993). Effects of heat and water stress on malt quality and grain parameters of schooner barley grown in cabinets. J. Cer. Sci., 18: 61 - 68.
- Madi, E., Antranikan, G., Ohmiya K. and Gottschu, G. (1987). Thermostable amyolytic enzymes from a new *Clostridium isolate*. Appl. Envir. Bacteriol., 53: 1661 - 1667.
- Miller, G. L. (1959). Use of dinitrosalicylic acid for determination of reducing sugar. Anal.Chem., 31(3):426-428
- Moura, G.S., Lanna, E.A.T., Donzele, J.L., Falkoski, D.L., Rezende, S.T., Oliveira, M.G.A. and Albino, L.F.T. (2016). Stability of enzyme complex solid-state fermentation subjected to the processing of pelleted diet and storage time at different temperatures. R.Bras.Zootec., 45(12):731-736.
- Muralikrishna, G. and Nirmala, M. (2005). Cereal  $\alpha$ -amylases - An overview. Carb.Pol. 60: 163-173.

- Niziolek, S. (1997). Beta-amylase production by some *Bacillus cereus*, *Bacillus megaterium* and *Bacillus polymyxa* (correction of polymaxa) strains. *Acta. Microbiol. Pol.*, 46(4):357 - 62.
- Obi, S. K. and Odibo, F. J. 1984. Partial purification and characterization of a thermostable Actinomycete  $\beta$ -amylase. *Appl. Environ. Microbiol.*, 47(3):571-5.
- Oyefuga, O. H., Adeyanju, M. M., Adebawo, O. O. and Agboola, F. K., (2011). Purification and some properties of  $\alpha$ -amylase from the nodes of sugar cane, *Saccharium officinarum*. *Int. J. Plant Physio. Biochem.*, 3(7): 117 - 124.
- Pervez, S., Afsheen, A., Samina, I., Nadir, N.S. and Shah, A.U. (2014). Saccharification and liquifaction of cassava starch: an alternative source for the production of bioethanol using amylolytic enzymes by double fermentation process. *B.M.C. Biotech.*, 14: 49 - 59.
- Prakash, O., Nivedita, J. and Panday, R. K. (2011). Effect of metal ions, EDTA and sulfhydryl reagent on soybean amylase activity. *As. J. Biochem.*, 6(3), 282 - 290.
- Prescott, S. C., Harley J. C. and Dunn, C. G. (2008). Saccharifying agents: Methods of production and uses. *Indust. Microb.*, 3: 836 - 885.
- Richardson, T. H., Tan, X., Frey, G., Callen, W., Cabell, M., Lam, D., Macomber, J., Short, J. M., Robertson, D. E. and Miller, C. (2002). A novel, high performance enzyme for starch liquefaction. Discovery and optimization of a low pH thermostable alpha amylase. *J. Biol. Chem.*, 277(29): 26501-7.
- Saini, R., Saini, H.S. and Dahiya, A. (2017). Amylases: Characteristics and industrial applications. *J. Pharma. and Phytochem.*, 6(4): 1865 - 1871
- Sattayasai, N. (2012). Protein purification, Chemical Biology, Prof. Deniz Ekinci (Ed). pp 7. In-Tech.
- Tapan, K. D., Malabendu, J., Priti, R. P. and Tanmay, B (2006). The effect of temperature, pH and salt on Amylase in *Heliodiaptomus viduus* (Gurney) (Crustacea: Copepoda: Calanoida). *Turk. J. Zool.*, 30: 187-195.
- Vengadaramana, A. (2013). Industrial important microbial alpha amylase on starch-converting process. *Sch. Acad. J. Pharm.*, 2(3): 209 - 211.