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# ULILIZATION OF SOME AGRICULTURAL BY-PRODUCTS FOR IMPROVING THE BIOSYNTHESIS OF *Aspergillus terreus* THERMOSTABLE AMYLOGLUCOSIDASE AND α-AMYLASE AND ITS USES FOR STARCH DIGESTION

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## ABSTRACT

The biosynthesis of *Aspergillus terreus* thermostable amyloglucosidase and α-amylase were improved greatly in this study, which the results revealed that: Ingredients of the medium No. (1) used in this study gave highest enzymes productivity. Three and two days incubation improved its biosynthesis, respectively. Maltose, glucose syrup (3%), zea glutein (0.28% as nitrogen content) supported highest enzymes productivity. 2.5% (v/v) inoculum size to volume of the fermentation media, 35°C, pH 5.0 and 225 rpm as agitation rate and 1:6.25 (v/v, vol of medium/vol of the flask) as aeration rate also supported highest enzymes production. PH 5.0 and 5.0 to 8.0 were found as the optimum pH for glucoamylase and α-amylase, respectively. Both enzymes were highest stable in the pH range between 4.0 to 8.0. 60°C and 70°C were found as the temperature optima for both enzymes, respectively. Both enzymes were highest stable up to 80°C, indicating that, these enzymes are thermostable enzymes. $K^{+}$, $Na^{+}$, $Mg^{+2}$ and $Ca^{+2}$ strongly induced glucoamylase activity, but $Fe^{+2}$ was found as slight activators of α-amylase. $Hg^{+2}$ and $Zn^{+2}$ inhibited both enzymes activity. Starch was found as the best substrate of these enzymes which showed highly affinity with those enzymes. Different starch substances were highly hydrolyzed with a mixture of *A. terreus* glucoamylase and α-amylase, which maximum hydrolysis was attained after 5 hours of hydrolysis. This is may be due to its thermostable character and its highly affinity between enzymes and substrate. Therefore, these enzymes were highest successful in similar biotechnological process.

**Keywords:** *Aspergillus terreus*, amyloglucosidase, α-amylase, thermostable, biosynthesis, submerged fermentation, starch digestion.

## INTRODUCTION

Amylolytic enzymes, also called α-glucanases, are involved in degradation of starch and related poly- or oligosaccharides. They occur widely in plants, animals and microorganisms; in most cases, more than one α-glucanases are produced by one organism. Degradation of starch to maltodextrins and finally to maltose by many bacteria is catalysed by α-amylase and is followed by hydrolysis to glucose by the action of either intra- or extracellular α-glucosidase (α-D-glucoside glucohydrolase, EC 3.2.1.20). Thermostable amylases are commercially important in various starch processing industries. Though the most widely used thermostable amylases

are produced by mesophilic microorganisms. *Bacillus licheniformis* (Dobreva *et al.*, 1994); *Bacillus megaterium* (Shady and Hassan, 1998) and *Bacillus subtilis* (Shady *et al.*, 1998). Glucoamylase, an enzyme capable of hydrolysing starchy substrates to glucose, has extensive uses in industry for the preparation of crystalline glucose or glucose syrup and beverage syrups. Also, in some applications, it is the sweetness of the product that is important. For this reason, considerable efforts have been made to produce it from various sources. Glucoamylase is an exo-acting enzyme that hydrolyzes 1, 4-linked α- D-saccharide chains with the release of D-glucose. The enzyme is also attacks α- (1-6) linkages of starch and accordingly it has received the most attention (Ghosh *et al.*, 1991; Janda *et al.*, 1997; DaSilva & Peralta, 1998; Shady, 1999 and Shady *et al.*, 2001).

The enzymes occur almost exclusively in filamentous fungi and far less in bacteria and yeasts. Recently, commercial glucoamylase is mainly obtained from fungi such as *Aspergillus* and *Rhizopus*. This enzyme was isolated from *A. terreus* (Ali & Hossain, 1991 and Ghosh *et al.*, 1991), *A. niger* (Ramadas *et al.*, 1996 and Metwally, 1998); *A. fumigatus* (Da Silva and Peralta, 1998 and Shady *et al.*, 2001). Methods of cultivation greatly influence the production and properties of the enzyme. Thus, now the most common methods used for the production of this enzyme are involved either solid-state or submerged cultivation (Ramadas *et al.*, 1996).

Therefore, the study reported here, deals with the production of amyloglucosidase and α-amylase by submerged fermentation. Some properties of these enzymes isolated from *Aspergillus terreus* are investigated. Saccharification of some starchy substances and glucose syrup production are also evaluated.

## MATERIALS AND METHODS

**Microorganism:**

*Aspergillus terreus* used in this study was obtained from Microbiol. Dept.; Soil, Water and Environ. Res. Institute, Agric. Res. Center, Giza, Egypt. The culture was maintained on potato-dextrose agar (PDA) slants and subcultured monthly.

**Submerged fermentation (SMF):**

One hundred ml portions of different fermentation media were dispensed in 500 ml Erlenmeyer flasks, sterilized at 121°C for 15 min and inoculated with spore suspension (1.25 ml) contained $10^7$ spores/ml, approximatly. Cultures were incubated with agitation (150 rpm) at 30°C for 4 days at pH 6.0. At the end of fermentation periods, the flasks were filtered and centrifuged at 8000 rpm for 15 min. The supernatants were collected and used as a source of amyloglucosidase and α-amylase enzyme.

