AR JOURNALS ADVANCED RESEARCH

International Journal of Phytomedicine
2018;10(4):175-180

Purification and characterization of α -glucosidase from *Penicillium chrysogenum*

Hamed Elshora[1*], Saida M Messgo[2], Mohsen E Ibrahim[3] and Mohammad W. Alfakharany[3]

Abstract

α-glucosidase (EC: 3.2.1.20) was isolated and purified from *Penicillium chrysogenum* Thom ATCC 10106 by ammonium sulphate precipitation (75%), DEAE-cellulose and Sephadex G-200. The specific activity was 140 units (U) mg^{-1} protein. The enzyme expressed a single band using SDS-PAGE and the molecular weight of the enzyme was nearly 43KDa. The optimal pH and temperature were 8 and 40ºC. The activation energy was 17.94 k J mol^{-1}. The optimal incubation time was 40 min. Glutamine, glutamic acid, cysteine, alanine, phenylalanine, glycine, methionine, asparagine enhanced the enzyme activity and cysteine was the best enhancer. However, cystine and arginine inhibited α-glucosidase activity. The V_{max} values were 48 and 38.1 U mg^{-1} protein with and without of cysteine, respectively. However, K_m values were 0.21 and 0.25 mM in absence and presence of cysteine, respectively.

Keywords: P chrysogenum; glucosidase; Purification; Amino acids; Kinetics

Introduction

α -glucosidase is an essential enzyme in the digestion of carbohydrates. It can release glucose by hydrolyzing linear as well as branched isomaltose oligosaccharides, causin g a postprandial hyperglycemia [1–3] . Diabetes mellitus is one of the most chronic endocrine disorder which affects the metabolism of carbohydrates. It includes a group of some metabolic diseases characterized by hyperglycemia, where blood sugar levels are increased either because the insulin does not produce from pancreas or cells does not response to produce insulin [4].

Some r eports revealed that a high postprandial plasma glucose level is considered more harmful than fasting blood glucose. Therefore, it is necessary to control the blood glucose level by inhibiting enzymes which are responsible for hydrolysis of carbohydrates, including α-glucosidase [5–7] . Thus, inhibition of α -glucosidase enzyme can decrease the postprandial of hyperglycemia and could perform an important role in the management of diabet es mellitus [8].

Inhibition of α -glucosidase system could help in reducing the rate of the digestion of carbohydrates [9]. This is of importance in diabetic people where low insulin levels prevent the fast clearing of extracellular glucose from the blood [10] . Acarbose and voglibose as inhibitors of α -glucosidase have been applied in diabetes treatment. However they exhibit side effects such as liver disorders, flatulence, abdominal pain, renal tumors, hepatic injury, acute hepatitis, abdominal fullness and diarrhea [11] .

Genus *Penicillium* is used as a source for many industrial enzymes such as α -glucosidase [3], laccase [12] and glutamine synthetase [13]. The present work aimed to purify and characterize α-glucosidase from *Penicillium chrysogenum*. Secondly, to study the influence of amino acids on α -glucosidase activity.

Material and methods

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).

*Correspondence:

[1]Department of Botany, Faculty of Science, Mansoura University, Dakahlia, Egypt

Full list of author information is available at the end of the article.

Received: 07 Sep 2018, Accepted:11 Nov 2018

Experimental

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

Growth medium

Modified Czapak´s dox agar (CDA)

This medium used for growth of *P. chrysogenum* at a final pH 7.3 was described by Eaton et al. [14]. includes the following in g/L: 2g sodium nitrate, 30g glucose, 1g potassium dihydrogen phosphate, 0.5g potassium, 0.5g chloride, 0.5g magnesium sulphate, 20g agar, 0.01g 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.

