

Optimization of Glucoamylase Production by Local Isolate of *Aspergillus niger* Using Agro-Industrial Substrates under Solid State Fermentation

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ABSTRACT

In this work, a local isolate of *Aspergillus niger* was used in the production of glucoamylase under Solid state fermentation (SSF). When *A. niger* grown on different agro-industrial wastes, wheat bran was the most promising fermentable substrate for glucoamylase production. Maximum activity of glucoamylase was recorded when the level moisture content was 1.5:1 (v/w) of wheat bran: distilled water. All additive carbon sources decreased the production of the enzyme, while, among organic and inorganic nitrogen sources, addition of 1% (w/w) calcium nitrate gave the best results of the production of the enzyme. The optimum inoculum size, temperature and pH for enzyme production was 1.5ml, 30°C and 5.5, respectively. The highest activity of the produced enzyme was recorded at 70°C and pH 5.5. Also, metal salts affected the activity of the enzyme. CuSO₄ gave maximum activity, while HgCl₂ made a huge decrease in the activity. The enzyme showed a great stability at 50°C and a moderate stability at 60°C. Also, the enzyme exhibited great stability at acidic condition of incubation.

INTRODUCTION

Glucoamylase is an important enzyme that used in the production of glucose by hydrolyzing starch and related polymers. Glucoamylase produces glucose by hydrolyzing the glucosidic linkages of starch. It hydrolyzes the non-reducing end of starch by cleaving the α -1,4glucosidic linkage, and by hydrolyzing the α -1,6 glucosidic, resulting in glucose as end product Mertens and Skory (2006). Glucoamylase is used for glucose production from starch and related polymers, which in form serve as a foodstock in fermentation processes such as production of ethanol or high fructose syrups Pavezzi *et al.* (2008) & Zambare (2010). In beer production, glucoamylase could be used for improving barley mash for the fermentation process Pavezzi *et al.* (2008).

Also, Glucoamylase has applications in pharmaceutical, baking and confectionery industries Pandey *et al.* (2000), in the textile and paper industries Pandey *et al.* (1996). Various species of *Aspergillus* are used for the production of glucoamylase Ellaiah *et al.* (2002); Sutthirak *et al.* (2005) and Nyamful *et al.* (2014). Glucoamylase produced from species of *Aspergillus* widely used in manufacture of glucose syrup are the most able with optimum temperature of 58-65°C, optimum pH of 4-4.5 for its activity and pH stability in a range of 3.5-5.5 Sauer *et al.* (2000).

A use of submerged fermentation in the production of amylases is quite expensive, because of its high energy consumption and its high costs for operation processing. Solid state fermentation (SSF) is used for reducing production costs. Also, it has low energy requirement, high product concentration, and reduced levels of catabolite repression. Also, it produces less waste water, resolves the problem of solid wastes disposal and requires a lower input in infrastructure and skill Baysal *et al.* (2003). Using of commercial carbon sources such as glucose and starch are not advantageous for amylases production since they are very expensive, so cheap and easily available agricultural wastes may be used. The aim of this research is to evaluate the potential of some agricultural materials as a substrate for production of glucoamylase from a local isolate of *A. niger* using SSF and to optimize the process parameters.

MATERIALS AND METHODS

Microorganism: A fungal isolate of *A. niger* was obtained from Microbiology Dept., Fac. Agric., Mansoura Univ., the fungal isolate was maintained and sub-cultured on potato dextrose agar (PDA) slants, and stored at 4°C.

Inoculum preparation: To each sporulated 5 days old PDA slant culture, 10 ml of sterilized water was added, and the surface of the culture was slightly scraped using a sterile wire loop. Spores per ml of the spore suspension were counted.

Agro-industrial wastes: Five agro-Industrial wastes namely, wheat bran, rice bran, wheat flour, corn flour and rice flour were collected from local markets of Mansoura City, Egypt.

Glucoamylase production in solid state fermentation (SSF): Five grams of each waste (wheat bran, rice bran, wheat flour, corn flour and rice flour) were added with 5 ml of distilled water to 250 ml Erlenmeyer flasks. Flasks were sterilized at 121°C for 20 min., cooled, inoculated with 1 ml of spore suspension having 1×10^6 spores/ml and incubated at 30°C for 3, 5 and 7 days.

Enzyme extraction: After SSF process, the fermented fungal substrate was mixed with 50 ml of distilled water and shaken for 30 minutes on 150 rpm. Mixture was filtered through double layer gauze. The filtrate was centrifuged at 5000 rpm for 15 min., the clear supernatant was used as crude enzyme.

Assay of enzyme activity: Enzyme activity was assayed by measuring the amount of glucose released after 5 minutes of enzymatic reaction by using 3,5-dinitrosalicylic acid (DNS) method Miller (1959). 0.1 ml of crude enzyme was added to 0.9 ml of starch solution 4% in 0.1 mM citrate buffer on pH 5.5 and incubated for 5 minutes at 50°C. The reaction was stopped by addition of 1.5 ml DNS solution, boiled for 5 minutes, cooled in ice water, and then read on spectrophotometer on 540nm. Glucose is used as standard curve for glucoamylase activity, one unit of glucoamylase defined as amount of enzyme released 1 μ mol of glucose per minute.

Factors affecting glucoamylase Production

Effect of initial moisture content on enzyme production: Wheat bran medium were adjusted by ten levels of moisture ranging from 0.5:1 to 5:1 (distilled

water : substrate, v/w), at interval 0.5. Fermentation medium were incubated at 30°C for 3, 5 and 7 days.