**Fermentation media:**

**Medium No. 1 basal medium (Mc Mahon *et al.*, 1997).**

It contains: (g/L) soluble starch, 30.0; tryptone, 5.0; yeast extract, 5.0; $CaCl_2.2H_2O$, 0.5; $MnCl_2.4H_2O$, 0.5; $MgCl_2.6H_2O$, 0.48 and $KH_2PO_4$, 1.0.

**Medium No.2 basal medium (Ramadas *et al.*, 1996).**

It contains (g/L): $KH_2PO_4$, 5.0; soluble starch, 50.0; KCl, 0.5; yeast extract, 10.0; $(NH_4)_2SO_4$, 5.0; $MgSO_4.7H_2O$, 0.5; $FeSO_4.7H_2O$, 0.01; $ZnSO_4.7H_2O$, 0.001 and $CuSO_4.5H_2O$, 0.001.

**Medium No. 3 basal medium (Uguru *et al.*, 1997).**

It contains (g/L): $KH_2PO_4$, 1.5; $K_2HPO_4$, 2.2; $MgSO_4.7H_2O$, 0.1; $FeSO_4.7H_2O$, 0.0025; $CaCl_2.2H_2O$, 0.025; yeast extract, 0.5 and soluble starch 20.0.

**Medium No. 4 basal medium (Metwally, 1998).**

It contains (g/L): $KNO_3$, 3.03; $KH_2PO_4$, 1.0; $MgSO_4$. $7H_2O$, 0.25; $CuSO_4.5H_2O$, 0.234 mg; $FeSO_4.7H_2O$, 6.3 mg; $ZnSO_4.7H_2O$, 1.1 mg; $MnCl_2.4H_2O$, 3.5 mg; $CaCl_2$, 46.7 mg.

**Enzymes assay:**

**Amyloglucosidase (AMG)** activity was determined by the method of Ramadas *et al.* (1996). The assay mixture consisted of 0.5 ml 1% soluble starch (BDH) as substrate, 0.4 ml 0.02 M sodium acetate buffer (pH 4.0) and 0.1 ml enzyme. After incubation for 10 min at 60°C, the reaction was stopped by adding 1.0 ml/dinitrosalicylic acid reagent and heating for 5 min in boiling water. After cooling, the total volume was made up to 6 ml with distilled water and the A540 measured using glucose as standard. One unit of amyloglucosidase activity was defined as the amount of enzyme that formed 1 μmole glucose in 1 min.

**α-Amylase**

activity in culture fluids was measured by iodine method as described by Hernandez and Pirt (1975), as follow: 2.5 ml of 0.4% soluble starch in phosphate buffer, pH 7.0 were mixed with 0.5 ml enzyme and incubated for 15 min at 50°C. The reaction was stopped by adding 1.0 ml of 1.0 N HCl. 0.5 ml from each tube mixed with 1.0 ml of a 0.2% iodine-0.4 % KI solution and 2 ml distilled water and allowed to stand 15 min at room temperature. The colour intensities were measured at 620 nm using spectrophotometer (Pye Unicam). One unit α-amylase activity was defined as the amount of enzyme, which caused a decrease of optical density by 0.05 in starch-iodine coloration under the assay conditions.

**Thermal and pH stability:**

Thermal stability of glucoamylase and α-amylase were determined by incubating the enzyme solution in 50 mM acetate buffer (at the optimum pH for each enzyme) without substrate for 2 hours at 30-90°C. The residual activity of the enzyme was examined under these conditions.

pH stability of both enzymes were assayed by determination the residual activity after incubating of each enzyme solution in an appropriate buffer (pH 3-9) at optimum temperature overnight. The residual enzyme activity was examined under these conditions.

**Starch digestion:**

Different starches substrates namely potato starch (PS), maize starch (MS), wheat starch (WS) and rice starch (RS) were employed using tap water to obtain 5%. The crude enzymes containing 35 units from *A. terreus* glucoamylase and α-amylase were used. The hydrolysis was conducted in 250 ml capacity conical flasks containing 200 ml of starch solution. The flasks were incubated in shaking water bath (80 rpm) at 70°C. Reducing sugars (as glucose) and total sugars in the hydrolyzed products were determined by the glucose oxidase method (Da Silva and Peralta, 1998) and phenol-sulphoric acid method (Hodge and Hofreiter, 1962), respectively. The degree of hydrolysis was expressed at the percentage of reducing sugar against the total sugars x 100.

## RESULTS AND DISCUSSIONS

**I- Factors controlling enzymes production:**

**1- Effect of different substrates:**

Amyloglucosidase and α-amylase are two enzymes used in the saccharification of starch contained materials. Therefore, the obtained results show the improved of these enzymes biosynthesis. Data presented in Table (1) show the effect of ingredients of different media on the production of *Aspergillus terreus,* glucoamylase or/and α-amylase and revealed that medium No. 1 showed highest productivity of both enzymes. Medium No. 3 also gave highest enzymes production, but was found in the second order. In contrast, *Aspergillus terreus* enzyme formation were found in lowest grade with the medium No. 4, this means that the composition of this medium didn't induce the enzyme biosynthesis, but repressed it.

**Table (1): Effect of different media on amyloglucosidase and α-amylase production.**

| Medium No. | Enz. activity (U/ml/min) | |
|---|---|---|
| | AMGase | ∝ - amylase |
| 1 | 19.0 | 14.3 |
| 2 | 13.9 | 11.1 |
| 3 | 15.9 | 12.1 |
| 4 | 8.5 | 6.5 |

**2. Effect of incubation period:**

Data illustrated in Fig (1) showed that the incubation period influences the overall enzyme yield. Thus, the enzymes activities increased significantly along with the incubation time, reaching a maximum of 25.6 units/ml at 3 days for amyloglucosidase production, but reached 17.6 units/ml at 2 days for α-amylase production. Thereafter, enzymes biosynthesis decreased sharply. Similar results were obtained by Pandey (1990); Krishna & Chandrasekaran (1996); Ramadas *et al.* (1996) and Shady *et al.* (2000 & 2001).