Potato-dextrose agar medium (PDA)

This medium used for the inoculum preparation at final pH 5.6 was that Vanderzant and Splittstoesser [15] . It included of the following in g/L: 20g dextrose, 4g potato extract, 15g 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. The volume of required acid for 100 ml of sterilized medium was approximately 1ml. The medium should not be heated after the addition of acid. Cultural characteristics 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

Extraction of intracellular α-glucosidase from P. chrysogenum

The culture was filtered by Whatman no. 1 filter paper. T he mycelial sample was washed with 100 mM phosphate buffer (pH 7). The washed mycelia were crushed in 100 mM phosphate buffer (pH 7) using homogenizer for 30 min and centrifuged for 20 min at 10,000 rpm. The produced supernatant was kept in refrigerator and used for α -glucosidase assay.

Purification of α-glucosidase enzyme

The obtained crude extract of α -glucosidase was precipitated with 80% ammonium sulphate. It was kept at 4ºC overnight then centrifuged at 6000 rpm for 20 min. The collected pellet was then suspended in 20 ml of 100 mM phosphate buffer (pH 7.5) followed by enzyme assay. Fifteen ml of the suspension were loaded on DEAE-cellulose column and the eluted enzyme was loaded again on Sephadex G-200 column. The α -glucosidase was eluted using phosphate buffer (pH 7.0) containing 1M NaCl.

Determination of protein content

Protein content was determined according to Bradford [16]. Five ml of diluted Coomassie-Brillant blue G-250 were mixed with 1 ml of enzyme extract and the mixture was put for one min in dark and the absorption was measured spectrophotometrically at 595 nm. Bovine serum Albumin (BSA) was used as standard.

SDS Polyacrylamide gel and activity staining of purified α-glucosidase

Purity of α -glucosidase enzyme was checked by SDS-PAGE [17]. The markers used were: chmotrypsinogene (25 KDa), glyceraldehyde 3-phosphate dehydrogenase (36 KDa), ovalbumin (45 KDa) and bovine serum albumin (66 KDa)

Estimation of kinetic parameters

The Michaelis-Menten´s constant (K_m) and the maximum velocity (V_{max}) were calculated by studying the influence of substrate concentration on α -glucosidase activity. Enzyme activity was determined using different substrate concentrations [S]. The Lineweaver-Burk plot (1/[V] vs. 1/[S] was then constructed. The K_m and V_{max} were determined for α -glucosidase from this graph.

Determination of the optimum pH for α-glucosidase activity

The optimal pH for enzyme activity was determined over pH range from 3 - 5 using sodium acetate / acetic, potassium phosphate buffer (pH 6, 7), Tris-HCl buffer (pH 8, 9) and sodium bicarbonate buffer (pH 10). From the obtained results a graph of enzyme activity vs. pH was plotted and the optimum pH for α -glucosidase was subsequently determined.

Effect of different concentrations of α-glucosidase on its activity

The experiment was performed to investigate the effect of various α -glucosidase concentrations (2, 3, 4, 5, 6 and 7 mg protein ml^{-1}) on its activity. The other factors affecting α -glucosidase activity were kept constant.

Effect of temperature on α-glucosidase activity

The optimum temperature of α -glucosidase was determined by measuring the activity at different temperatures (10, 20, 30, 40, 50, 60 and 70 ºC). The other factors which affect α -glucosidase activity were kept constant.

Effect of various amino acids on α-glucosidase activity

The effect of different amino acids on α -glucosidase activity were studied. The tested amino acids were glycine, glutamic, glutamine, cysteine, alanine, phenylalanine, methionine, asparagine, arginine and cystine. The amino acids were examined at 10 mM in the assay mixture then the enzyme activity was determined.

Results

Purification of α-glucosidase from P. chrysogenum

α - glucosidase was isolated from *P. chrysogenum* and purified using ammonium sulphate precipitation (75 %), DEAE- cellulose and Sephadex G-200. The final specific activity value was 140, with 127.3- fold. The yield of purification was 41.9. El-Shora et al. [18] reported purification of α - glucosidase of *Penicillium notatum* with 27.8-fold. The purity of α - glucosidase was confirmed by SDS-PAE which showed a single band with molecular weight of 55 KDa. Also, lower specific activity 0.7 units mg^{-1} protein and purification - fold of 45.5 were observed for the enzyme from *Xanthophyllomyces dendrohous* [19] .

