

# Partial Characterization of Glucoamylase Crude Enzyme Produced by *Aspergillus* and *Rhizopus* Strains

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**ABSTRACT---** *Glucoamylase is widely used in the food industry to produce high glucose syrup, and also in fermentation processes for production of beer and ethanol. In this work glucoamylase enzyme produced by six fungal strains were previously isolated from different Sudanese soils, crop seeds, rotten fruits and pieces of moistened bread designated as Aspergillus awamori (A1 and A7), A. niger (A10), A. tamari (Aw), A. terreus (At) and Rhizopus oryzae (R3) in a liquid culture was evaluated and characterized. The maximum temperature of glucoamylase activity are found to be in the range of 60-70°C and at pH value of 5.0-6.0 and the activity of all enzymes from all isolates increased with increase of the concentration of soluble starch. Also the activity of the enzyme from all isolates under study increase with increase of time reaction.*

**Keywords---** Characterization, Glucoamylase, *Aspergillus* & *Rhizopus* isolates

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## 1. INTRODUCTION

Glucosylase ( $\alpha$ -1,4-glucan glucohydrolase, amyloglucosidase) is exoenzyme of great importance for saccharification of starchy materials and other related oligosaccharides. Glucoamylase consecutively hydrolyzes 1,4- $\alpha$ -glycosidic bonds from the non-reducing ends of starch and 1,6- $\alpha$ -glucosidic linkages in polysaccharides yielding glucose as the end-product, which in turn serves as a feedstock for biological fermentations (Gupta, et al., 2003; Norouziyan, et al., 2006). This enzyme is used in the baking industry, the brewing Process, and the most important application of glucoamylase is the production of high glucose syrups. also have various applications in major areas of food processing, animal nutrition, fermentation biotechnology, paper making, fabric industries and in whole grain hydrolysis for the alcohol industry (Selvakumar et al., 1996; Zambare, 2010). On the basis of the importance of glucoamylase, the present study has been taken to characterize the crude GA extract obtained without further purification in terms of optimal pH and reaction temperature, as well as thermo-stability. In a previous work (Abdalwahab et al. 2012) we reported the selection of six strains viz. *Aspergillus awamori* (A1 and A7), *A. niger* (A10), *A. tamari* (Aw), *A. terreus* (At) and *Rhizopus oryzae* (R3) because they produce high glucoamylase activity when grown on rice flour medium. The present study has been taken to partial characterization of the crude enzyme.

## 2. MATERIAL AND METHODS:

### *Inoculum preparation*

The growth medium contained (% w/v) 0.5 g of rice flour, 100 ml of distilled water in 500 ml Erlenmeyer flasks and then was sterilized by autoclaving at 121°C and 15 lb/square inch for 15 min. after cooling the flasks, inoculum was prepared taking a loop full of spores of the chosen fungal isolates (from a 7 days old culture grown in PDA slant) was inoculated separately in the flask. These cultures propagated in a rotary incubator shaker (100 rpm) for 24 h at 30°C. 5 mL of these vegetative inocula were then inoculated in 250 mL flasks containing 45 mL of the growth medium. These cultures propagated in a rotary incubator shaker (100 rpm) for 3 days at 45°C

### *Isolation of glucoamylase*

The samples were then centrifuged at 5000 rpm to remove mycelia. The supernatant fluids were collected and used for assaying of amylase activity, the supernatant was carefully collected and stored under refrigerated conditions as to crude enzyme assay.

### *Assay of Glucoamylase*

The enzyme assayed by the method of Bernfeld (1951) as described by Pestana and Castillo (1985) using starch as substrate. One unit of enzyme activities was expressed as one mg of glucose liberated per one ml. of culture supernatant at pH 7.5, 40°C and in one minute. Extensive screening was carried out by measuring residual glucose and glucoamylase activity.

### **Protein determination**

The protein content was determined by the method of Bradford (1976) with bovine serum albumin as the protein standard. The specific activity of amylolytic enzyme was taken as units/mg protein. Glucose standard curve was measured according to (Muhammad et al., 2011).

### **Partial characterization of glucoamylase**

#### **Effect of Temperature on activity:**

The effect of temperature on enzyme activity was studied in a range between 40 and 90°C using thermostatically controlled water bath incubator and oven at pH 6.0. The Specific activities for the six isolates were determined at each temperature.