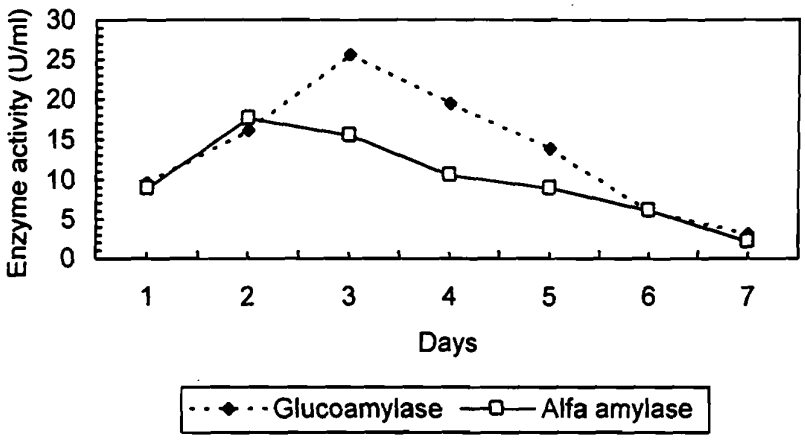


**Fig. (1): Effect of incubation period on *A. terreus* amyloglucosidase and ∝-amylase production.**

**3. Effect of different carbon sources:**

After fermentation for the optimum incubation periods for both enzymes, data presented in Table (2) showed the effect of different carbon and energy sources on enzyme production. Maltose, soluble starch, fructose and yellow dextrin were found as the most inducers for both enzymes biosynthesis. Maximum enzymes productivity were found with maltose which induced the enzyme formation. Other carbon sources repressed both enzymes synthesis. These results show clearly that these enzymes were constitutive in their nature and induced greatly with its substrates or carbohydrates contained materials. Shady & Hassan, (1998); Shady *et al.* (2000) and Mansoure & Saber (2001) reported similar results.

**Table (2): Effect of different carbon sources on enzymes production by *A. terreus*.**

| Carbon sources | Enzy. activity (U/ml/min) | |
|---|---|---|
| | AMGase | ∝ - amylase |
| Glucose | 12.2 | 10.7 |
| Sucrose | 13.1 | 11.2 |
| Galactose | 11.9 | 11.0 |
| Lactose | 12.4 | 12.0 |
| Arabinose | 14.9 | 10.3 |
| Maltose | 29.1 | 23.6 |
| Xylose | 11.5 | 10.8 |
| Fructose | 25.1 | 22.3 |
| Sorbitol | 14.9 | 11.2 |
| Manitol | 11.3 | 10.6 |
| Sol. Strach *C | 25.9 | 27.8 |
| Yellow dextrin | 22.0 | 20.9 |

*C = control

**4. Effect of some agricultural by-products:**

Data presented in Table (3) show that, agricultural by-products presented in Table (3) induced glucoamylase as well as α-amylase production.The results also show that the two enzymes were highly produced with the use of all agricultural by-products, this means that all these materials were induced the biosynthesis of both enzymes. Glucose syrup was found as highly induced both enzymes formation, which enzymes productivity reached 40.3 and 37.5 units/ml for glucoamylase and α-amylase, respectively. These results may be due to their contents of these substances of adequate amounts of essential nutrients as well as its content of minerals which induced the biosynthesis of *A. terreus* amylolytic enzymes. Thus, these enzymes were constitutive ones and induced with its substrates. These results are in agreement with those obtained by Fadel (2000) and Mansour & Saber (2001).

**Table (3): Effect of some agricultural by-products on enzymes production .**

| Agric. by-products (1% Conc.) | Enz. activity (U/ml/min) | |
|---|---|---|
| | AMGase | ∝ - amylase |
| Corn starch | 34.9 | 26.2 |
| Potato starch | 33.9 | 25.8 |
| Crushed rice | 30.0 | 27.6 |
| Glucose syrup | 40.3 | 37.5 |
| Sugar cane molasse | 38.2 | 29.9 |
| Vinase | 37.5 | 28.6 |

**5. Effect of different concentrations of glucose syrup:**

From the results presented in Fig. (2), it could be observed that amyloglucosidase as well as α-amylase biosynthesis were highly affected with glucose syrup concentration presented in the fermentation media. Maximum enzymes productivity were found at 3% concentration, which, the enzyme biosynthesis rose steadily with the increasing of glucose syrup concentration up to 3%. Higher or lower concentrations resulted in a sharp fall in production levels of both enzymes, which decreased sharply. Selim *et al.* (1998) found that 1% maltose induced the biosynthesis of α-amylase. Malhotra *et al.* (2000) found that 2% of starch enhanced the enzyme synthesis. Similar results were reported by Mansour and Saber (2001).