Elution profile of purified α-glucosidase from Sephadex G-200

Studying the elution profile of α -glucosidase (Fig. 1) from Sephadex G-200 revealed that the highest activity of α - glucosidase was recorded in fraction no. 10. The purification was confirmed by SDS-PAGE (Fig. 2) which revealed the presence of a single band at 43 KDa revealing the purity of the enzyme.

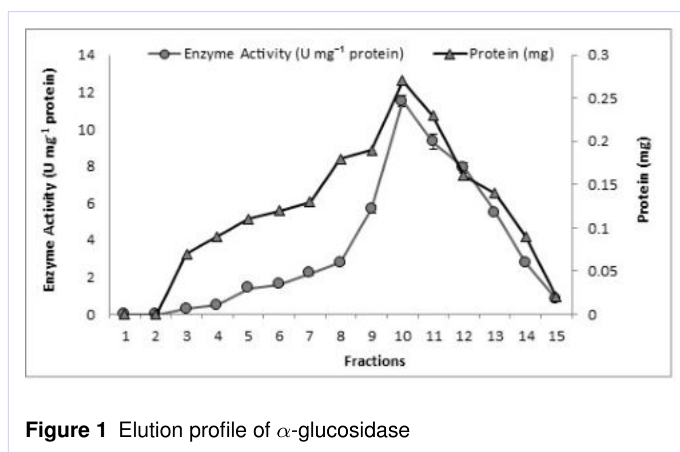

Figure 1 Elution profile of α-glucosidase

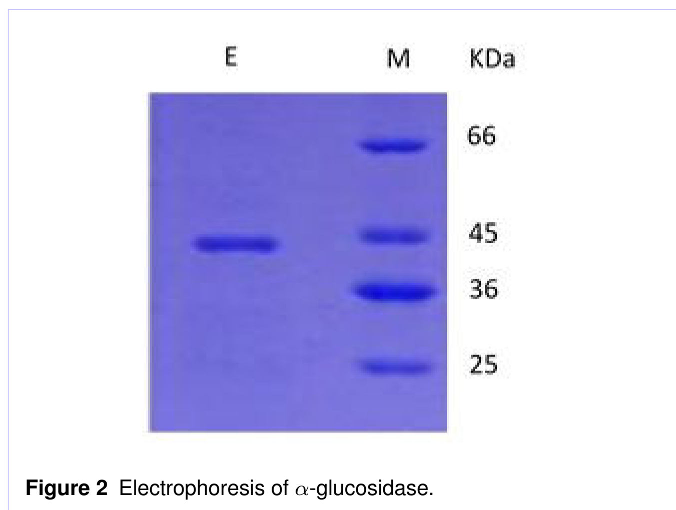

Figure 2 Electrophoresis of α-glucosidase.

Effect of pH on α-glucosidase activity from P. chrysogenum

The results in F ig. 3 showed an increase of α -glucosidase activity with increasing the pH value up to pH 8 after which the activity decreased gradually to reach 17.2 U mg^{-1} protein at pH 10. Thus, the optimal pH for the enzyme was 8.0. However, the pH of α - glucosidase was 6 from *Mortierella alliacea* [20] and *Rhizopium* [21] . Lower pH of 5.5 was found for α - glucosidase from *Xanthophyllomyces dendrohous* [19]. Also, pH 4.5 was reported for α - glucosidase from *Thermoascus aurantiacus* [22] . The characteristics of ionizable side of chains of amino acids depend on pH. Thus, enzyme activity usually varies with pH changes. At extreme pH, the tertiary structure of α -glucosidase protein might be disrupted and the denaturation of protein took place. At moderate pH values where the tertiary structure is not disrupted the α -glucosidase activity may be dependent on the degree of the ionization of certain amino acid side chains and the pH profile of the enzyme may suggest the identity of those residues [23].