Effect of additional carbon sources on enzyme production: Production of glucoamylase by *A. niger* on wheat bran medium was optimized by supplementation with (1% w/w) of different sources of carbon; glucose, fructose, lactose, mannitol, starch, sucrose, sorbitol, arabinose, galactose and maltose. Control flask contained wheat bran medium without any addition of carbon source. Flasks were incubated at 30°C, and glucoamylase activity was measured after 3, 5 and 7 days.

Effect of additional nitrogen sources on enzyme production: Wheat bran medium was supplemented with (1% w/w) of organic and inorganic additional nitrogen sources. Control flask contained basal medium without addition of nitrogen source. Flasks were incubated at 30°C, and enzyme activity was measured after 5 and 7 days.

Effect of inoculum size on enzyme production: Fermentation media was inoculated by 0.5, 1.0, 1.5, 2.0, 2.5 and 3 ml of spore suspension (1ml contains 1×10^6 fungal spores). Flasks were incubated at 30°C for 5 and 7 days.

Effect of incubation temperature: It was studied by incubating the fermentation media at 25, 30, 35 and 40°C for 5 and 7 days, to select the optimum temperature for glucoamylase production.

Effect of initial pH: The fermentation media having different pH ranging from 3-7 with pH 0.5 interval were incubated at 30°C for 5 days.

At all parameters, the optimum factor achieved by the previous tests was kept constant for the consecutive experiment.

Factors affecting enzyme activity:

Optimum temperature: It was determined by measuring the activity of the enzyme at pH 5.5 at temperature varying from 30 to 90°C.

Optimum pH: Enzyme activity was measured at 70°C in range of pH from 3-7 using phosphate buffer and citrate buffer.

Effect of metal salts: It was evaluated by adding some metal salts in concentrations of 5 and 10 μ mole to reaction mixture.

pH stability was measured by incubating the enzyme at pH range from 3 to 7 at 4°C and then the residual enzyme activity was measured at different times interval between 0 and 72 hours.

Thermal stability was determined by pre-incubation of glucoamylase in presence and absence of CaCl_2 at different temperature degrees 50, 60 and 70°C at pH 5.5 in absence of substrate. Aliquots were removed at different times intervals between 0 and 72 hours, and immediately cooled on ice. Residual enzymatic activities were assayed in the treated enzyme solutions.

RESULTS AND DISCUSSION

The variation of glucoamylase production with SSF using five agro-industrial wastes as nutrient medium anchorage for *A. niger* under SSF through 3, 5 and 7 days of incubation at 30°C shown in data in Fig.

(1). Maximum production of glucoamylase by *A.niger* was recorded when wheat bran was used as agro-industrial waste followed by rice bran, wheat flour, rice flour and corn flour. Similar results were obtained by Anto *et al.* (2006); Kaur *et al.* (2003) and Nyamful *et al.* (2014), they reported that wheat bran is suitable agro-industrial waste for the production of glucoamylase. Also results showed that the maximum production of glucoamylase by *A. niger* under SSF using wheat bran was 451.29 IU/g substrate after 5 days of incubation. Ezugwu *et al.* (2015) reported that the highest peaks of glucoamylase were obtained after 4 days of incubation. The decrease in the production, observed after 5 days may be because of the reduced consumption of nutrient materials, toxic substances accumulation and the proteolysis of glucoamylase. From data it can concluded that wheat bran could be a rich source of nutrients for the production of glucoamylase by *A. niger*, and it could be helpful in reducing the production cost.

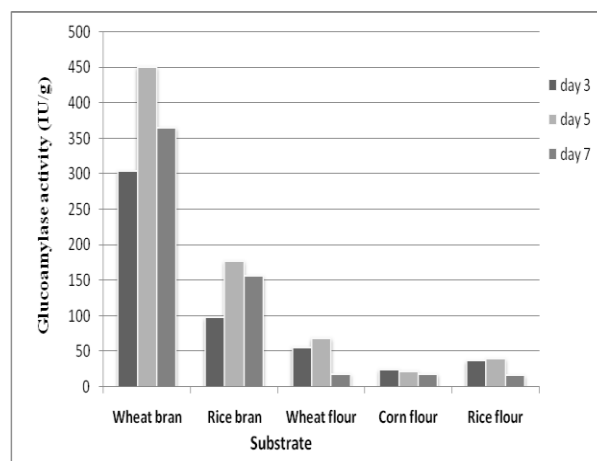


Fig. 1. Effect of different agro-industrial wastes on glucoamylase production by *A.niger* under SSF process.

Effect of initial moisture content on glucoamylase production

Results in Fig.(2) showed that the optimal moisture level with wheat bran to achieve highest enzyme production was found to be 1.5: 1 (v/w), which is 60.28% of moisture, the enzyme achieved 498.7 UI/g substrate after 5 days of incubation. The enzyme production decreased below and above. Chimata *et al.* (2010) reported that 70% of moisture level gave the highest production of amylases by *Aspergillus* sp. using wheat bran. Kundu and Das (1970) mentioned that enzyme production was inhibited when moisture content is below determined optimal moisture level, and there was great enzyme diffusion from the substrate if moisture content was above the optimum level. The enzyme decreases in production with high level of moisture is due to the decrease in the porosity of the substrate, resulting in decrease in gas exchange, while the decrease of enzyme production at low moisture levels is due to the fungal weak growth and lower swelling degree of substrate which resulting in a decreases in enzyme production. The subsequent experiment for the enzyme production was carried out with initial moisture content of 1.5: 1 of moisture ratio.

