Research Article

Kinetic Parameters of Purified β-glucosidase from the Seeds of *Tamarindus indica*

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ABSTRACT

Tamarind seed is an underutilized byproduct of the tamarind pulp industry. Only a small portion of the seed, in the form of tamarind kernel powder (TKP), is used as a sizing material in the textile, paper and jute industries. Though many applications of this seed are possible, there have been hardly any other uses for it including using it as an additive in food formulations. The major industrial use of the seeds is in the manufacture of Tamarind Kernel Powder (TKP). It is prepared by decorticating the seed and pulverising the creamy white kernels. In India, TKP is used as a source of carbohydrate for the adhesive or binding agent in paper and textile sizing and weaving. The present study is aimed at determining the kinetic parameters of purified β-glucosidase isolated from germinated tamarind seeds. The kinetic parameters of the purified enzyme revealed that optimum incubation time was 15 mins. β-glucosidase enzyme showed a maximum activity at pH pH 5.0 and showed stability from pH 5.0 to pH 7.0. The enzyme showed highest activity at 30^{O} C, and the enzymes were stable up to 40^{O} C. β-glucosidase exhibited Km value of 121 µM and Vmax value of 5.26nmoles/min. β-glucosidase activity increased in the presence of $MnCl_2$, $FeSO_4$, $AgNO_3$ and $HgCl_2$ at 10mM concentration. Enhancement of glucosidase activity was brought about by the addition of $MnCl_2$, $CaCl_2$, $CoCl_2$, $ZnCl_2$ and $MnSO_4$.

Keywords: Tamarind kernel powder, β-glucosidase, p-nitrophenyl-β-D-glucoside, ρ-nitrophenol, endoglucanases.

INTRODUCTION

The *Tamarindus indica* L. tree belonging to family Caesalpinaceae is found in tropical and subtropical regions of the world[1,2]. It is grown extensively in the dry tracts of Central and South Indian States for its sour fruit pulp, which is used extensively in the local confectionary industry and is a common article of trade in India.

Research on cellulase in higher plants has attempted to correlate its activity with various physiological processes including leaf abscission, pollen tube elongation, growth responses induced by hormones and fruit softening[3]. The three components of fungal cellulases (endoglucanase, exoglucanase and β-glucosidase) are believed to function synergistically[3]. A general accepted enzymatic mechanism distinguishes their actions. Endoglucanases initiate attack by randomly cleaving β-1, 4-glucosidic bonds and thus creating shorter-length cellulose chains, neighboring exoglucanases start degrading these chains at the nonreducing termini, thus generating cellobiose and glucose residues, finally, β-glucosidases cleave cellubiose to form glucose units. However, as cellulose hydrolysis proceeds, its rate decreases and eventually ceases before all the substrate is hydrolyzed.

β-Glucosidases (E.C. 3.2.1.21) represent a group of ubiquitously expressed, hydrolytic enzymes, which catalyze the hydrolysis of β-*O*-glucosidic linkages between β-D-glucose and an aglycone or another sugar. β-Glucosidases (β-D-glucoside glucohydrolases; EC 3.2.1.21) occur in plants, fungi, mammals and microorganisms and are subject of much recent research due to the key role these enzymes play in biological processes and potential biotechnological applications[4]. Plant β-glucosidases play an important role in defense against pests[5,6] phytohormone activation[7-11], lignifications[12] and cell wall catabolism[13]. β-glucosidases are key enzymes in the release of aromatic compounds from glucosidic precursors present in fruits and fermentating products. Indeed, many natural flavor compounds, such as monoterpenols, C-13 norisoprenoids, and skimate-derived compounds, accumulate in fruits as flavorless precursors linked to mono- or diglycosides and require liberation by enzymatic or acidic hydrolysis[14,15].

The present investigation is aimed at characterization of β-glucosidases from Tamarind seeds during germination. by determining its *Km, Vmax*, pH and temperature optima and stability as well as the effect of metal ions on its activity. This enzyme from other sources are unsuitable because of high cost. The enzyme β-glucosidases obtained from agricultural product such as the seeds of Tamarind is a heat stable enzyme and can be commercially exploited for the production of the enzyme. In the current study, β-glucosidases of *Tamarindus indica* seeds has been examined as a new source for producing these enzymes.