#### **Effect of pH on Activity:**

Enzyme activity as function of pH was tested using three buffers: (a) 0.2 M KCl - HCl the pH range 2.0 to 3.0 (b) 0.2 M acetate in pH range 3.0 to 6.0 and (c) 0.2 M phosphate in pH range 6.0 to 8.0. The activity was determined at 70°C for *Aspergillus awamori* (A<sub>7</sub>), *A. niger* (A<sub>10</sub>) and *A. terreus* (A<sub>1</sub>) and at 60°C for *A. tamaris* (A<sub>w</sub>), *A. awamori* (A<sub>1</sub>) and *Rhizopus oryzae* (R<sub>3</sub>).

#### **Effect of Substrate Concentration on Activity:**

The effect of substrate concentration on enzyme activity was studied using different concentrations of soluble starch 0.5%, 1.0%, 1.5%, 2%, 2.5%, 3.0% and 3.5%. The amylolytic enzyme activity of each strain was determined at each concentration using 0.2 M acetate buffer (pH 5.0) for all strains except for *A. awamori*. The pH of the buffer was adjusted at the value of 6.0, and incubated at temperature 70°C for *Aspergillus awamori* (A<sub>7</sub>), *A. niger* (A<sub>10</sub>) and *A. terreus* (A<sub>1</sub>) and at 60°C for *A. tamaris* (A<sub>w</sub>), *A. awamori* (A<sub>1</sub>) and *Rhizopus oryzae* (R<sub>3</sub>).

#### **Effect of Reaction Time:**

This was studied using different reaction times at 15 minute's intervals; 15, 30, 45, 60, 75, 90, 105 and 120 min. The amylolytic enzyme activity was determined at each time interval for each strain. The reaction mixture consisted of 1 ml of 1% soluble starch and 1 ml of 0.2 mol / l acetate buffer (pH 5.0) for all strains except for *A. awamori*. The pH of the buffer was adjusted at the value of 6.0 and incubated at temperatures as above.

#### **Storage Stability:**

Crude enzyme of each of the six strains was kept separately in sample bottles 20 ml of each at -20°C, 4°C and 25°C for six weeks. Every week the enzyme activity was assayed for the three temperatures.

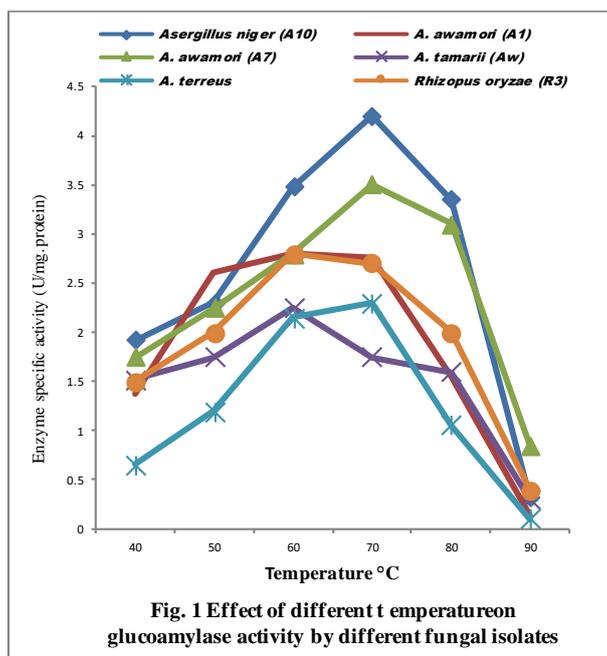
## **3. RESULTS AND DISCUSSION**

### **Effect of Temperature on Glucoamylase Activity by Different Fungal Isolates:**

The activity of the crude glucoamylase enzyme from the six isolates was investigated in temperature range 40-90°C Fig 1. Glucoamylase crude enzyme from *Aspergillus awamori* (A<sub>1</sub>), *Rhizopus oryzae* (R<sub>3</sub>), and *Aspergillus tamaris* (A<sub>w</sub>) showed maximum activity at 60°C with specific activities 2.77, 2.76 and 2.26 U/mg. protein, in order. At 90°C, these enzymes lost 91, 85 and 97%, in order of their original activity.