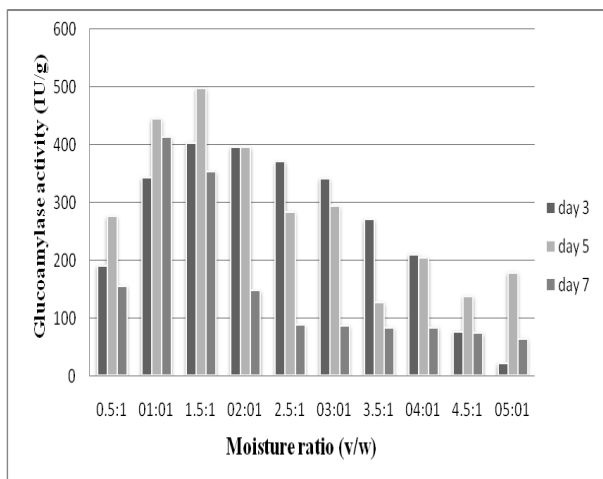


Fig. 2. Effect of moisture content on glucoamylase production by *A. niger* using wheat bran medium under SSF process.

Effect of additive carbon sources on glucoamylase production

Fig. (3) showed the effect of ten carbon sources addition on glucoamylase production. Data showed that all additive sources of sugars decreased the enzyme production through different incubation periods. Wheat bran without any additive of carbon source gave highest enzyme production 509.08 IU/g at 5 days of incubation.

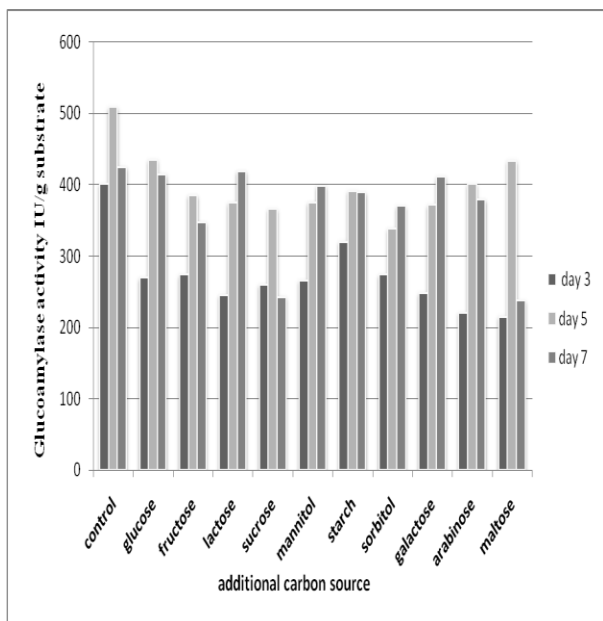


Fig. 3. Effect of carbon sources addition on glucoamylase production by *A. niger* using wheat bran under SSF process.

The reduction in enzyme production caused by sugars addition may be due to catabolite repression. Similar results were obtained by Alva *et al.* (2007), they found that glucose and other metabolizable substrates have repressed glucoamylase production by catabolite repression. Anto *et al.* (2006) reported that addition of maltose, glucose, lactose and starch to wheat bran media decreased glucose production, while sucrose addition caused slightly increase.

Effect of additive nitrogen source on glucoamylase production

Some nitrogen additives decreased the production of glucoamylase such as beef extract and ammonium nitrate, while most of nitrogen additives increased the enzyme production as it shown in Fig.(4). Calcium nitrate, peptone and ammonium sulphate were the highest nitrogen source additives. After 5 days of incubation, calcium nitrate gives the maximum increase glucoamylase, it recorded 676.39 IU/g, increasing the enzyme production by 32.2%, while peptone increased the production by 26.5% recording 647.05 IU/g, and ammonium sulphate recorded 625.74 IU/g increasing the enzyme production by 22.3%. Anto *et al.* (2006) found that addition of peptone and ammonium sulphate to wheat bran medium increased enzyme production, while casein and ammonium chloride decreased enzyme production.

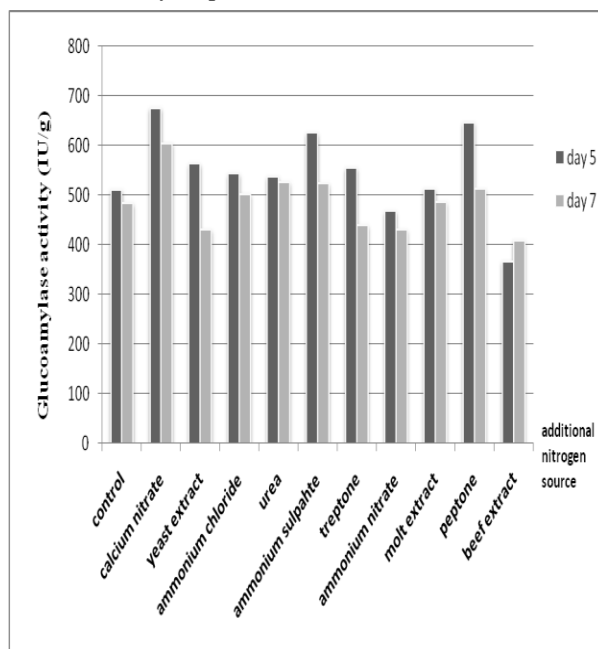


Fig. 4. Effect of nitrogen sources addition on glucoamylase production by *A. niger* using wheat bran under SSF process.