MATERIALS AND METHODS

Materials

The seeds of *Tamarindus indica* were collected from local areas of Bangalore district, Karnataka State, India. Acrylamide, N – N'- methylene bisacrylamide, Coomassie brilliant blue R-250, p–nitrophenyl–β–D–glucoside, Acetone purchased from Sigma Chemical Company,

St.Louis, MO, U.S.A. All other chemicals used were of analytical grade.

Plant Material

The seeds of *Tamarindus indica* were collected using random sampling technique (RST) from local areas of Bangalore district, Karnataka State, India. After dehulling the fruits, equal samples of seeds were combined to give one bulk population sample from which sub samples were taken for test. Collected seed samples were dried in the sunlight for 24 hrs. After removing immature and damaged seeds, the dried matured seeds were washed under tap water, dried and stored in plastic containers or refrigerator until further use.

Preparation of the crude enzyme

Twenty grams of 25 days old germinated seeds was blended in 100 ml of 0.01M sodium acetate buffer pH 5.6 containing 0.5M NaCl. This was then centrifuged for 20 min at 10,000 rpm. The homogenate obtained was used as crude enzyme extract.

Enzyme assay

β-glucosidase assay is based on the measurement of the amount of p-nitrophenol formed. The enzyme reaction was initiated by adding 0.25 ml of the extract to 0.75 ml of 1.2 mM p-nitrophenyl-β-D-glucoside (PNPG) in 10Mm acetate buffer, pH 5.6 and incubated at 37° C for 30 mins. The reaction was stopped by adding 4.0 ml of 0.1 M sodium hydroxide. The amount of p-nitrophenol liberated is measured at 440 nm. One enzyme unit corresponds to 0.5µmoles of ρ-nitrophenol/min.

Kinetic parameters

Effect of time

Effect of time on the purified β-glucosidase from the seeds of *Tamarindus indica* was carried out by incubating the purified β-glucosidase with p–nitrophenyl–β–D–glucoside for different time interval and assayed as described above.

Effect of enzyme concentration

The velocity of the reaction was monitored with different concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 µg) of the purified β-glucosidase from the seeds of *Tamarindus indica*.

Km and Vmax

The purified β-glucosidase was assayed at varying concentration of p–nitrophenyl–β–D–glucoside. Km and Vmax for β-glucosidase were determined by constructing LB- plot.

pH Optima

The effect of pH on the activity of the purified β-glucosidase was studied using the 100 mM buffers of different pH, (citrate buffer pH 2.0 - 6.0, phosphate buffer pH 6.5 - 7.5, Tris - HCl buffer pH 8.0 - 9.0 and carbonate buffer pH 9.5 and 10). The activity was measured using p–nitrophenyl–β–D–glucoside prepared from these buffers.

Temperature optima

The optimum temperature was determined by assaying the β-glucosidase activity at different temperatures ranging from 7^O C, 15^O C, 23^O C, 30^O C, 40^O C, 50^O C 60^O C, 75^O C, 85^O C and 95^O C using p–nitrophenyl–β–D–glucoside for 30 mins at different temperatures.

pH Stability

The purified β-glucosidase was pre-incubated with 100 mM buffers of different pH (citrate buffer pH 3.0 - 6.0, phosphate buffer pH 6.5 - 7.5, Tris - HCl buffer pH 8.0 - 9.0 and carbonate buffer pH 9.0 - 10) for 1 hrs at 4^O C. A known aliquots from the incubated samples were removed and assayed using p–nitrophenyl–β–D–glucoside as a substrate at an optimum pH 4.8.

Temperature Stability

The purified β-glucosidase was pre-incubated at different temperatures ranging from 7^O C to 95^O C for 30 minutes, rapidly cooled to 0^O C and assayed using p–nitrophenyl–β–D–glucoside at an optimum temperature 40^O C.