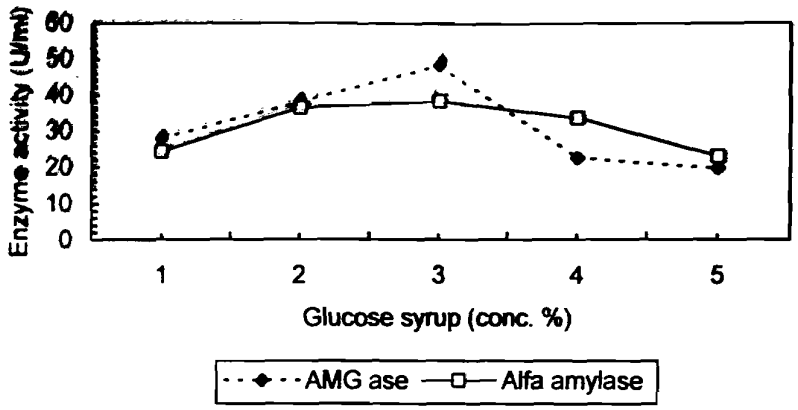


**Fig. (2): Effect of different concentration of glucose syrup on amyloglucosidase and α-amylase production by *A. terreus*.**

**6. Effect of different nitrogen sources:**

Table (4) show that *A. terreus* glucoamylase as well as α-amylase were affected markedly by the presence of nitrogen sources in the fermentation media. Zea glutein was more suitable for enzyme formation than the other ones followed by yeast extract and corn steep liquor. Whereas, ammonium chloride was found as the lowest one, which repressed greatly the enzyme production. This means that organic nitrogen sources enhanced and induced the enzyme biosynthesis, reversibly with inorganic ones. This is may be due to their contains minerals or/and growth substances. Consequently, the permeability of mycelium to release enzymes were affected greatly. Pandey *et al.* (1994) could improved the glucoamylase production by *A. niger* under solid-state fermentation by adding ammonium salts. Fadel (2000) found that urea was more suitable for enzyme formation.

**Table (4): Effect of different nitrogen sources on enzymes production by *A. terreus*.**

| Nitrogen sources | Enz. activity (U/ml/min) | |
|---|---|---|
| | AMGase | α - amylase |
| Ammonium nitrate | 26.8 | 23.0 |
| Ammonium sulfate | 26.0 | 22.2 |
| Ammonium chloride | 25.1 | 23.1 |
| Ammonium phosphate dihydrogen | 27.3 | 23.5 |
| Potassium nitrate | 30.2 | 24.1 |
| Sodium nitrate | 33.3 | 37.0 |
| Ammonium oxalate | 32.4 | 26.1 |
| Ammonium tartarate | 30.1 | 25.4 |
| Asparagine | 36.0 | 48.0 |
| Casein hydrolysate | 31.3 | 24.8 |
| Yeast extract | 33.3 | 35.6 |
| Peptone | 34.2 | 36.7 |
| Y. extract + peptone *C | 49.6 | 46.4 |
| Tryptone | 33.7 | 36.3 |
| Beef extract | 45.4 | 37.0 |
| Corn steep liquor | 46.2 | 47.8 |
| Zea glutein | 59.3 | 48.7 |

*C = control

**7. Effect of different concentration of zea glutein:**

As shown in Fig. (3), it could be concluded that the addition of zea glutein up to 0.28% concentration (as nitrogen content) to the production media induced and stimulated both enzymes formation by *A. terreus*. Maximum enzymes productivity reached 73.1 and 66.3 units/ml for glucoamylase and α-amylase, respectively. Thereafter, enzyme biosynthesis decreased sharply. Mansour and Saber (2001) report that, corn steep liquor up to 0.315% as nitrogen content induced amylolytic enzymes formation by *A. terreus*.

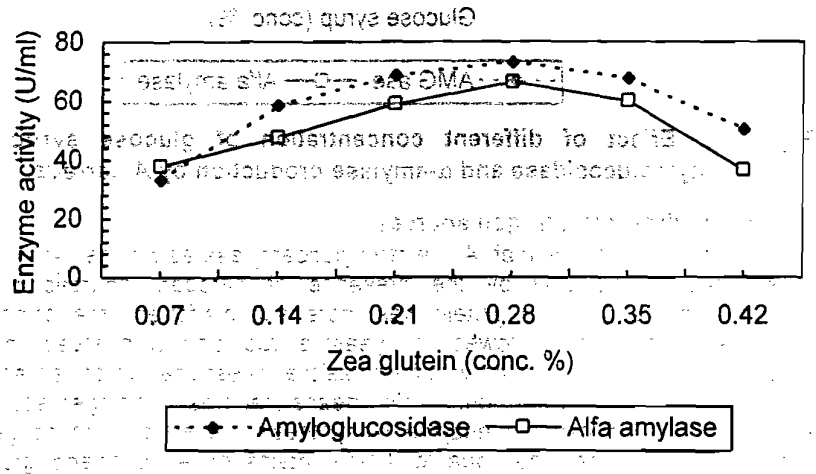


**Fig. (3): Effect of different concentration of Zea glutein on amyloglucosidase and α-amylase production by *A. terreus*.**

**8. Influence of inoculum size:**

In the present results (Fig. 4), spores of *A. terreus* was used as inoculum with different concentration and the results show that the varied inoculum size of *A. terreus* spore suspension had remarkable effect on enzyme production. The results also show that, higher numbers of spores had no advantage for amyloglucosidase as well as α-amylase production. A. 2.5% (v/v inoculum size to volume of the fermentation media) was found as the most suitable for greatest production of both enzymes. Above this concentration, decreased in enzymes productivity were obtained, which the enzyme yield was less than that with 2.5%. Pandey (1990) and Mansour & Saber (2001), found that 5% inoculum size was found as the most suitable for amyloglucosidase formation.

**9. Effect of incubation temperature:**

As the results illustrated in Fig. (5), both enzymes productivity increased gradually with greatest amounts with the raising of incubation temperature up to 35°C. Above or below this temperature, enzymes

biosynthesis decreased sharply. These results are in agreement with those obtained by Krishna & Chandrasekaran (1996) and Mansour & Saber (2001).