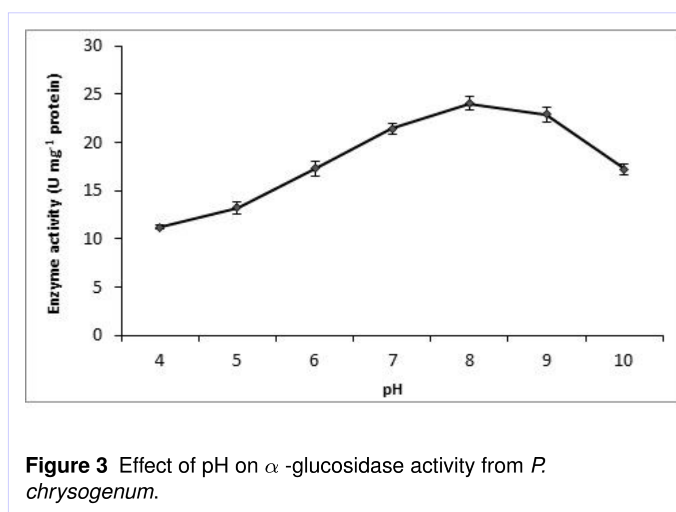

Figure 3 Effect of pH on α -glucosidase activity from *P. chrysogenum.*

Table 1 Purification of α -glucosidase from *P. chrysogenum*

Purification Step	Protein (mg)	Activity (U)	Specific activity (U mg^{-1} protein)	Purification fold	Yield (%)
Crude extract	220	234	1.1	1.0	100
Ammonium sulphate (75%)	118	164	1.30	1.18	70.1
DEAE – Cellulose	1.8	135	75.0	86.2	57.7
Sephadex G-200	0.7	98	140	127.3	41.9

*Effect of temperature on α-glucosidase from P. chrysogenu*m

The results in Fig. 4 indicate that increasing the incubation temperature resulted in continuous increment in the enzyme activity up to 40ºC where α -glucosidase activity was 22.2 units mg^{-1} protein followed by continuous decline at the higher temperatures and it reached 7.0 units mg^{-1} protein at 70ºC. However, the optimal temperatures of the enzyme from *Rhizopium* and *Mortierella alliacea* were 30ºC and 55ºC, respectively [20, 21] . Increasing the temperature leads to increase in the inherent energy of the enzyme system. Thus, more enzyme molecules can obtain the necessary activation for the reaction to take place. However, at certain point the increase of the reaction would be equal to the reduction in the reaction rate because of tertiary structure destruction by elevated temperature [24]. At this critical point α -glucosidase activity is at its maximum and this temperature is called optimal temperature.

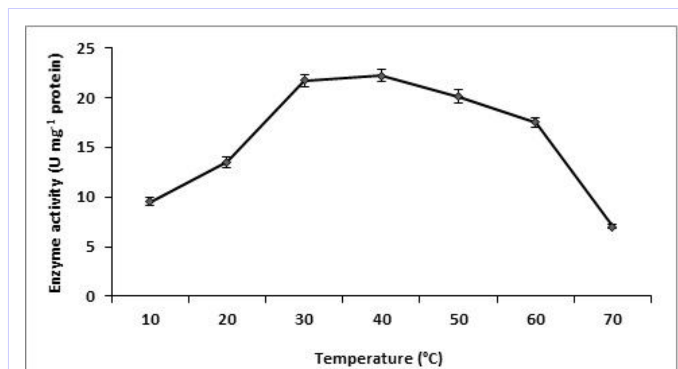

Figure 4 Effect of temperature on α -glucosidase activity from *P. chrysogenum*.

*Arrhenius plot of α-glucosidase from P. chrysogenu*m

Plotting log v against 1/(t+273) $\times$ 10^{-3} (Fig. 5) resulted in straight line from which the slope was taken and used for calculating the activation energy. The activation energy of α -glucosidase was 17.94 k J mol^{-1}