Effect of salts

The different metal salts, SDS and EDTA were used to test the effect on the activity of the pure enzyme. The salts used were $MgSO_4$, $CaCl_2$, NaCl, $FeSO_4$, $HgCl_2$, $CuSO_4$, $MnCl_2$, $Pb(CH_3COO_2)$, $ZnSO_4$, and $Ag(NO_3)_2$. The assay for purified β-glucosidase was carried out by pre-incubating with 1 mM concentration of different salts for 30 mins at room temperature and then assayed as described earlier.

RESULTS AND DISCUSSION

In the present study, the activity of β-glucosidases increased during the first and third week of germination (data not shown). The activity of the enzyme were high on 6^{th} day which gradually decreased from 7^{th} to 12^{th} day and almost stable for about 5 days between 13^{th} to 19^{th} day and again the activity was high on 21^{st} to 23^{rd} day of germination (data not shown). By the end of 25^{th} day, the shoot starts developing with well grown leaves. The seeds during this stage almost detach from the shoot and falls down. The size of the seed is reduced on 25^{th} day when compared to the seed during initial stage of germination. The observed variation in β-glucosidase activity during germination of tamarind seed indicates that the enzyme produced by the source is utilized for the development of the seeds.

The 25^{th} day old seeds were collected and were further extracted for purification of the enzymes. All the purification procedures were performed at 4-10°C unless otherwise stated (data not shown).

Effect of Time on Activity

The effect of time on activity was determined by incubation of reaction mixture for different time intervals.

Optimum incubation time was 15 mins. (Fig 1). For all further experiments, the optimum time of incubation was maintained.

Effect of Enzyme concentration on Activity

The effect of enzyme concentration (10 – 120 µg) on activity was determined by incubation of reaction mixture for one hour. Optimum enzyme concentration was found to be 50 µg (Fig 2).

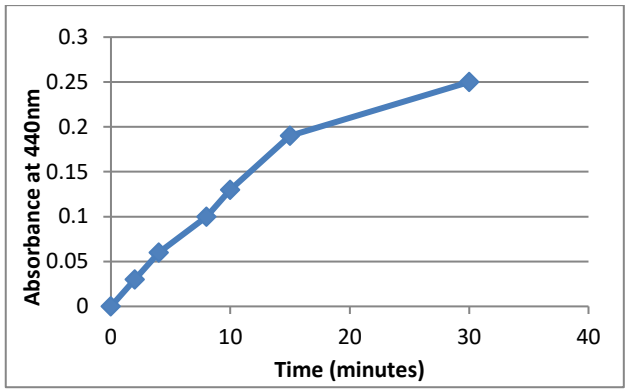


Figure 1: Effect of time on β-glucosidase activity

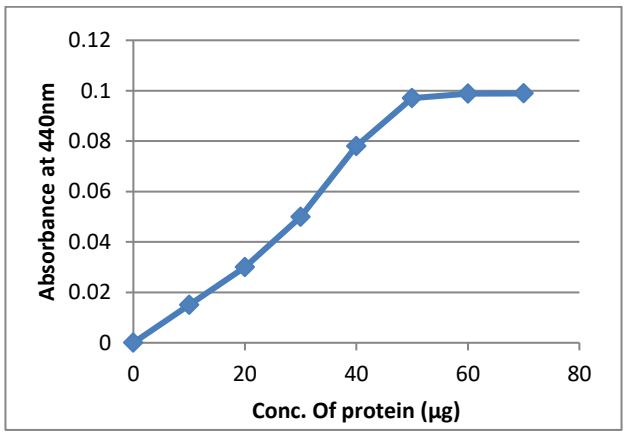
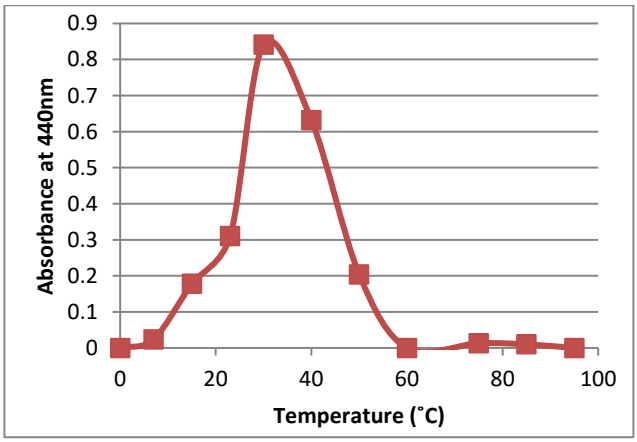