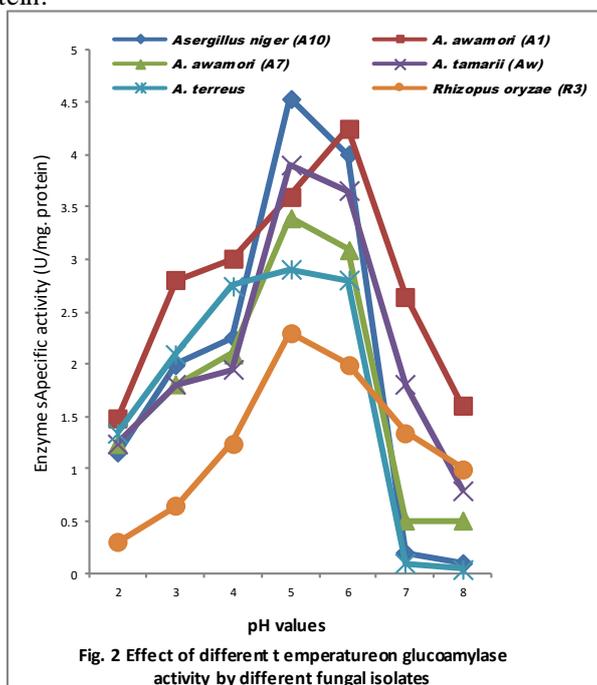
The glucoamylase from *Aspergillus awamori* (A<sub>7</sub>), *A. niger* (A<sub>10</sub>) and *A. terreus* (A<sub>1</sub>), showed maximum performance at 70°C with specific activities 3.24, 4.11 and 2.23 U/mg. protein in order. These enzymes were stable in temperature range 40-80°C but at 90°C lost 77, 94 and 95% of their activities in order.

Thus, the results concluded that the crude enzyme is moderately temperature stable. It is therefore worthwhile to consider means to stabilize the enzyme under storage conditions. These results generally agree with those of Deb *et al.* (2013) working on *Bacillus amyloliquefaciens* P-001. However, the maximum activity of *A. niger* (Coral and Colak, 2000) was found to be in the range of 45-55°C.



**Effect of pH on Glucoamylase Activity by Different Fungal Isolates:**

Fig. 2 shows the effect of pH on the activity of glucoamylase of the six strains. Optimal pH for glucoamylase activity from *Aspergillus awamori* (A<sub>7</sub>), *A. niger* (A<sub>10</sub>), *A. tamarii* (A<sub>w</sub>), *A. terreus* (A<sub>t</sub>) and *Rhizopus oryzae* (R<sub>3</sub>) was 5.0 with specific activities of 3.29, 4.54, 3.37, 2.85 and 2.24 U/mg. protein in order. For *A. awamori* (A<sub>1</sub>), the optimum pH is 6.0 with specific activity 4.22 U/mg. protein.



It is clear that *A. niger* (A<sub>10</sub>) and *A. awamori* (A<sub>7</sub>) have sharp pH optima while *A. terreus* (A<sub>t</sub>) has the broadest optimum in the range of 4.0-6.0. This fact makes this enzyme very suitable for use where pH control is not very strict or expensive. The other species seems to have gradual increase and decrease in the pH range.

**Effect of Incubation Time on Glucoamylase Activity by Different Fungal Isolates:**

It is seen in fig. that the glucoamylases from the six isolates showed an increase in starch hydrolysis with time and to varying degrees. Thus, *Aspergillus niger* (A<sub>10</sub>) displayed the highest rate of hydrolysis followed in decreasing order by *A. tamarii* (A<sub>w</sub>), *A. awamori* (A<sub>1</sub>), *A. awamori* (A<sub>7</sub>), *A. terreus* (A<sub>t</sub>) and finally *Rhizopus oryzae* (R<sub>3</sub>). This result clearly indicates the products of the reaction do not show inhibitory effect on the glucoamylase activity.