Effect of inoculum size on glucoamylase production

Flasks were inoculated with different inoculum size (0.5, 1.0, 1.5, 2.0, 2.5 and 3 ml) to study the inoculum size effect on glucoamylase production. Data in Fig.(5) showed that glucoamylase production increased with increasing the inoculum size and reached its maximum with 1.5 ml of inoculum. Further increasing of inoculum led to decrease the glucoamylase production. However, the higher inoculum size may cause an increase in moisture level causing a decrease in enzyme production. On the other hand, with low size of inoculum, it may prolong the time needed for the fermentation process to produce a desired product Murthy *et al.*(2009).

Effect of incubation temperature on glucoamylase production

Data in Fig. (6) showed that production of glucoamylase recorded the highest value at 5 days of incubation on 30°C and recorded 756.46 IU/g, then decreased at 7 days to 511.16 IU/g, while enzyme production decreased with incubation at 25°C, whereas

incubation at temperature over 30°C caused a big fall in the production of glucoamylase. The optimum temperature of the enzyme production was 30°C for 5 days. These results are in agreement with Uma and Nasrin (2013), they found that 30°C was optimum for the production of glucoamylase from *A. niger*.

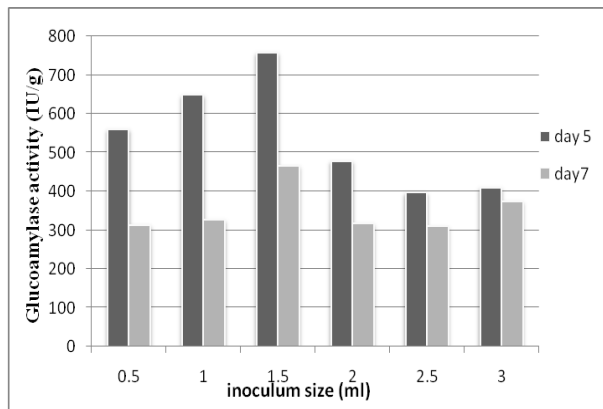


Fig. 5. Effect of inoculum size on glucoamylase production by *A. niger* by using wheat bran under SSF process.

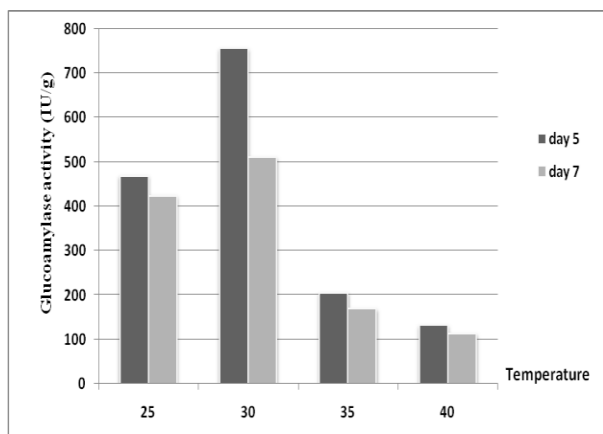


Fig. 6. Effect of incubation temperature on glucoamylase production by *A. niger* by using wheat bran under SSF process.

Effect of initial pH on glucoamylase production

pH is one of the most important factors which affecting the growth and enzyme production during solid state fermentation processes. Results in Fig.(7) showed that pH 5.5 was the optimum initial pH for the enzyme production, which the enzyme activity recorded 799.4 IU/g after 5 days of incubation. Enzyme production decreased if initial pH is higher or lower the optimum pH value; this is due to the high sensitivity of metabolic activities of *A. niger* to changes in pH. Anto *et al.* (2006) reported that initial pH 5 was optimum for glucoamylase production by *Aspergillus* sp. Isolate HA-2. Also, Parbat and Singhal (2011) found that the optimum initial pH of glucoamylase produced by *A. oryzae* grown on agro-industrial products was 5.0, while Kumar *et al.* (2013) mentioned that pH 6.0 is the optimum pH for glucoamylase production by *A.oryzae* on wheat bran.

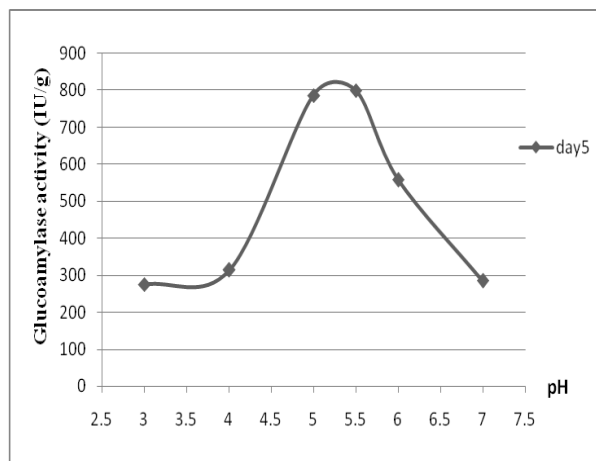


Fig. 7. Effect of initial pH on glucoamylase production by *A. niger* by using wheat bran under SSF process.

