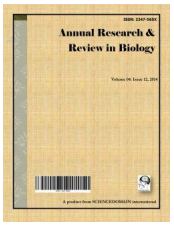

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Activators and Inhibitors of *α*-glucosidase from *Penicillium chrysogenum*

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Authors' contributions

This work was carried out in collaboration between all authors. Author HMES designed the study, managed the literature searches, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MEI and MWA managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

α-glucosidase (EC: 3.2.1.20) from *Penicillium chrysogenum* Thom ATCC 10106 was induced by GSH at the lower concentrations. H_2O_2 was inhibitor at all tested concentrations and the IC_{50} was 92.2%v/v. AMP, ADP and ATP enhanced the activity revealing that *α*-glucosidase is endothermic enzyme. The chelating agents are ethylenediaminetetraacetate (EDTA), *α*-*α*-dipyridyl and *o*-phenanthroline inhibited the enzyme. IC_{50} for these three compounds were 7.1, 10.2 and 10.9 mM, respectively. The highest activity of *α*-glucosidase was recorded at 150 mM phosphate buffer. Mannitol as polyol protected the enzyme against heat inactivation. The five sugars trehalose, lactose, raffinose, glucose and sucrose protected *α*-glucosidase against thermo-inactivation at 60ºC. Also, sarcosine as a product of glycine provided *α*-glucosidase with appreciable thermostability at 60ºC.

Keywords: P. chrysogenum; glutathione; adenosine compounds; chelating agents; trehalose; mannitol.

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1. INTRODUCTION

α-glucosidase can produce glucose from linear and branched isomaltose oligosaccharides through hydrolysis process and the production of glucose might result in postprandial hyperglycemia. One of therapeutic approach to treat diabetes is the reduction of postprandial hyperglycemia through the inhibition of enzymes responsible for carbohydrate hydrolysis such as *α*-glucosidase [1,2].

Inhibition of *α*-glucosidase helps to reduce the rate of digestion of carbohydrates [3,4]. This is of importance in diabetic people where low insulin levels prevent the fast clearing of extracellular glucose from the blood [5]. Acarbose and voglibose as inhibitors of *α*-glucosidase have been applied in treatment of diabetes. However, they exhibit side effects such as liver disorders, flatulence, abdominal pain, renal tumors, hepatic injury, acute hepatitis, abdominal fullness, and diarrhea [6,7].

Thus, the aim of the present work to study the thermostability, activators and inhibitors of *α*-glucosidase from *Penicillium chrysogenum*.

2. MATERIALS AND METHODS

2.1 Materials

Glycerol and other chemicals mentioned in the investigation were purchased from Sigma Chemicals (Sigma Aldrich, Steinheim, Germany). All media cultures including Plate Count Agar (PCA) and others were obtained from Merck Company (Merck, Darmstadt, Germany).

2.2 Experimental Organism

Penicillium chrysogenum Thom ATCC 10106 was provided from Salwa A. Khalaf, Prof. of Microbiology, Botany Department, Faculty of Science, Zagazig University, Egypt.

2.3 Growth Medium

2.3.1 Modified Czapak dox agar (CDA)

This medium used for growth of *P. chrysogenum* at a final pH 7.3 was described by Eaton et al. [8] includes the following in g/L: 2 g sodium nitrate, 30 g glucose, 1 g potassium dihydrogen phosphate, 0.5 g Potassium chloride, 0.5 g magnesium sulphate, 20 g agar, 0.01 g ferrous sulfate and 1L distilled water. Boil to dissolve the medium completely. The medium was sterilized by autoclaving at 15 Ibs pressure (121ºC) for a period of 15 min. The medium was mixed well and poured into sterile petri plates. Cultural characteristics observed after an incubation at 25ºC - 30ºC for 48-72 h and were kept in the refrigerator at 4ºC for storage.