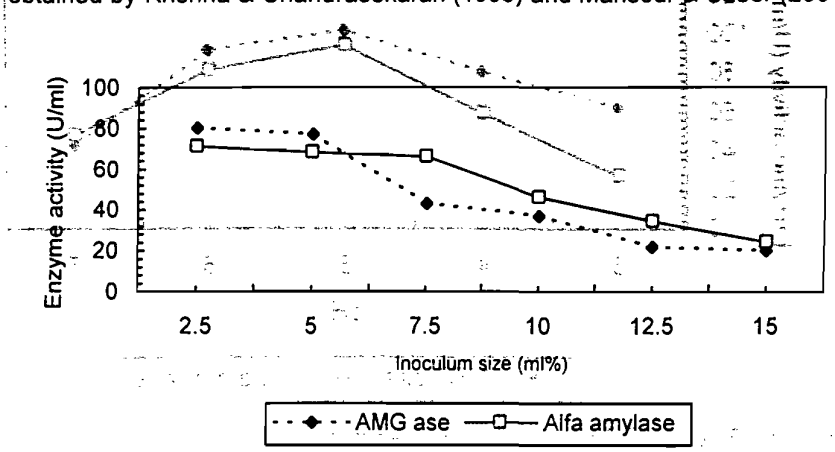


**Fig. (4): Influence of inoculum size on enzymes production by *A. terreus*.**

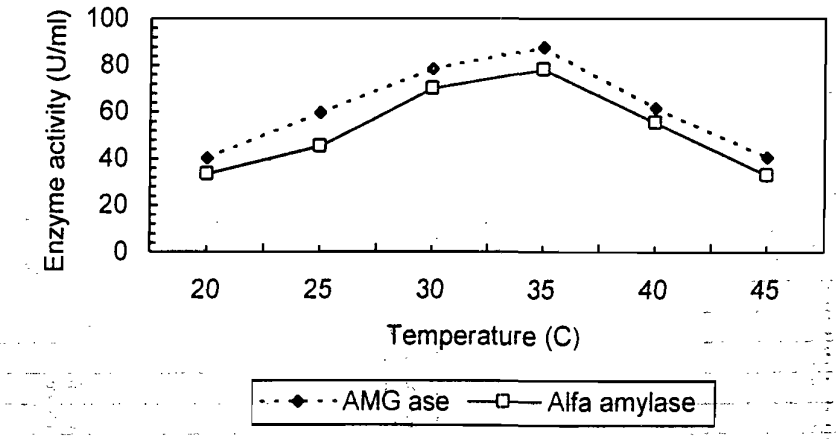


**Fig. (5): Effect of incubation temperature on enzymes production by *A. terreus*.**

**10. Effect of initial pH:**

The pH value of culture affected the permeability of enzyme (Fadel, 2000). The results present in Fig. (6) show that the enzymes productivity reached its maximum at pH 5.0. Enzymes formation is affected clearly and decreased sharply above or below the previous degree of pH. Fadel (2000) found that pH 6.0 was found as the optimum initial pH for glucoamylase production. Mansour and Saber (2001) reported similar results.

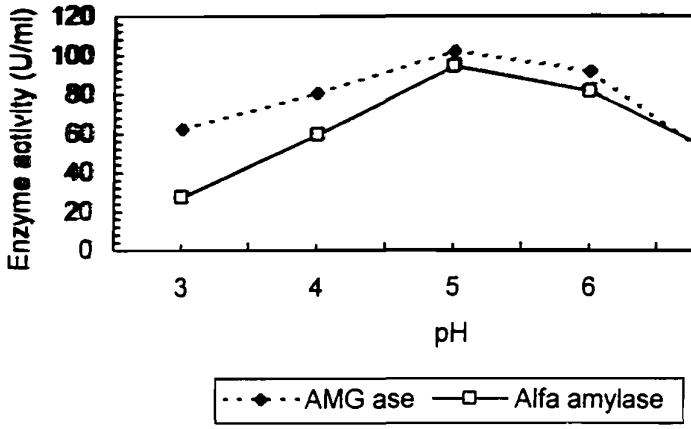


**Fig. (6): Effect of initial pH on enzymes production by *A. terreus*.**

**11. Effect of agitation rate:**

Results in Table (5) show the effect of agitation rate on both enzymes production, which the agitation rate changed between 100 to 300 rpm/min. It could be concluded that, agitation rate affected greatly both enzymes synthesis, which they increased greatly up to 225 rpm/min. thereafter, enzymes synthesis decreased sharply. This means that, submerged fermentation increased the biosynthesis of amylolytic enzymes with much more than static culture. These results are in agreement with those reported by Ramadas *et al.* (1995) and Mansour and Saber (2001).

**Table (5): Effect of agitation rate on enzymes production by *A. terreus*.**

| Agitation rate (rpm) | Enz. activity (U/ml/min) | |
|---|---|---|
| | AMGase | ∝ - amylase |
| 100 | 65.7 | 54.5 |
| 125 | 70.8 | 68.6 |
| 150 *C | 106.3 | 91.3 |
| 175 | 118.3 | 105.8 |
| 200 | 122.6 | 112.0 |
| 225 | 129.8 | 116.3 |
| 250 | 109.8 | 95.2 |
| 275 | 80.3 | 60.1 |
| 300 | 36.1 | 38.7 |

*C = control

**12. Effect of aeration rate:**

Results in Table (6) show the effect of aeration rate on enzymes biosynthesis by *A. terreus*. The results indicated that there is greatly variation in both enzymes formation in relation with aeration rate. Enzymes productivity reached its maximum at 1:6.25 (v/v, volume of medium/vol. Of

the flask). An other aeration rates, decreased the enzyme productivity. Mansour and Saber (2001) found that 1:5.0 (v/v) is the suitable aeration rate for highest enzyme production.