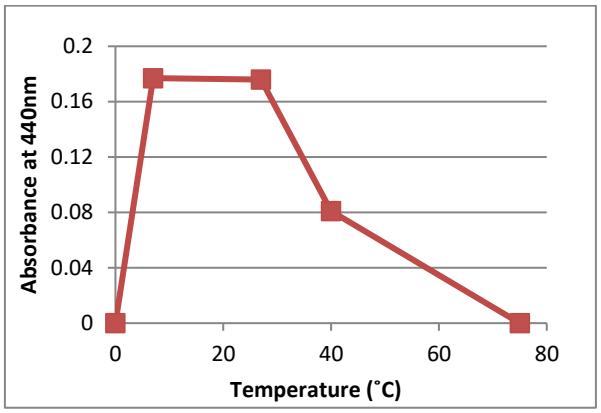
Figure 2: Effect of enzyme concentration on β-glucosidase activity.

Temperature Optima and Stability

β-glucosidase enzyme showed highest activity at 50^{O} – 70^{O} C and 30^{O} C, and the enzymes were stable up to 40^{O} C and 35^{O} C, respectively (Fig 3 a & b).



(a)



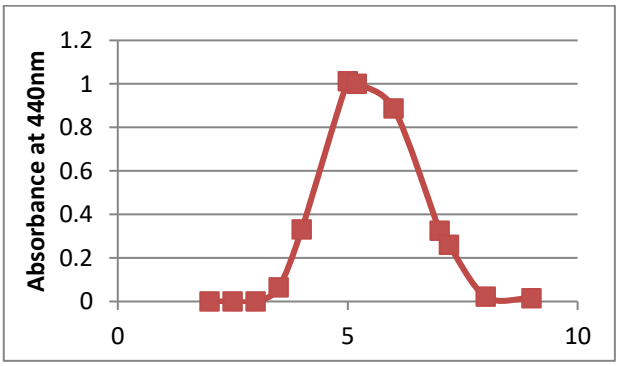
(b)

Figure 3: Effect of temperature on a) activity and b) thermal stability of the enzyme β-glucosidase.

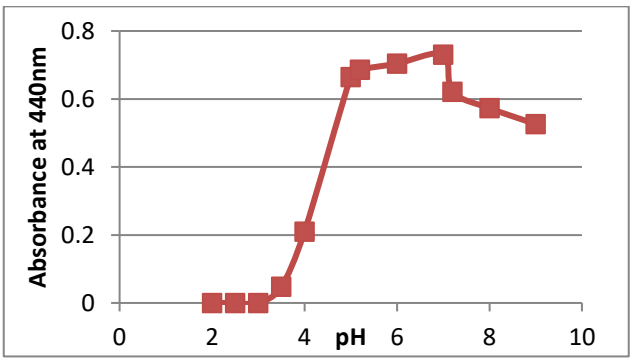
pH Optimum and Stability

β-glucosidase enzyme showed a maximum activity at pH pH 5.0 and showed stability from pH 5.0 to pH 7.0 (Fig 4 a & b). In case of rye, the glucosidase activity was precipitated when the pH of the crude enzyme extract was adjusted to 5.0, in contrast to maize and wheat glucosidase activities which remain in the supernatants[16,17]. The pIs of maize, wheat, and rye glucosidases were 5.2, 5.1–5.6 and 4.9–5.1, respectively.[16,17]

The optimum pH of the tamarind seed glucosidase were comparable to results reported for maize and wheat glucosidases but the optimum temperature was lower than that for the maize enzyme, 50^{O} C[16,17.]



(a)



(b)

Figure 4: Effect of pH on a) activity and b) pH stability of the enzyme β-glucosidase.