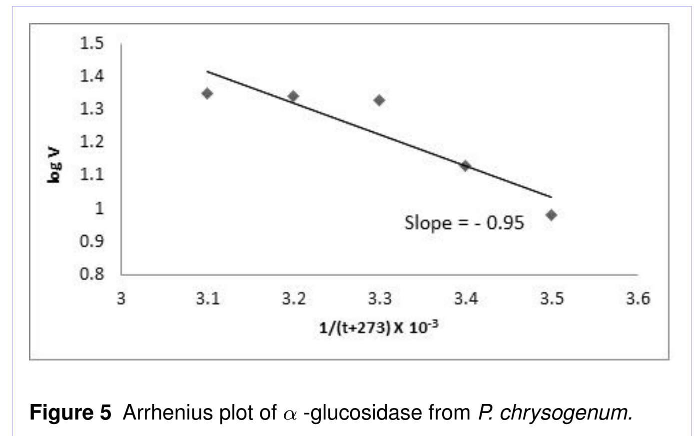

Figure 5 Arrhenius plot of α -glucosidase from *P. chrysogenum.*

*Effect of incubation time on α-glucosidase activity from P. chrysogenu*m

The influence of incubation time on α -glucosidase activity was studied (Fig. 6). An increment of α -glucosidase activity was observed with the increase of the incubation time from 20 min to 40 min followed by continuous reduction of the activity at 60, 80 and 100 min. There are two parts on this figure. Firstly, in the initial interval of time where the amount of substrate is transformed, it seems that the transformation is in direct proportional to the length of time for which the reaction has been carried out. After the optimal time the reaction started to decline and the enzyme substrate is present in excess and this was due to loss of the activity after a period of time. This may be due to the influence of temperature of incubation on the tertiary structure of the enzyme or to the formation of some products or side-product of the reaction, which inhibits the enzyme activity [25].

Effect of different amino acids on α-glucosidase activity from P. chrysogenum.

The amino acids glycine, glutamic acid, cysteine, glutamine, alanine, phenylalanine, methionine and asparagine enhanced the enzyme activity (Fig. 7). However, arginine and cyst ine inhibited the activity. The inhibition by the se two amino acids might be due to prevention of ES complex.

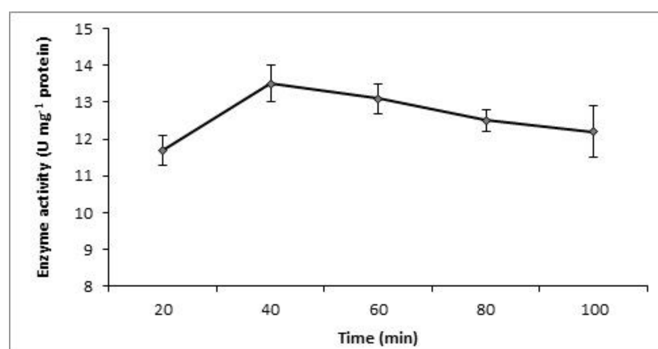

Figure 6 Effect of incubation time on α -glucosidase activity from *P. chrysogenum*.

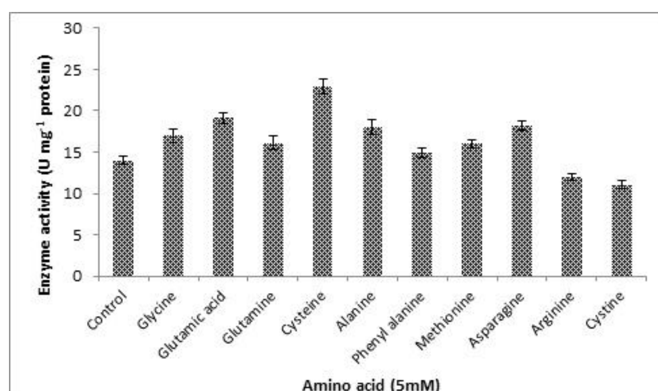

Figure 7 Effect of various amino acids on α -glucosidase activity from *P. chrysogenum*.

Effect of cysteine on α-glucosidase activity from P. chrysogenum.