Factors affecting enzyme activity

Optimum pH of enzyme activity

As indicated in Fig. (8), it could be noticed that the optimum pH of enzyme activity was 5.5. Enzyme activity decreased with lower or higher pH values, even though the enzyme do well at pH range of 4.5 - 6.5, while enzyme activity highly decreased at very low and at high pH values. Bagheri *et al.* (2014) found that glucoamylase produced by *A. niger* was gave highest enzyme activity at pH 5.0. Sutthirak *et al.* (2005) reported that pH 4.5 was the optimum for the activity of glucoamylase produced by *A. niger*, the enzyme lost its activity rapidly after 5 min of incubation at 60°C and pH 5.5, and completely inactivated at pH 6.5. Koç and Metin (2010) and Kumar *et al.*(2013) reported optimum pH 6.0 for glucoamylase produced by *A. flavus* and *A. oryza*. Optimum pH for glucoamylase produced by *A. oryzae* was 6.0 Zambare (2010), while Nahid *et al.* (2012) found acidic pH range 4.5-4.7 for glucoamylase produced by *A. niger*.

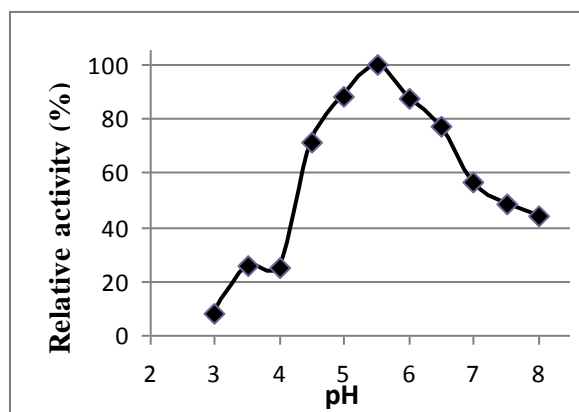


Fig. 8. Effect of pH of reaction mixture on the activity of glucoamylase produced by *A. niger* by using wheat bran under SSF process.

Optimum temperature of enzyme activity

Fig. (9) showed the effect of reaction mixture incubation temperature on glucoamylase activity. Data showed that glucoamylase activity increased within the increases in temperature from 30°C to 70°C, then decreased with the rises of temperature degree. The

optimum temperature of enzyme activity was 70°C. Results are in agreement with Bagheri *et al.* (2014), they found that glucoamylase produced by *A. niger* exhibited optimal activity in a narrow range of temperature around 70°C and it lost its activity rapidly above this temperature. Results were in agreement with those obtained by Norouzian *et al.*(2006), they found that 70°C was the optimum temperature of glucoamylase activity. Results demonstrated that at 60°C the activity of the enzyme lost about 9.33% compared with activity at optimum temperature. There was huge decrease in enzyme activity with raising the temperature degree over 70°C, at 80°C the enzyme activity dropped 57.84% compared to the activity on optimum temperature, whereas there is almost no activity at 90°C. The increase in glucoamylase activity with the increases of temperature may happen because of the change in the enzyme conformation which brings the essential residues to close proximity for catalysis. Koç and Metin (2010) mentioned that temperature of 60°C was the optimum for glucoamylase produced by *A. flavus*, Sutthirak *et al.*(2005) reported that 60°C is optimum for glucoamylase produced by *A. niger*. Ezugwu *et al.* (2015) found that optimum temperature of GluAgCSV4 and GluCSV11 were 50°C and 55°C. On the other hand, Optimum temperature for gluamylase produced from *A. niger* was 40°C Jebor *et al.* (2014). El-Gendy (2012) reported that the optimum temperature range of glucoamylase produced by *A. niger* was observed at 50-60°C.

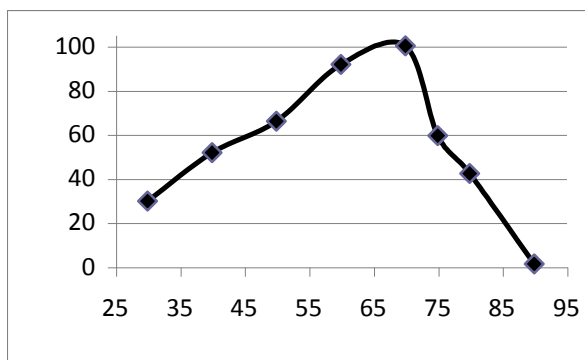


Fig. 9. Effect of reaction mixture incubation temperature on the activity of glucoamylase produced by *A. niger* by using wheat bran under SSF process.

Effect of metal salts on glucoamylase activity:

Adding of some metal salts enhance the activity of group amylases, whereas others may reduce enzyme activity. Data in Fig. (10) showed the effect of some metal salts with concentrations of 5 and 10 µmol on the activity of glucoamylase. Data showed that most of metal salts increased enzyme activity in concentration of 5 µmol, while the effect of 10 µmol vary from salt to another. CuSO₄ gave highest increase in the enzyme activity in concentration of 5 µmol, it raised the activity by 53.97%. Also addition of CaCl₂ and FeSO₄ made good increases in the activity of the enzyme, while HgCl₂ and EDTA caused a huge decrease in the activity of the enzyme in both 5 and 10 µmol. Deb *et al.* (2013) reported that most of amylases are known to be metal dependant. Ezugwu *et al.* (2015) found that Mn⁺², Zn⁺², Fe⁺² and Ca⁺² enhanced the activity