2.3.2 Potato-dextrose agar medium (PDA)

This medium used for the inoculum preparation at final pH 5.6 was that of Vanderzant and Splittstoesser [9]. It included the following in g/L: 20 g dextrose, 4 g potato extract, 15 g agar and 1L distilled water. Boil to dissolve the medium completely and sterilize by autoclaving at 15 Ibs pressure (121ºC) for 15 min. Mix well before dispending. One ml of acid was added per 100 ml of cooled and sterile medium and the medium should not be heated after the addition of the acid. Cultural characteristics were recorded after incubation at 25ºC to 30ºC days. Rate of recovery is considered as 100% for the growth of *P. chrysogenum* on Sabouraud Dextrose Agar.

2.4 Effect of Reduced Glutathione (GSH) on *α*-glucosidase Activity

The effect of reduced glutathione was tested at various concentrations (5, 10, 15, 20, 25 and 30 µmole) in the reaction mixture and the enzyme activity was measured.

2.5 Effect of H_2O_2 on *α*-glucosidase Activity

The effect of H_2O_2 on *α*-glucosidase from *P. chrysogenum* was examined. H_2O_2 was examined at various concentrations (10, 20, 30, 40 and 50% v/v) in the assay medium followed by determination of the enzyme activity.

2.6 Effect of Adenosine Compounds on *α*-glucosidase Activity

The effect of adenosine compounds (AMP, ADP and ATP) on *α*-glucosidase activity was investigated at different concentrations (0.2, 0.4, 0.6, 0.8 and 1 mM) in the reaction mixture followed by determination of *α*-glucosidase activity.

2.7 Effect of Chelating Agents on *α*-glucosidase Activity

EDTA was tested at 2, 4, 6, 8 and 10 mM. α-α-dipyridyl and o-phenanthroline were tested at 5,10, 15, 20, 25 and 30 mM. Each of the three

compounds was included in the assay medium at the various concentrations followed by determination of α-glucosidase activity.

2.8 Effect of Ionic Strength on *α*-glucosidase Activity

The effect of the ionic strength on *α*-glucosidase was tested using 50, 100, 150, 200 and 250 mM phosphate buffer followed by determination of *α*-glucosidase activity.

2.9 Effect of Different Sugars on Thermostability of *α*-glucosidase Activity at 60ºC

The thermostability of *α*-glucosidase was tested at 60ºC with and without 10 mM of sucrose, glucose, raffinose and lactose. The stabilization factor at 60ºC was calculated.

2.10 Effect of Sarcosine on Thermostability of *α*-glucosidase Activity at 60ºC

The effect of sarcosine on *α*-glucosidase activity at 60ºC was investigated. Sarcosine was tested at different concentrations (2, 4, 6, 8 and 10 mM) in the assay mixture followed by measuring of the enzyme activity.

2.11 Effect of Mannitol and Trehalose on Thermostability of *α*-glucosidase at 60ºC

The influence of mannitol as polyol and trehalose as disaccharide on the thermostability of *α*-glucosidase was studied at 60ºC. The two compounds were tested at various concentrations (2, 4, 6, 8 and 10 mM) and the stabilizing factor was calculated.

All the data in the present investigation are the mean of three replicates ± standard error (SE).

3. RESULTS AND DISCUSSION

3.1 Effect of Reduced Glutathione (GSH) on *α*-glucosidase Activity

The activity of *α*-glucosidase (Fig. 1) was increased by increasing of GSH concentration up 10 µmol where the activity was 37 units mg^{-1} protein and then the activity decreased gradually and reached 23 units mg^{-1} protein at 30 µmol. The activation of α-glucosidase by this compound was possibly due to protection of its sulfhydryl groups during the enzyme reaction. In support, GSH activated other enzymes such as urease [10] and protease [11].