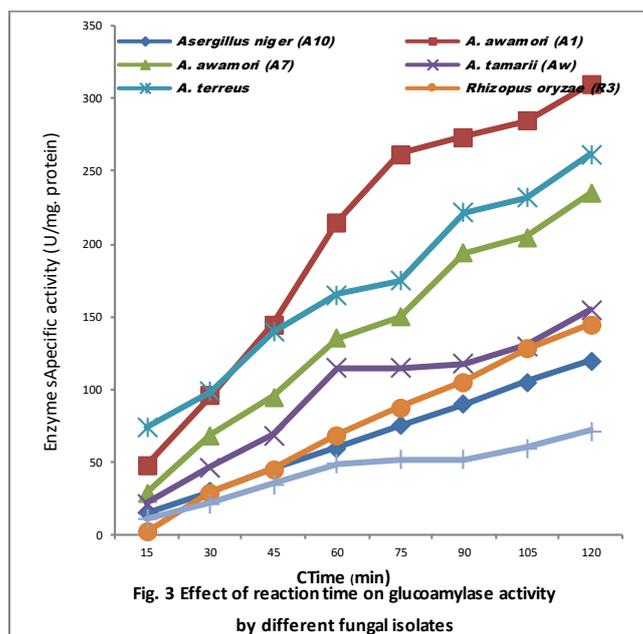
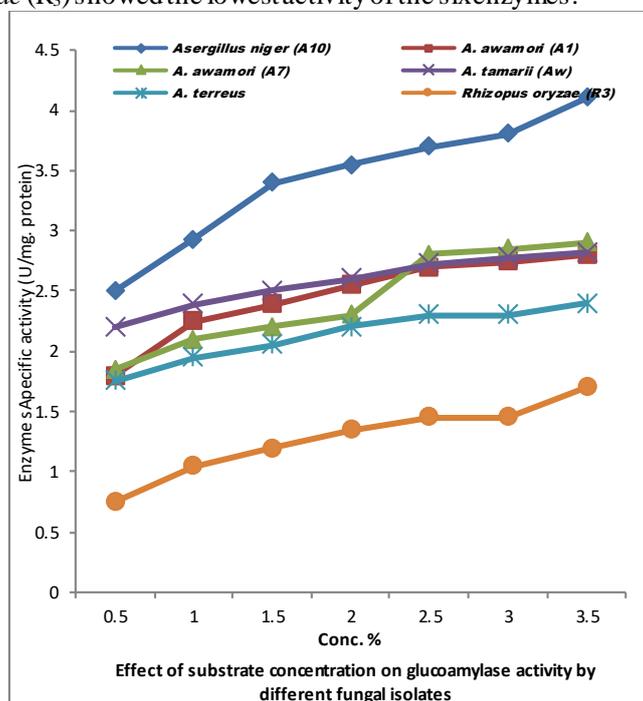


Fig. 3 Effect of reaction time on glucoamylase activity by different fungal isolates

#### Effect of Substrate Concentration on Glucoamylase Activity:

The activity of the glucoamylase from the six isolates was investigated as a function of starch concentration. It is seen that in fig. 4 all the enzymes showed gradual increase in activity as in exp. 4.4.3. *A. niger* (A<sub>10</sub>) showed the highest level of activity while, *Rhizopus oryzae* (R<sub>3</sub>) showed the lowest activity of the six enzymes.