of GluAgacs V4, while Co⁺² inhibited the enzyme activity. On the other hand, the activity of GluAgSV11 was enhanced by Ca⁺² and Co⁺², while Fe⁺², Zn⁺², Pb⁺² and Mn⁺² completely inactivated the enzyme. Glucoamylase produced by *A. flavus* was activated by Mn⁺², Ca⁺² and Co⁺² and inhibited by Fe⁺² and Zn⁺² Koç and Metin (2010). Activity of glucoamylase produced by *Rhizopus nigricans* was enhanced by Mn⁺² and significantly reduced by K⁺², Fe⁺², Zn⁺², Cu⁺² and Ca⁺² supplementation. Supplementation of Mn⁺², Ca⁺² and Fe⁺² increased the activity of *Rhizopus oligosporus* SK5 mutant Kareem *et al.*(2014). The inhibition of some metal salts on glucoamylase may be due to the formation of complexes between the enzyme and metals preventing the enzyme from binding to the substrate Aziz and Ali (2012).

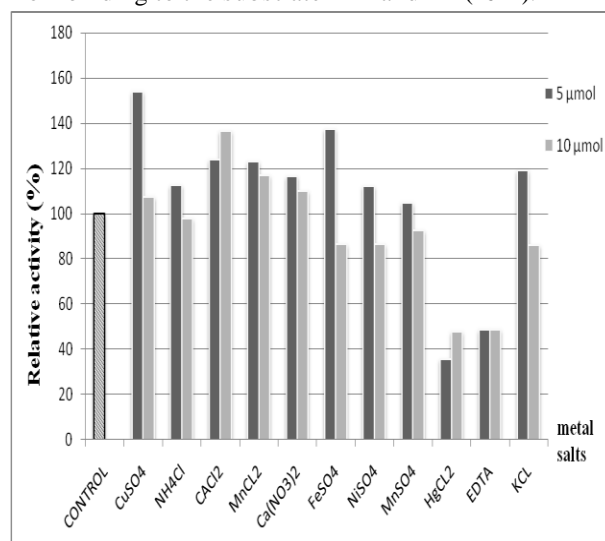


Fig. 10. Effect of different metal salts on *A. niger* glucoamylase activity. The activity was measured at 70°C and pH 5.5 for 5 min.

pH stability of glucoamylase

The residual enzyme activities were assayed after incubating the enzyme at different values of pH from 3-7 for 72 hours. Data illustrated in fig (11) showed that the enzyme activity dropped with time at different pH values. Data showed that glucoamylase enzyme is stable at pH 3 and 4 for 24 hours. At pH 3, the enzyme was very stable; it lost just 10.10% of its activity after 24 hours, 14.98% after 48 hours and 20.09% after 72 hours. The enzyme has reasonable stability at pH values of 5.5, 6 and 7.

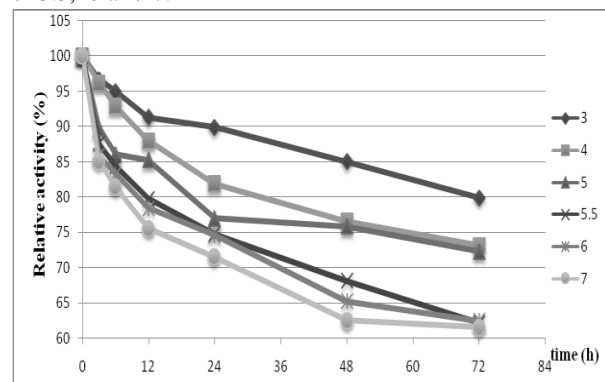


Fig. 11. Effect of pH on the stability profiles of glucoamylase produced by *A. niger*.

Temperature stability of glucoamylase

The enzyme solution, in the presence and absence of CaCl₂ were exposed to different temperature range between 50 and 70°C for different intervals.

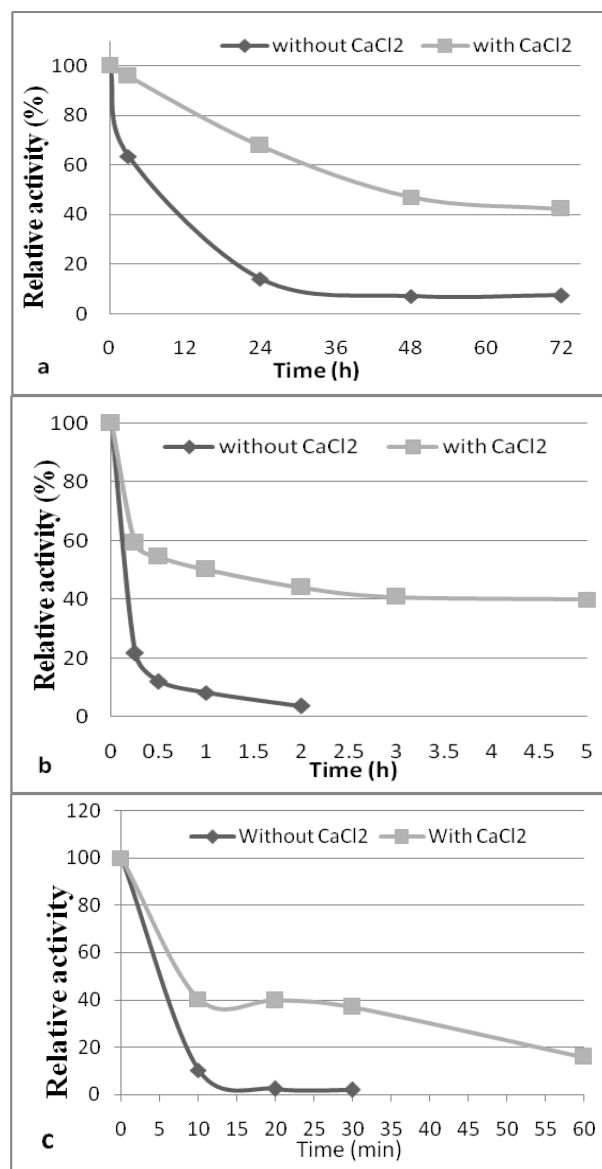


Fig. 12. Effect of storage at 50°C (a), 60°C(b) and 70°C(c) on glucoamylase stability.