3.2 Effect of Hydrogen Peroxide on *α*-glucosidase Activity

Increasing H_2O_2 concentration (Fig. 2) inhibited *α*-glucosidase activity in concentration-dependent manner. The activity reached 2.8 U mg^{-1} protein at 50% (v/v) H_2O_2. IC_{50} of H_2O_2 was 29.2% v/v. This inhibition could be attributed to oxidation of sulfhydryl groups of the enzyme. It seems likely that H_2O_2 inhibited other enzymes including pullulanase [12]. Also, H_2O_2 inhibited Krebs cycle enzymes [13].

3.3 Effect of Adenosine Compounds on *α*-glucosidase Activity

Both AMP and ADP (Fig. 3) increased *α*-glucosidase activity up to 0.8 mM then declined at 1 mM to reach 16.8 and 26 units mg^{-1} protein at 1 mM of AMP and ADP, respectively. ATP increased *α*-glucosidase activity at the lower concentrations up to 0.6 mM where the activity was 28 units mg^{-1} protein followed by reduction at 0.8 and 1 mM to reach 23 and 19 units mg^{-1} protein, respectively. This activation suggested that *α*-glucosidase reaction is endothermic. The adenosine compounds induced other enzymes such as protease [14].

3.4 Effect of Chelating Agents on *α*-glucosidase Activity

Increasing EDTA concentration (Fig. 4) resulted in continuous decrease of *α*-glucosidase activity in a concentration- dependent manner.IC_{50} of EDTA was 7.1 mM. Continuous reduction of *α*-glucosidase activity was observed with increasing *α*-*α*-dipyridyl concentration (Fig. 5). It was noticed that at 30 mM the enzyme activity reduced to 0.2 units mg^{-1} protein. IC_{50} of *α*-*α*-dipyridyl was 10.2 mM. *o*-phenanthroline as chelating agent inhibited *α*-glucosidase activity (Fig. 6). The inhibition was concentration-dependent. It was remarkable that at 30 mM the activity was 0.5 units mg^{-1} protein representing 3.8% of the control value. IC_{50} of *o*-phenanthroline was 10.9 mM. The inhibition by the three chelating agents indicates that *α*-glucosidase is metalloenzyme.

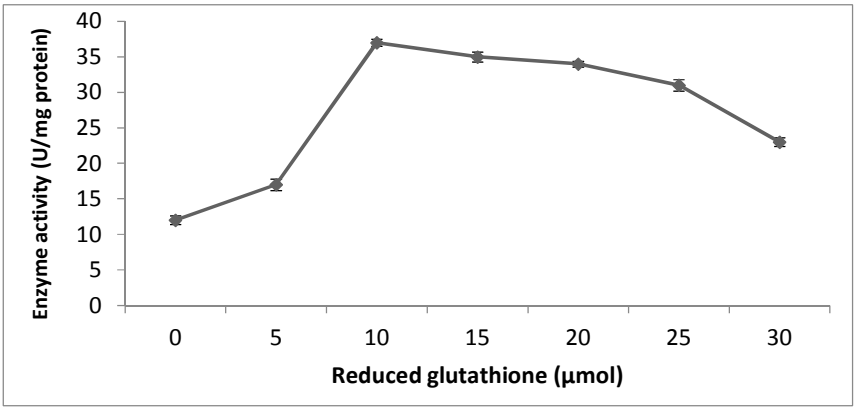

Fig. 1. Effect of reduced glutathione (GSH) on *α*-glucosidase activity from *P. chrysogenum*