**Table (6): Effect of aeration rate on enzymes production by *A. terreus*.**

| Volume of medium : Volume of flask | Enzyme activity (U/ml/min) | |
|---|---|---|
| | AMGase | ∝ - amylase |
| 1 : 12.50 | 91.6 | 80.6 |
| 1 : 8.33 | 120.1 | 93.3 |
| 1 : 6.25 | 132.3 | 125.1 |
| 1 : 5.00 *C | 128.1 | 114.3 |
| 1 : 4.16 | 96.2 | 91.3 |
| 1 : 3.13 | 75.3 | 59.0 |

*C = control

**II- Enzyme properties:**

**1. pH optima:**

The pH profile of both enzymes was investigated using different buffers with various pH values at 50°C (Fig. 7). Glucoamylase had an optimum pH of 5.0. But, ∝-amylase had a broad pH optimum from 5.0 to 8.0. On either side of these values, activity diminished markedly, especially at the lower pH. Ghosh *et al.* (1991); Mamo *et al.* (1999); Shady *et al.* (2000) and shady *et al.* (2001) reported similar results.

**2. pH stability:**

*A. terreus* glucoamylase was stable over a pH range of 3.0-8.0, which, maximum optimal activity was retained in the pH range 4.0-6.0 and 60% at pH 3.0 (Fig. 8) and showed 20% loss of activity at pH 4.0. ∝-Amylase was markedly destabilized at pH values < 5.0 or > 8.0, but was unaffected at pH 6.0-7.0. These observations were reported by Dobreva *et al.* (1994) and Shady *et al.* (2001).

**3. Temperature optima:**

*A. terreus* glucoamylase was highly active over a broad temperature range of 30-70% °C with optimal activity at 60 °C (Fig. 9). At 65 and 70 °C optimal activity was reduced by about 10 and 20% from maximum activity, respectively. ∝-Amylase had an optimum temperature of 70 °C. Thereafter, enzyme activities decreased sharply. Ghosh *et al.* (1991); Ugura *et al.* (1997); Mamo *et al.* (1999) and Shady *et al.* (2001) reported similar observation.

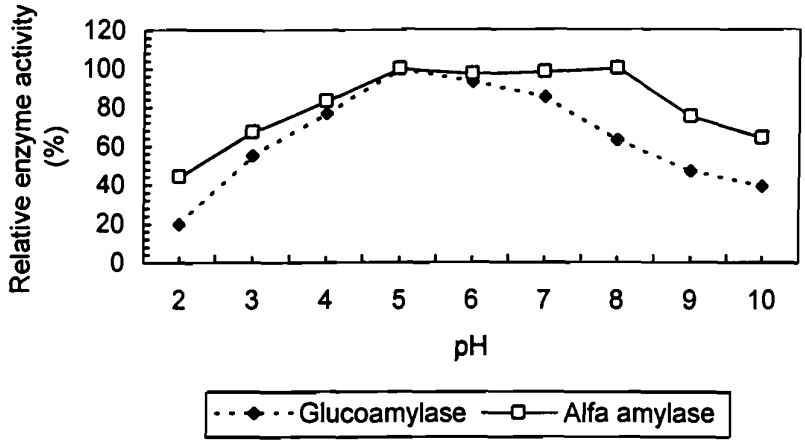


**Fig. (7): Effect of pH on enzyme activities.**

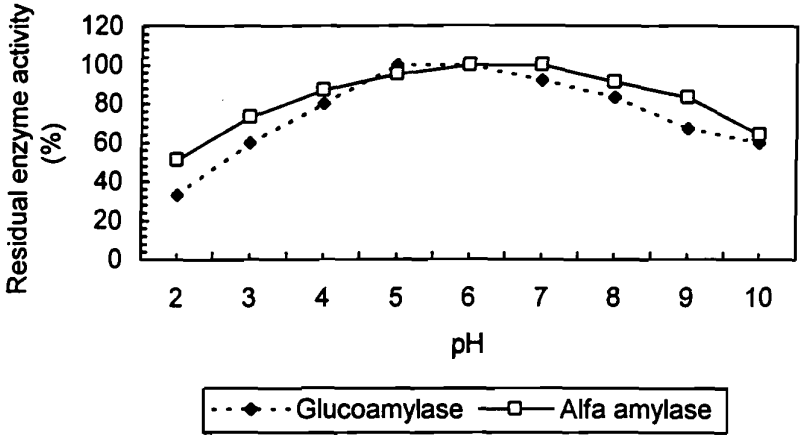


**Fig. (8): pH stability of enzymes.**

**4. Thermal stability:**

It was found from the results illustrated in (Fig. 10) that both enzymes were completely stable up to 70 °C and reduced little of their activities (15%) at 80°C. Remarkably destabilized were found above 80°C. These observations showed that these enzymes were thermostable enzymes and successful in different starch biotechnological processes required thermostable enzymes. These observation are similar to those obtained by Ghosh *et al.* (1991); Janda *et al.* (1997); Ugura *et al.* (1997) and Shady *et al.* (2001).