From data illustrated in Fig (12) it could be noticed that the enzyme exposed to 50°C did not lose pronounced activity during the first 2.5 hours in the presence of CaCl₂. It lost 4.16% of its activity after 3 hours and 32.20% after 24 hours. The loss in enzyme activity increased by increasing time of exposure. The enzyme solution exposed to 60°C lost 40.86% and 45.60% of its activity after 15 and 30 min of exposure. On the other hand, enzymes exposed to 70°C for 10, 20 and 30 min lost 96.30, 97.67 and 98.00% of its activity. It could be stated that glucoamylase showed high thermal stability at temperatures below 50°C and the enzyme was sensitive to temperature above 60°C. Also data showed that the presence of CaCl₂ (10µmol) has made the enzyme more stable at tested temperature. Similar results were obtained by Sutthirak *et al.* (2005),

they found that *A. niger* glucoamylase activity decreased during prolonged incubation at 60°C

Interesting observation was that the enzyme showed and attained maximum activity at 70°C and showed optimum activity under acidic conditions. This make the produced glucoamylase as useful for various industrial applications like starch liquefaction.

REFERENCES

- Alva S.; J. Anupama; J. Savla; Y.Y. Chiu; P. Vyshali; M. Shruti; B.S. Yogeetha, D. Bhavya; J. Purvi; K. Ruchi; B.S. Kumudini and K.N. Varalakshmi (2007). Production and characterization of fungal amylase enzyme isolated from *Aspergillus* sp. JG12 in solid state culture. *Afr. J. Biotechnol.*, 6:576-581.
- Anto, H.; U.B. Trivedi and K.C. Patel (2006). Glucoamylase production by solid-state fermentation using rice flake manufacturing waste products as substrate. *Bioresour. Technol.*, 97(10):1161–1166.
- Aziz, G.M. and H.M. Ali (2012). Purification and characterization of amylase from local isolate *Pseudomonas* sp. SPH4. *J. Biotechnol. Res. Center*, 5(1):69-79.
- Bagheri, A.; R. Khodarahmi and A. Mostafaei (2014). Purification and biochemical characterisation of glucoamylase from a newly isolated *Aspergillus niger*: Relation to starch processing. *Food Chem.*, 161:270-278.
- Baysal, Z.; F. Uyar, and C. Aytakin (2003). Solid State Fermentation for production of Alfa-Amylase by a hermotolerant *Bacillus subtilis* from hot-spring water. *Process Biochemistry*, 38:1665-1668.
- Chimata M.K.; P. Sasidhar and S. Challa (2010). Production of extracellular amylase from agricultural residues by a newly isolated *Aspergillus* sp. In solid state fermentation. *Afri. J. Biotechnol.*, 9(32):5162-5169
- Deb, P.; S.A. Talukdar; K. Mohsina; P.K. Sarker and S.A. Sayem (2013). Production and partial characterization of extracellular amylase enzyme from *Bacillus amyloliquefaciens* P-001. *Springer Plus*, 2(154):1-12.
- El-Gendy, M.M.A. (2012). Production of glucoamylase by Marine endophytic *Aspergillus* sp. JAN-25 under optimized solid-state fermentation conditions on agro residues. *Aust. J. Basic Appl. Sci.*, 6(4):41-54.
- Ellaiah, P.; K. Adinarayana; Y. Bhavani; P. Padmaja and B. Srinivasula (2002). Optimization of process parameters for glucoamylase production under solid state fermentation by a newly isolated *Aspergillus* species. *Process Biochemistry*, 38: 615-620.
- Ezugwu, A.L.; S.O.O. Eze and F.C. Chilaka (2015). A study of the optimal conditions for glucoamylases obtained from *Aspergillus niger* using amylopectin from cassava starch as carbon source. *Afric. J. Biotech.*, 14(36):2693-2702.