Since cysteine expressed the highest activation of α -glucosidase, it was decided to test the effect of 5 mM cysteine in presence of various concentrations of substrate. The results in Fig. 8 demonstrated that α -glucosidase activity increased with increasing the substrate concentrations in presence of cysteine. V_{max} values were 48 and 38.1 units mg^{-1} protein with and without of cysteine, respectively. K_m values were 0.21 and 0.25 mM. The K_m of α -glucosidase from *Thermoascus aurantiacus* was 0.07 μM whereas the V_{max} 318 U mg^{-1} protein [22]. Cysteine activated α -glucosidase and this may be due to the fact that cysteine contains SH-group which may keep the –SH group of α -glucosidase . The V_{max} values were 38.1 and 48 U mg^{-1} protein in absence and presence of cysteine, respectively. However, K_m values were 0.25 and 0.21 mM with and without cysteine, respectively. Therefore, it appeared that cysteine activated α-glucosidase through lowering the K_m of the enzyme for its substrate. Cysteine activated other enzyme s such as asparaginase [26], protease [27] and tyrosi nase [28]. These results reveal that SH-group of α -glucosidase may be protected by cysteine during the incubation time.

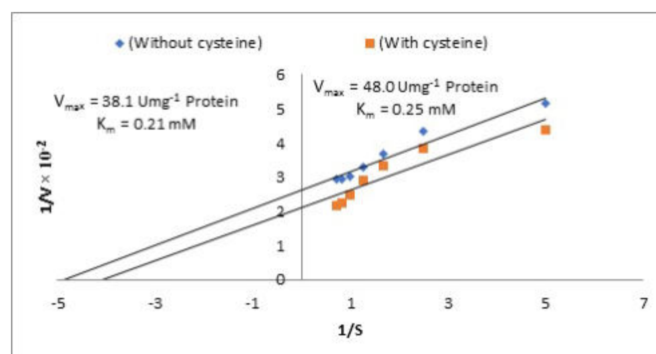

Figure 8 Reciprocal of substrate (1/S) concentration against reciprocal of velocity (1/V $\times$ 10^{-2}) with and without cysteine.

Conclusion

α -glucosidase was successfully purified from *Penicillium chrysogenum* to homogeneity and with appreciable specific activity. The enzyme can be activated by cysteine and other amino acids. The results also suggest using arginine and cystine as inhibitors of α -glucosidase.

Author contributions

Hamed M. Elshora, Mohsen E. Ibrahim and Mohammad W. Alfakharany**.** Performed the experiments together and participated in manuscript for publications.

Conflicts of interest

None.

Author details

[1]Department of Botany, Faculty of Science, Mansoura University, Dakahlia, Egypt. [2]Laboratory for Research on Medicinal and Aromatic Plants, Science and Life Faculty, University of Blida, Algeria. [3]Department of Botany, Faculty of Science,, Port Said University, Port Said, Egypt.

References

[1] Bhat M, Zinjarde SS, Bharagava SY, Kumar AR, Joshi BN. Antidiabetic activity of Gnidia glauca and Dioscorea bulbifera: Potent amylase and glucosidase inhibitors Evi Based Complement Alternative Medical; 2012. Available from: 10.1155/2012/929051.

[2] Lin AHM, Nichols BL, Quezada-Calvillo R, Avery SE, Sim L, Rose DR, et al. Unexpected high digestion rate of cooked starch by the Ct-maltase-glucoamylase small intestine mucosal -glucosidase subunit. PLoS One. 2012;5:354–373.

[3] El-Shora HM, Ibrahim ME, Alfakharany MW. Activators and Inhibitors of -glucosidase from Penicillium chrysogenum. Annu Res and Rev in Biol. 2018;24:1–9.

[4] West IC. Radicals and oxidative stress in diabetes. Diab Med. 2000;3:171–180.

[5] Hiroyuki F, Tomohide Y, Kazunori O. Efficacy and safety of Touchi extract, an -glucosidase inhibitor derived from fermented soybeans, in non-insulin-dependent diabetic mellitus. The J of Nutritional Biochem. 2001;6:351–356.

[6] Kim WJ, Lee SW, Park SW, Kim YH, Yun SC, Lee JY, et al. Randomized comparison of everolimus-eluting stent versus sirolimus-eluting stent implantation for de novo coronary artery disease in patients with diabetes mellitus (Essence-Diabetes). Circulation. 2011;8:886–892.