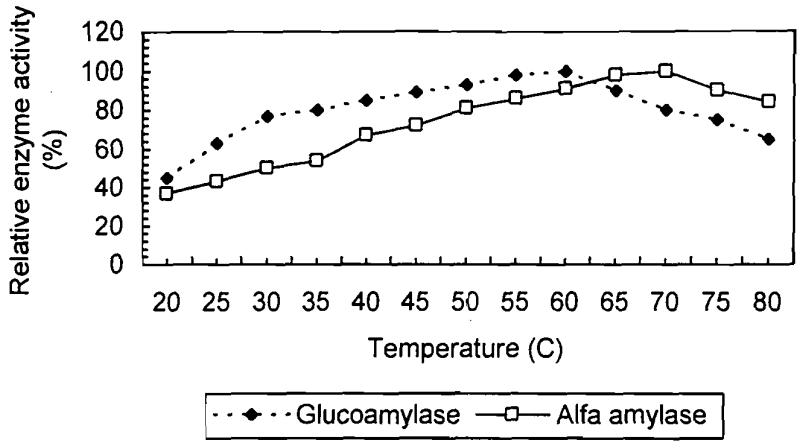


**Fig. (9): Effect of temperature on enzymes activities.**

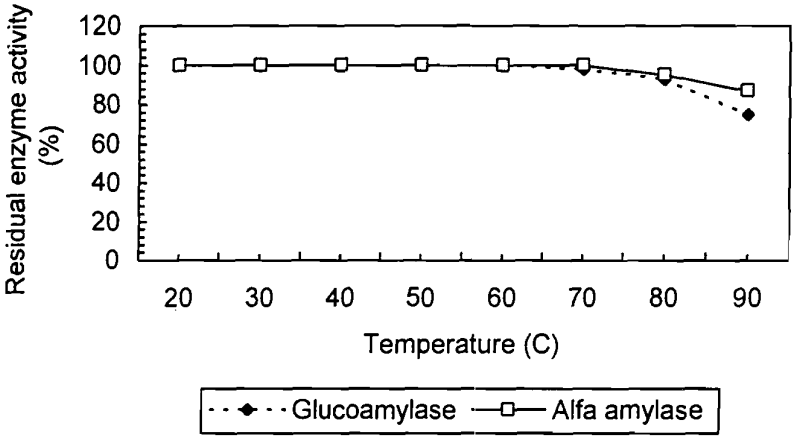


**Fig. (10): Thermal stability of enzymes.**

**5. Effect of different ions:**

When investigating the effect of metal ions on the activities of both enzyme with 10 mmole $L^{-1}$ as a concentration of these ions, data presented in Table (7) showed that the enzyme was strongly activated by $K^{+}$, $Na^{+}$ $Mg^{+2}$ and $Ca^{+2}$ which increased glucoamylase activity by 15%, 10%, 35% and 29%, respectively. Other metal ions such as $Hg^{+2}$, $Cu^{+2}$, $Zn^{+2}$ inhibited the enzyme activity. While $Mn^{+2}$ + $Fe^{+2}$ inhibited the enzyme activity to some extent.

Slight stimulation of $\alpha$-amylase activity was observed with $Fe^{+2}$. While, $Cu^{+2}$, $Mg^{+2}$ and $Mn^{+2}$ were inhibitory. The presence or absence of $Ca^{+2}$ did not affect enzyme activity. Strongly inhibited was observed with

$Hg^{+2}$ and $Zn^{+2}$. These results are in agreement with these obtained by Ghosh *et al.* (1991); Janda *et al.* (1997); Mamo *et al.* (1999); Malhotra *et al.* (2000) and Shady *et al.* (2001).

**Table (7): Effect of different ions on enzymes activities.**

| Metal ions | Relative enzyme activity % | |
|---|---|---|
| | Glucoamylase | ∝ - Amylase |
| None | 100 | 100 |
| Na Cl | 110 | 103 |
| K Cl | 115 | 105 |
| $Mg Cl_2$ | 135 | 93 |
| $Mn Cl_2$ | 82 | 87 |
| $Ca CL_2$ | 129 | 100 |
| $Fe SO_4$ | 72 | 105 |
| $Cu SO_4$ | 55 | 88 |
| $Hg Cl_2$ | 42 | 54 |
| $Zn Cl_2$ | 48 | 42 |

**6. Substrate specificity of enzymes:**

The substrate specificity of both enzymes was studied by using a number of di-, tri- and polysaccharide substrate to a final concentration of 1% for all the substrates. Glucoamylase activity was assayed by the glucose oxidase method (Da Silva and Peralta, 1998). The results are shown in Table (8) in terms of relative values for starch. The results indicated that glucoamylase hydrolyzed the high molecular weight (HMW) substrates such as starch, amylopectin and amylose more rapidly than the low MW substrates maltose and maltotriose. This results may be due to lower affinity of this enzyme for low MW substrate than for highly plomerized glucan.

The results also indicated that ∝-amylase was able to hydrolyze starch and polysaccharides substrates more rapidly that maltotriose and maltose. This is due to its highly affinity between ∝-amylase and these substrates. Ghosh *et al.* (1991); Ali *et al.* (1994) and Shady *et al.* (2001) reported similar results.