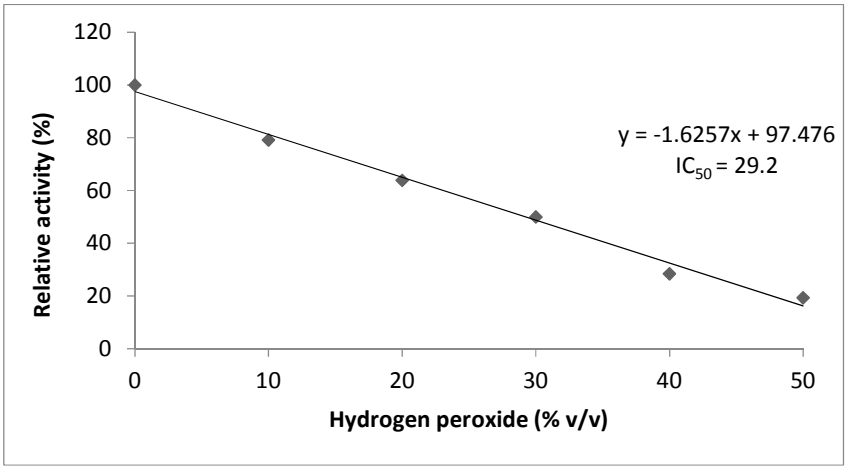

Fig. 2. Effect of H_2O_2 on *α*-glucosidase activity from *P. chrysogenum*

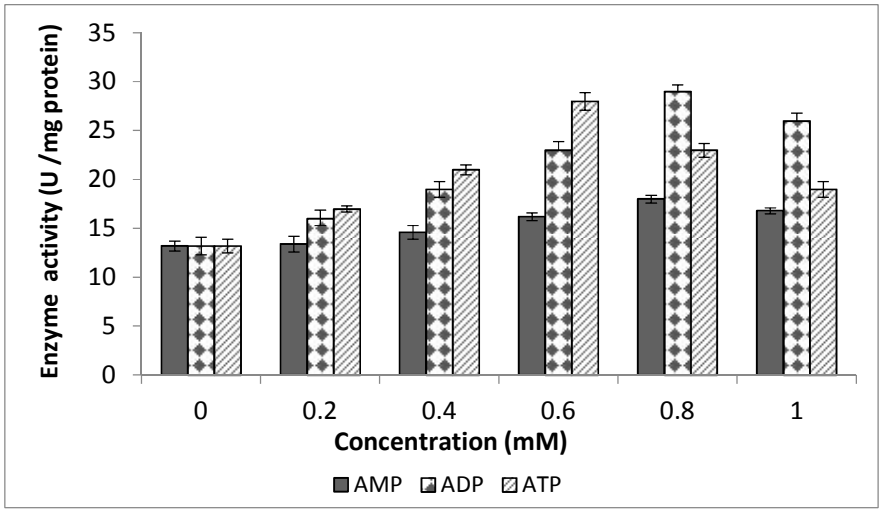

Fig. 3. Effect of adenosine compounds on *α*-glucosidase activity from *P. chrysogenum*