- Jebor, M.A.; M. Zahra; Z.M. Ali and B.A. Hassan (2014). Purification and characterization of the glucoamylase from *Aspergillus niger*. Int. J. Curr. Microbiol. Appl. Sci., 3(1): 63-75.
- Kareem, S.O.; I. Akpan; T.O.S Popoola and L.O. Sanni (2014). Purification and characterization of thermostable glucoamylase from *Rhizopus oligosporus* SK5 mutant obtained through UV radiation and chemical mutagenesis. Int. J. Niger. Soc. Exp. Biol., 26 (1): 19–24.
- Kaur, P.; H.S. Grewal and G.S. Kocher (2003). Production of α -amylase by *Aspergillus niger* using wheat bran in submerged and solid state fermentations. Indian J. Microbiol., 43: 143-145.
- Koç, O. and K. Metin (2010). Purification and characterization of a thermostable glucoamylase produced by *Aspergillus flavus* HBF34. Afr. J. Biotechnol., 9(23): 3414-3424.
- Kumar, M.S.; M.V.V.C Lakshmi and V. Sridevi (2013). Production and optimization of Glucoamylase from wheat bran by *Aspergillus oryzae* NCIM1212 under Solid State Fermentation. Int. J. Appl. Innov. Eng. Manage., 2(10):318-323.
- Kundu, A.K. and S. Das (1970). Production of amylase in liquid culture by a strain of *Aspergillus oryzae*. Appl. Microbiol., 19:598-603.
- Mertens, J.A. and Skory, C.D. (2006). Isolation and characterization of a second glucoamylase gene without a starch binding domain from *Rhizopus oryzae*. Enzyme and Microbial Technology, 40: 874-880.
- Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem., 31(3):426-428.
- Murthy, P. S.; Madhava M. Naidu, and P. Srinivas, (2009). Production of α -amylase under solid-state fermentation utilizing coffee waste. J. Chem. Technol. Biotechnol., 84: 1246–1249.
- Nahid, P., M. Vossoughi; R. Roostaazad and M. Ahmadi (2012). Production of glucoamylase by *Aspergillus niger* under solid state fermentation. IJE Transactions B: Applications, 25(1):1-7.
- Norouzian, D; A. Akbarzadeh; J.M. Scharer and M.M. Young (2006). Fungal glucoamylases. Biotechnol. Adv., 24: 80-85.
- Nyamful, A.; E. Moses; E.G. Ankudey and M.Y. Woode (2014). Solid state fermentation of *Aspergillus niger* menacolla and *Rhizopus menacolla* for glucoamylase production on agricultural residues. Int. J. Sci. and Res. Publications, 4(6): 1-4.
- Pandey, A.; P. Nigam; C.R. Soccol; V.T. Soccol; D. Singh and R. Mohan (2000). Advances in microbial amylases. Biotech. App. Biochem., 31: 135-152.
- Pandey, A.; P. Selvakumar and L. Ashakumary (1996). Performance of a column bioreactor for glucoamylase synthesis by *Aspergillus niger* in SSF. Process Biochemistry, 31: 43–46.
- Parbat R. and B. Singhal (2011). Production of Glucoamylase by *Aspergillus oryzae* Under Solid State Fermentation Using Agro Industrial Products. Int. J. Microbiol. Res., 2(3): 204-207.
- Pavezzi, F.C.; E. Gomes and R. Silva (2008). Production and characterization of glucoamylase from fungus *Aspergillus awamori* expressed in yeast *Saccharomyces cerevisiae* using different carbon sources. Brazilian J. Microbiology, 39:108-114.
- Sauer, J.; B.W. Sigurskjold; U. Christensen; T.P. Frandsen; E. Mirgorodskaya and M. Harrison (2000). Glucoamylase: structure/function relationships, and protein engineering. Biochim. Biophys. Acta., 93:1543:275.
- Sutthirak, P.; S. Dharmstithi and S. Lertsiri (2005). Effect of glycation on stability and kinetic parameters of thermostable glucoamylase from *Aspergillus niger*. Process biotech., 40(8):2821-2826
- Uma Maheswari N and Nasrin Sulthana LS (2013). Production and optimization of glucoamylase from agro waste by using *Aspergillus niger* and *Aspergillus flavus*. Int. J. Chem. Pharm. Sci., 4(1):34-38.
- Zambare, V. (2010). Solid state fermentation of *Aspergillus oryzae* for glucoamylase production on agro-residues. Int. J. Life Sci., 4:16-25.

إنتاج إنزيم الجلوكوأميليز بواسطة عزلة محلية من فطر أسبيرجيليس نيجر بواسطة تخمر الحالة الصلبة على ردة القمح محمد عبدالله العوضى سليم قسم الميكروبيولوجي – كلية الزراعة – جامعة المنصورة

تم استخدام عزلة محلية من الفطر أسبيرجيليس نيجر في إنتاج إنزيم الجلوكوأميليز بواسطة تخمر الحالة الصلبة. وبعد تجربة الإنتاج على عدة مخلفات كان أعلى إنتاج للإنزيم على مخلف ردة القمح بعد 5 أيام من التخمر. وكانت نسبة الرطوبة المثلى 1:0.5 مقدره بوزن : حجم من الردة والماء. انخفض إنتاج الإنزيم باستخدام كل مصادر الكربون الإضافية المستخدمة، في حين تمت تجربة مصادر نيتروجين عضوية ومعدنية بنسبة 1% وكانت نترات الكالسيوم أكثر مصادر النيتروجين زيادة في إنتاج الإنزيم. في حين كان حجم اللقاح الأمثل لإنتاج الإنزيم 1.5 مل من اللقاح ودرجة الحرارة المثلى كانت 30°م والأس الهيدروجيني عند 5.0. وقد أعطى الإنزيم المنتج أكثر نشاط عند 70°م وعند أس هيدروجيني 5.0. وقد لوحظ تأثير الإنزيم بإضافة الأملاح المعدنية ففي حين كانت كبريتات النحاس أكثر الأملاح زيادة في نشاط الإنزيم، كان كلوريد الزنك أكثرها في تثبيط الإنزيم. وقد أعطى الإنزيم درجة ثبات حرارية عالية عند تحضينه على درجة حرارة 50°م وثبات جيد عند تحضينه على درجة حرارة 60°م. كما لوحظ أن للإنزيم ثبات عالي عند تحضينه في درجات الحموضة العالية.