***Table (8): Substrate specificity of enzymes.***

| Substrate | Relative enzyme activity % | |
|---|---|---|
| | Glucoamylase | ∝ - amylase |
| Starch | 100 | 100 |
| Amylopectin | 95 | 98 |
| Amylose | 87 | 93 |
| Glucogen | 82 | 85 |
| Maltose | 79 | 15 |
| Maltotriose | 44 | 25 |
| Sucrose | 0 | 0 |
| Cellobiose | 0 | 0 |
| Trehalose | 0 | 0 |
| Lactose | 0 | 0 |

**III- Starch digestion:**

Table (9) shows the hydrolysis of four different starches namely potato starch (PS); maize starch (MS), wheat starch (WS) and rice starch (RS) using 5% (W/V) of substrate contained crude enzymes about 35 unit of both enzyme, then incubated at the optimum temperature (70 °C) in shaking water both, the digestion was followed at intervals of 30 min and the digestion percent was calculated. The results show that the rate of digestion for these substrates is variant with the incubation time as well as with the differ of substrate. Progressive increases in different starch digestion reaching its maximum after five hours of incubation. These mixed enzymes (glucoamylase and α-amylase) seems to exhibit a good effect on liquefaction and saccharification of these starchy substances. Fadel (1999) reported similar results.

**Table (9): Digestion of different starches by *A. terreus* glucoamylase and ∝ - amylase, using 5% of different substrates contained 35 units from each enzyme.**

| Time of hydrolysis (hour) | % Digestion | | | |
|---|---|---|---|---|
| | PS | MS | WS | RS |
| 0.5 | 19.8 | 15.4 | 9.5 | 5.5 |
| 1.0 | 27.5 | 24.5 | 16.6 | 11.6 |
| 1.5 | 42.9 | 36.6 | 27.9 | 18.4 |
| 2.0 | 53.4 | 47.0 | 36.8 | 26.9 |
| 2.5 | 63.0 | 56.0 | 47.5 | 33.5 |
| 3.0 | 68.9 | 63.6 | 56.0 | 40.0 |
| 3.5 | 77.5 | 67.8 | 64.9 | 46.5 |
| 4.0 | 85.6 | 73.4 | 73.0 | 53.0 |
| 4.5 | 90.0 | 78.0 | 79.6 | 57.0 |
| 5.0 | 90.5 | 83.0 | 83.0 | 65.0 |
| 5.5 | 92.0 | 86.6 | 87.0 | 69.7 |
| 6.0 | 93.6 | 92.0 | 89.0 | 73.4 |

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# استخدام بعض النواتج الثانوية الزراعية لتحسين إنتاج الأميلوجلوكوسيديز والألفا أميليز الثابت حراريا من الأسبرجلس تيريس واستخدامه فى تحلل النشا

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اتجه العلماء فى الآونة الأخيرة نحو التحلل البيولوجى للنشا باستخدام إنزيمات الأميليز الميكروبية بدلا من التحلل الكيماوى واستخدام الحرارة العالية حيث تلعب هذه الإنزيمات دورا حيويا فى عمليات التسكر للمواد النشوية ونظرا لأهمية إنزيم الأميلوجلوكوسيديز جنبا إلى جنب مع إنزيم الألفا-أميليز والذى يحلل المواد النشوية من الطرف الغير مختزل منتجا بذلك سكر الجلوكوز وكذلك شراب الجلوكوز الهام بيوتكنولوجيا كمادة محلية فى مثل هذه الصناعات ولذلك فقد هدفت هذه الدراسة إلى إنتاج هذه الإنزيمات الهامة صناعيا ودراسة خصائصها وكذلك استخدامها فى عمليات التسكر الإنزيمى لبعض المواد النشوية وقد أوضحت الدراسة النتائج التالية:

١- وجد أن البيئة رقم ١ هى الأفضل من بين البيئات المختبرة لإنتاج هذه الإنزيمات.

٢- ثلاث أيام ويومين تحضين هما المثاليين لإنتاج الجلوكوأميليز والألفا أميليز على الترتيب.

٣- المالتوز وشراب الجلوكوز (٣% تركيز فى البيئة ) وجلوتين الأذرة (٠,٢٨% كنسبة نيتروجين فى البيئة) حثت الميكروب على الإنتاج العالى لهذه الإنزيمات.

٤- ٢,٥% حجم اللقاح إلى حجم بيئة الإنتاج و٣٥ م° و pH ٥ و٢٢٥ لفة/دقيقة كمعدل هز و ١: ٦,٢٥ (حجم البيئة إلى حجم دورق التخمير) كمعدل تهوية قد حث على الإنتاج العالى لهذه الإنزيمات.

٥- pH ٥ و٥-٨ هى المثاليين لنشاط هذه الإنزيمات على الترتيب.

٦- وجد أن هذه الإنزيمات ذو درجة ثبات عالية فى المدى من درجات الـ pH من ٤-٨.

٧- ٦٠، ٧٠ °م هى المثاليين لنشاط هذه الإنزيمات على الترتيب.

٨- أظهر كلا الإنزيمين ثبات عالى تجاه درجات الحرارة حتى ٨٠ °م.

٩- حثت بعض العناصر مثل البوتاسيوم والصوديوم والماغنسيوم والكالسيوم على نشاط الجلوكو أميليز بدرجة ملحوظة فى حين كان للحديد تاثير منشط ضئيل على الألفا أميليز وقد ثبط الزنبقيك والزنك نشاط كلا الإنزيمين.

١٠- نجح الإنزيم فى تحليل العديد من المواد النشوية وإن كانت النشا هى أفضل مادة تفاعل لهذه الإنزيمات.

١١- نجح الإنزيم فى تحليل نشا البطاطس والأذرة والقمح والأرز بدرجة تحلل عالية وصلت ذروتها بعد ٥ ساعات تحليل وهذا يرجع لخاصية ثبات هذه الإنزيمات تجاه درجات الحرارة فضلا عن التوافق العالى بين الإنزيم ومواد التفاعل النشوية مما يؤكد أهمية هذه الإنزيمات الثابتة حراريا فى مثل هذه الصناعات البيوتكنولوجية الهامة.