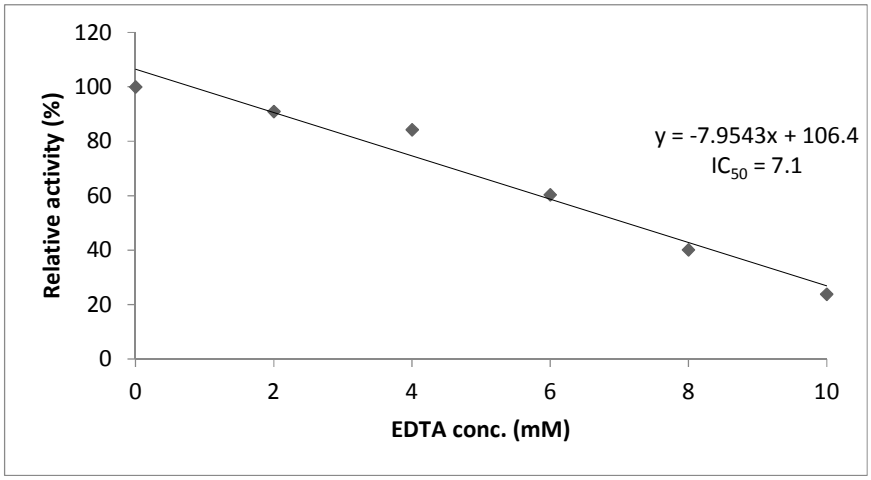

Fig. 4. Effect of EDTA on *α*-glucosidase activity from *P. chrysogenum*

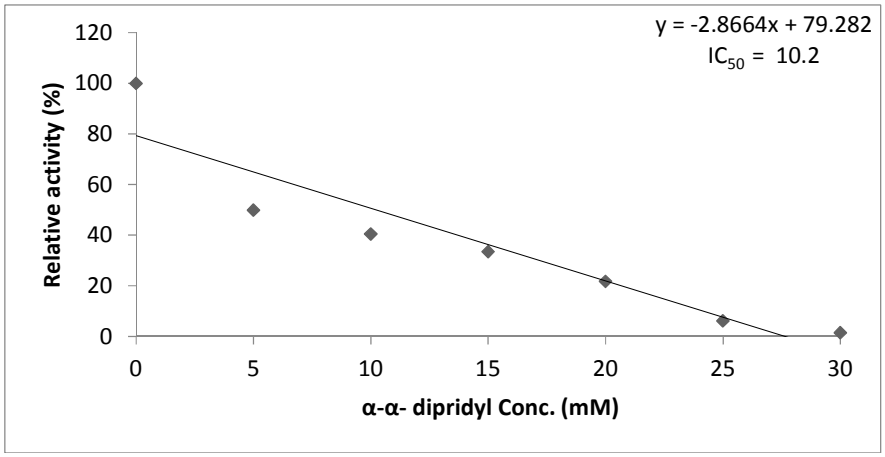

Fig. 5. Effect of *α*-*α*-dipyridyl on *α*-glucosidase activity from *P. chrysogenum*

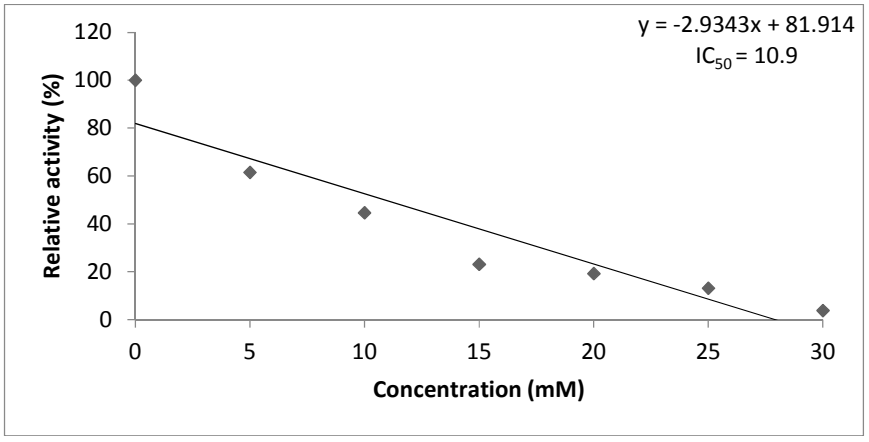

Fig. 6. Effect of *o*-phenanthroline on *α*-glucosidase activity from *P. chrysogenum*

3.5 Effect of Ionic Strength on *α*-glucosidase Activity

The results illustrated in Fig. 7 showed an increase in *α*-glucosidase activity with the increase of buffer concentration up to 150 mM which was the optimum concentration after which the activity declined gradually at 200 and 250 mM. At 250 mM the enzyme activity was 11 units mg-1 protein.

3.6 Effect of Mannitol and Trehalose on Thermostability of α-glucosidase at 60ºC

The stabilizing effect of mannitol and trehalose for *α*-glucosidase at 60ºC was increased in concentration-dependent manner (Fig. 8). Mannitol was the better stabilizing compound of *α*-glucosidase at 60ºC. The values of the stabilizing factor were 4.5 and 2.3 using 10 mM for mannitol and trehalose, respectively. Polyols are known compounds with their multiple hydroxyl groups and they function in protection and stabilization of enzymes. Polyols have the capability to form hydrogen bonds which play a key role in supporting and stabilizing the native confirmation of protein. The high capability of the polyols such as mannitol in the formation of hydrogen bonds should play the most important role in stabilizing of *α*-glucosidase against thermal stress and increasing the degree of organization of water molecules as reported for other enzymes [15,16,17].