[7] Souza D, Sales PMD, Simeoni PM, Silva LA, Silveira EC, Magalhaes D, et al. Inhibitory activity of -glucosidase by plant extracts from the Birazilian cerrado. Planta Medica. 2012;78:393–399.

[8] Hirsh AJ, Yao SY, Young JD, Cheeseman CI. Inhibition of glucose absorption in the rat jejunum: a novel action of alpha-D-glucosidase inhibitors Gastroenterol; 1997.

[9] Bakkalbai E, Mente O, Artik N. Food ellagitannins-occurrence, effects of processing and storage. Critical Rev in Food Sci and Nutri. 2009;3:283–298.

[10] Ye R, Jung DY, Jun JY, Li J, Luo S, Ko HJ, et al. Grp78 heterozygosity promotes adaptive unfolded protein response and attenuates diet-induced obesity and insulin resistance. Diabetes. 2010;1:6–16.

[11] Shobana S, Sreerama YN, Malleshi NG. Composition and enzyme inhibitory properties of finger millet (Eleusine coracana L.) seed coat phenolic: Mode of inhibition of -glucosidase and pancreatic amylase. Food Chem. 2009;4:1268–1273.

[12] El-Shora HM, Youssef M, Salwa AK. Inducers and inhibitors of laccase from Penicillium. Biotechnol. 2008;7:35–42.

[13] El-Shora HM, Khalaf SA. Characterization of glutamine synthetase from Penicillium cyclopium. Ann of Microbiol. 2002;55:263–294.

[14] Eaton AD, Clesceri LS, Greenberg AE. Standard Methods for the Examination of Water and Waste water. Washington, USA: American Public Health Association; 1998. 20th Ed.

[15] El-Shora HM, Hegazy R. Marrow. J. of Plant Production. 2014;5:295–303. Man. Uni.

[16] El-Shora HM, Abou-El-Wafa GS, Kadhim A. Purification and biochemical characteristic of protease from the red seaweed Pterocladia capillacea. Intern J of Curr Microbiol of App Sci. 2016;5:297–308.

[17] Warangkar SC, Khobragade CN. Purification, characterization and effect of thiol compounds on activity of the Erwinia carotovora asparaginase. Enzyme Research. 2010;.

[18] Lizotte PA, Henson CA, Duke SH. Purification and characterization of pea epicotyl -amylase. Plant Physiol. 1990;92:615–621.

[19] Palmer J. Attention in visual search: Distinguishing four causes of set- size effects. Curr Direction in Psychological Sci. 1995;4:118–123.

[20] Palmer T. Understanding Enzymes 2nd Ed. Ellis Horwood Publisher; 1985.

[21] Carvalho AF, Boscolo M, Silva RD, Ferreira H, Gomes E. Purification and characterization of the alpha-glucosidase produced by thermophilic fungus Thermoascus aurantiacus CBMAI 756. J of Microbiol. 2010;4:452–459.

[22] Berthelot, Delmotte FM. Purification and characterization of an alpha-glucosidase from Rhizobium sp. (Robinia pseudoacacia L.) strain USDA 4280. App Environmen Microbiol. 1999;7:2907–2911.

[23] Tanaka Y, Aki T, Hidaka Y, Furuya Y, Kawamoto S, Shigeta S, et al. Purification and characterization of a novel fungal alpha-glucosidase from Mortierella alliacea with high starch-hydrolytic activity. Biosci, Biotechnol and Biochem. 2002;66:2415–2423.

[24] Marn D, Linde D, Lobato M. Purification and biochemical characterization of an -glucosidase from Xanthophyllomyces dendrorhous. Yeast. 2006;2:117–125.

[25] El-Shora HM, Metwally AM, Salwa AK. Essential groups and stability of -glucosidase of Penicillium notatum. Ann of Microbiol. 2009;59:285–291.

[26] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970;227:680–685.

[27] Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochem. 1976;72:248–254.

[28] Vanderzant C, Splittstoesser DF. Compendium of methods for the microbiological examination of food. Washington, D.C.: American Public Health Association; 1992. 3rd ed.