Km and Vmax

β-glucosidase exhibited Km value of 83 µM and Vmax value of 3.84 nmoles/min, respectively. The *V*max values of the rye glucosidase for DIBOA-Glc and DIMBOA-Glc were four to five times higher than those for lactam glucosides, HBOA-Glc and HMBOA-Glc, while the *K*m values were 1.4 to 1.7 times smaller. These results indicated that the *N*-4-hydroxy group is important for the exhibition of higher activity in the enzymes and it has a larger influence on the *V*max value than *K*m value, as has been shown for maize and wheat glucosidases[16]. On the other hand, the influence of 7-methoxy group on the rye glucosidase was different from that on the other two glucosidases.

Effect of Metal Ions

Large increase in β-glucosidase activity was observed in the presence of $MnCl_2$, $FeSO_4$, $AgNO_3$ and $HgCl_2$ (Fig 5) at 10mM concentration. The optimum concentration of $MnCl_2$, $FeSO_4$, $AgNO_3$ and $HgCl_2$ for maximum activation was 10mM. The addition of $CaCl_2$ and NaCl in concentrations of 10 and 1 mM caused gradual decrease in β-glucosidase activity. SDS resulted in a decrease in the activity of β-glucosidase.

Among the metal ions, Ag and Cu_2 were strong inhibitors although Cu_2 reportedly has no effect on the maize glucosidase[17].

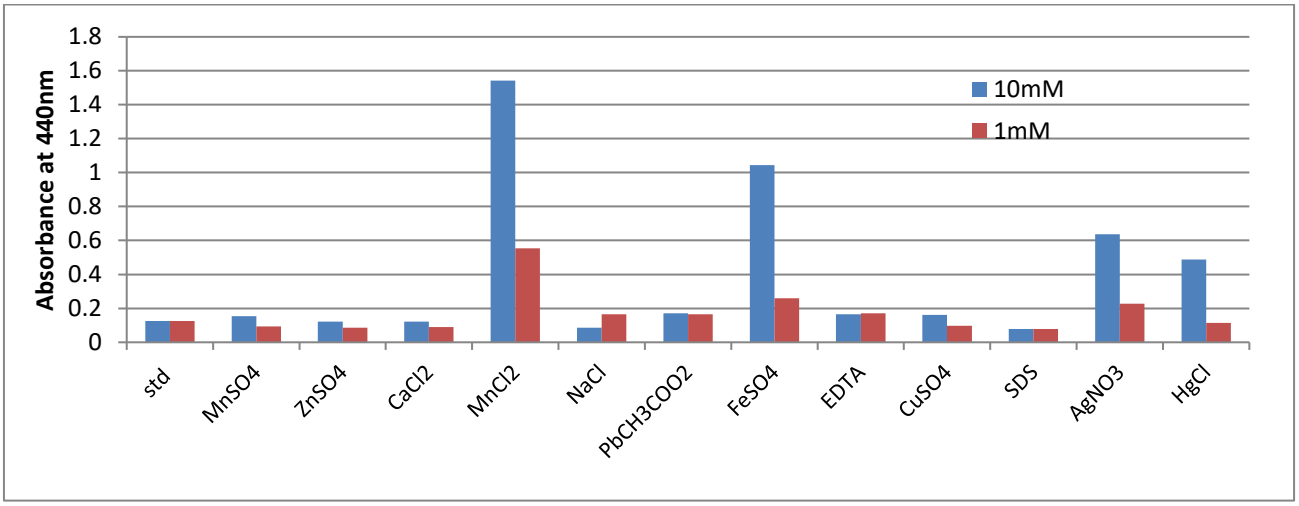


Figure 5: Effect of salt on activity of β-glucosidase

The kinetics of Tamarind seed β-glucosidase for the hydrolysis of p–nitrophenyl–β–D–glucoside are in similar order of magnitude to those of other plant enzyme.

CONCLUSION

In plants, β-glucosidases (EC 3.2.1.21) and related glycosidases play roles in many biological processes, including defense, lignification, phytohormone activation and cell-wall modification (Esen, 1993). Their physiological functions depend upon their location and substrate-specificity.

The enzyme β-glucosidases obtained from agricultural product such as the seeds of Tamarind is a stable enzyme and can be commercially exploited for the production of the enzyme. β-glucosidases of Tamarind seeds has been examined as a new source for producing these β-glucosidases, a member of glycosyl hydrolases.

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