Trehalose partially protected the purified *α*-glucosidase from denaturation at 60ºC. Trehalose stabilized other enzymes such as ribonuclease A at elevated temperature [18,19,5]. However, the stabilizing effect of additives is not considered an absolute effect to be applied for all enzymes because it depends on many factors including enzyme nature, the degree of enzyme interaction with the additive and its hydrophobic character [20,21].

3.7 Effect of Various Sugars on Thermostability of *α*-glucosidase at 60ºC

This experiment was done to investigate the effect of various sugars on the stability of *α*-glucosidase activity at 60ºC. The tested sugars were sucrose, glucose, raffinose and lactose and they were examined at 10 mM. The results in Fig. 9 show that sucrose was the best sugar which protected *α -glucosidase from* denaturation at 60ºC. The stabilizing factors for the examined sugars were 1.6, 0.9, 0.7 and 0.3 for sucrose, glucose, raffinose and lactose, respectively. The stabilization of α-glucosidase by these compounds might be due to retaining the osmotic of the solution. It was reported that raffinose protected glucose oxidase from *Cladosporium oxysporum* against denaturation at 60ºC [22].

3.8 Effect of Sarcosine on Thermostability of *α-glucosidase at 60ºC*

An increment in *α*-glucosidase activity (Fig. 10) was observed at the lower concentrations of sarcosine up to 6 mM which was the optimal concentration after which the activity declined at 8 and 10 mM. The stabilizing effect of sarcosine for *α*-glucosidase might be due to retaining the configuration of the enzyme molecules [12].

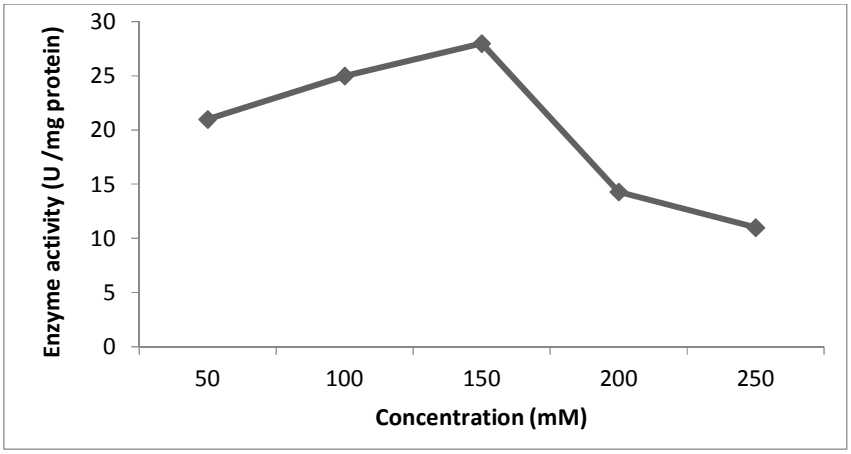

Fig. 7. Effect of ionic strength on *α*-glucosidase activity from *P. chrysogenum*