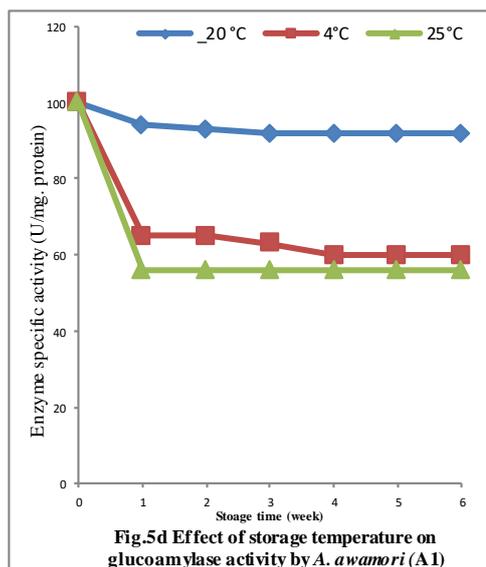
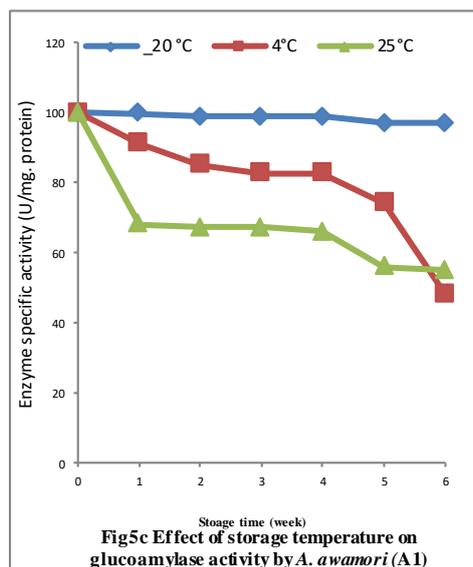
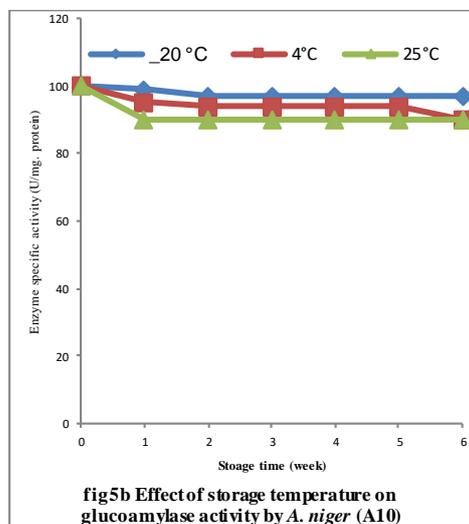
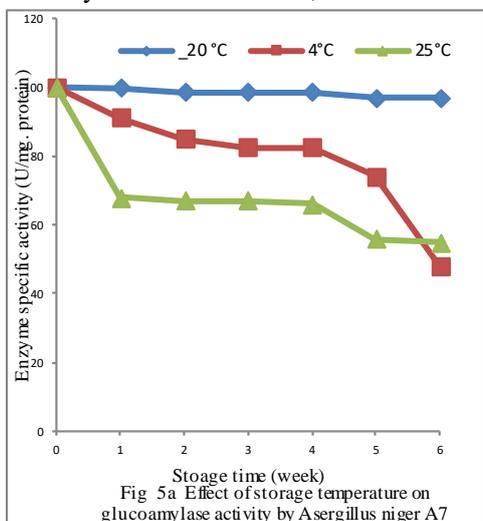
#### Effect of Storage Temperature on the Glucoamylase Activity:

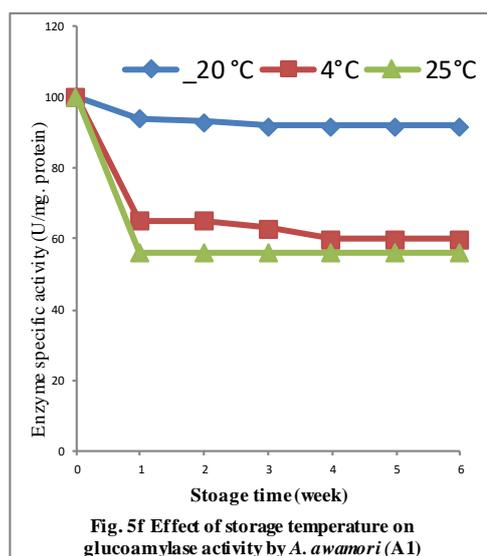
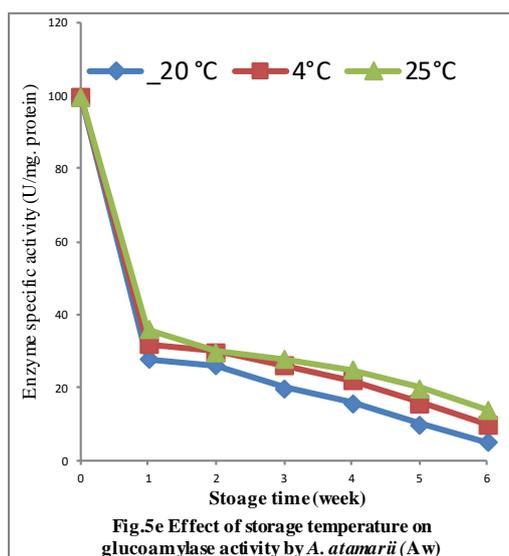
Figs.5 (a-f) show the effect of the storage temperature on the crude enzyme of the six isolates. It is seen that no significant difference in the loss of activity of crude enzyme from *A. niger* (A<sub>10</sub>) and *Rhizopus oryzae* (R<sub>3</sub>) at -20 and the enzymes retained up to 92 and 91% respectively of their activity after six weeks. At the higher temperature of 4 and 25°C, these enzymes are still relatively stable. At these temperatures and after six weeks the enzyme from *A. niger* (A<sub>10</sub>) retained about 84 and 85% respectively of its activity and that from *Rhizopus oryzae* (R<sub>3</sub>) retained about 89 and 79% of its activity too respectively. This result is in agreement with the finding of Deb *et al.* (2013) working on *Bacillus amyloliquefaciens* P-001. He reported that crude glucoamylase enzyme from this bacterium showed no significant loss of activity on storage at room temperature.