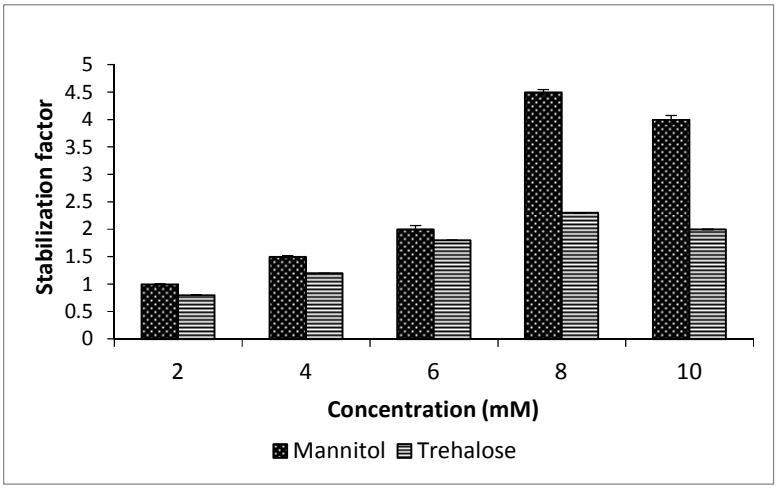

Fig. 8. Effect of mannitol and trehalose on thermostability of *α*-glucosidase from *P. chrysogenum* at 60ºC

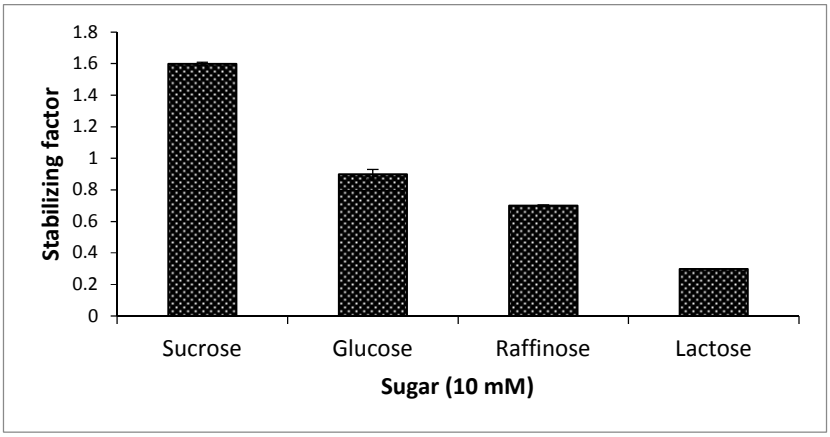

Fig. 9. Effect of various sugars on thermostability of *α*-glucosidase from *P. chrysogenum* at 60ºC

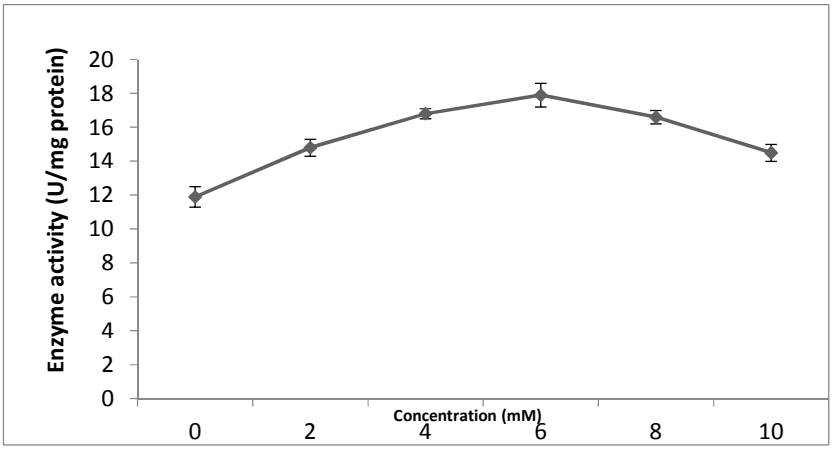

Fig. 10. Effect of sarcosine on thermostability of α-glucosidase from P. chrysogenum at 60ºC

4. CONCLUSION

The results of the present investigation revealed that GSH and adenosine compounds including AMP, ADP and ATP could be used in the activation of *α*-glucosidase from *P. chrysogenum*. Also, mannitol, trehalose, sugars and sarcosine could be applied for protection of *α*-glucosidase at higher temperatures over the optimum in the industrial purposes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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