Crude enzyme from *A. awamori* (A<sub>1</sub> and A<sub>7</sub>) on storage at -20°C showed little loss of their activities and they retained about 90 and 92% respectively of their activities after six weeks, while at 4 and 25°C these enzymes lost their activities rapidly. After six weeks storage and at 4°C the enzymes from *A. awamori* (A<sub>1</sub> and A<sub>7</sub>) retained about 43 and 44% respectively of their activity and at 25°C, they retained 48 and 53% respectively of their activities.

*A. terreus* (A<sub>t</sub>) crude enzyme showed little loss of its activity on storage at -20°C. It retained 86% of its activity after six weeks. At 4 and 25°C it showed sharp decrease in its activity and after the first week it retained about 65 and 55% respectively of its activity and then the activity was stable to some extent and the enzyme retained about 54 and 51% of its activity at these temperatures after six weeks, respectively.

*A. tamarii* (A<sub>w</sub>) crude enzyme showed sharp decrease in activity on storage at -20, 4 or 25°C after six weeks of storage that the enzyme retained about 4, 8 and 11% of its activity.





#### 4. CONCLUSION

The six isolates were found to be thermophilic with their glucoamylases operating at 60- 70°C. Of special importance among the six strains is *Aspergillus niger* (A<sub>10</sub>) for its enzyme does not need special condition for storage and also *A. terreus* (A<sub>10</sub>) for its broadest pH optima for activity.

#### 5. REFERENCES

- Abdalwahab, S. A., Ibrahim, S. A. and Dawood, E. S. (2012). Culture condition for the production of glucoamylase enzyme by different isolates of *Aspergillus spp.* *International Food Research Journal* 19 (3): 1261-1266
- Bernfeld, P. (1951). Enzyme of starch degradation and synthesis. *Advan. Enzymol.*, Vol. 12. pp. 382-428, F.F. Nord ed., Interscience Publishers, Inc. New York.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. *Analytical Biochemistry* 72: 248-254.
- Coral G, Colak O (2000). The isolation and characterization of glucoamylase enzyme of *Aspergillus niger* natural isolate-Turk. *J. Biol.*, 24: 601-609.
- Deb, P.; Talukdar, S.A.; Mohsina, K.; Sarker P. K. and Saye, S. A. (2013). Production and partial characterization of extracellular amylase enzyme from *Bacillus amyloliquefaciens* P-00. *SpringerPlus* 2013, 2:154
- Gupta R, Gigras P, Mohapatra H, Goswami VK, Chauhan B (2003) Microbial  $\alpha$ -amylases: a biotechnological prospective. *Process Biochem* 38:1599–1616
- Mertens, J.A.; Skory, C.D. (2006). Isolation and characterization of a second glucoamylase gene without a starch binding domain from *Rhizopus oryzae*. *Enzy. Microbia. Technol.*, 40, 874-880.
- Muhammad, J. A., Muhammad, G., N. M., Nyla, J., Saqib, H., Hadri, M. I., Zahid A. and Dawood, A. 2011. Hyper production of glucoamylase by *Aspergillus niger* through the process of chemical mutagenesis. *International Journal of the Physical Sciences* Vol. 6(26), pp. 6179-6190.
- Norouzian, D.; Akbarzadeh, A.; Scharer, J.M.; Young, M.M. Fungal glucoamylases. *Biotechnol. Adv.* 2006, 24, 80–85.
- Pestana, F. and Castillo, J. (1985). Glucoamylase production by *Aspergillus awamori* on rice flour medium and partial characterization of the enzyme. *MIRCEN Journal of Microbiology and Biotechnology* 1, 225-237.
- Zambare, V. Solid state fermentation of *Aspergillus. oryzae* for glucoamylase production on agro residues. *Int. J. Life Sci.* 2010, 4, 16–25.