Journal of Global Biosciences
ISSN 2320-1355
Volume 5, Number 4, 2016, pp. 3892-3901
Website: www.mutagens.co.in
E-mail: submit@mutagens.co.in
researchsubmission@hotmail.com

Research Paper

EXTRACTION AND PARTIAL PURIFICATION AND KINETIC STUDY OF BETA AMYLASE FROM SWEET POTATO

Manisha N.Chalse, Dipali.K.Suryawanshi, Rohini.B.Kharat , Tejeswini.S.Mundhe , Pooja.P.Shinde , Kirti.B.Jogdand and Rajeshwari .V.Rathod.

Abstract

Enzyme activity plays a key role in each stage of plant development. Beta amylase is a starch degrading enzyme which was extracted from Sweet potato and partially purified. Beta amylase is different from alpha amylase, Beta amylase act on starch molecule from non reducing end, it yields maltose after hydrolysis of starch. Sweet potato is a rich source of Beta amylase present in tuberous root of Sweet potato .Beta amylase was extracted and precipitated by ammonium sulphate salt and partially purified by dialysis bag. Beta conformation was determined by polarimeter. The optical rotation was observed over at time span past the achroicpoint. Rotation was found to be 0.045º which was different from alpha amylase. Thin layer chromatography was also performed by using glucose, alpha amylase, maltose, Beta amylase as sample spot. Kinetic study of Beta amylase was also performed. The optimum pH and temperature was found to be 5.4 and 60ºc.The Km and Vmax value for Beta amylase with soluble starch was found to be 5mg/ml and value of Vmax 1.5mg/ml. The enzyme activity calculated was found to be 112µg/ml/0.5ml The molecular weight of partially purified enzyme was 6.5KDa determined by Sodium Dodecyl Sulphate. Polyacrylamide gel electrophoresis Beta amylase is the most common enzyme for industrial purpose.

Key words: Sweet potato, Beta amylase, Extraction, partial purification, kinetic study.

INTRODUCTION

Sweet potato, Botanical Name- *lpomoeabatatas*, is a dicotyledonous plant that belong to the family convolvulaceae .It is a perennial; plant mainly grown as an annual. This species (*lpomoeabatatas*) was first described by Linnaeus,(1) as convolvulus batatas. However Lamarck (2)classified this species within the genus *lpomoea.* Sweet potato has skin colour runs from pink to dark red. there are 400 varieties of sweet potato. There are two different varieties of Sweet potato, firm and soft. Sweet potato is nutritious and provides energy and appreciable amount of vitamins and minerals.

Approximately 80-90% of the dry matter is made up of carbohydrates which consists mainly of starch and sugars. Sweet potato are cultivated wherever there is enough water to support their growth. optimal rainfall for growth range between 750and 2000mm. The sweet potato is a warm season growing plant annually requiring 20-25˚C average temperature and full sunlight for optimal development. Sweet potato tubers are mainly an energy source due to their high carbohydrates content which account for 80-90% of the dry weight. Carbohydrates consists of starch, sugar, small amount of pectins , hemicelluloses and cellulose. Starch is the

main carbohydrates and is very resistant to amylase hydrolysis. Tuber are a poor protein source, as they contain about 4% of crude protein they have low content of fiber, fat.
Sweet potato got over 400% of our daily need for vitamin A in one medium spud as well as loads of fiber and potassium. Many studies have suggested that the increasing consumption of plant foods like Sweet potato decreases the risk of obesity, diabetes, heart disease and healthy all mortality while promoting a healthy complexion, increased energy and overall lower weight V.A.Prassana,(3).Sweet potatoes are considered low on the glycemic index scale and recent research suggests they may reduce episodes of low blood sugar and insulin resistance in people with diabetes. Sweet potato roots contained high amount of extractable amylolytic enzyme and use the outer tissue extract to hydrolyze starch in Sweet potato mashes. In addition to high starch content Sweet potato roots have a high content of `Amylase`. The native amylase have important and well documented influence in Sweet potato storage and processing. Amylase play an important role in digestion of starch and the combined action of α-and β-amylase is more effective than the action of α –β amylase alone. Starch is an important industrial compound and its by product have extensive uses in industries such as of food,pharmaceutics and other J.F.Carlos(4).

Amylase:
Amylase is a general term for several different enzymes that hydrolyse starch also called a Diastase. Amylase found in both plants and animals..
Amylase hydrolyzes starch , glycogen and dextrin to form in all three instance glucose, maltose and the limit-dextrins. All amylase are glycoside hydrolyses and act on α-1,4-glycosidic bonds. Amylase is involved in anti-inflammatory reaction such as those caused by release of histidine and similar substances.

Alpha- Amylase:
The α-Amylase alternative names:1,4-α-D-glucan glucanohydrolase; glycogenase. α-amylase tends to be faster acting than β- amylase. α- amylase also found in plant, animal and fungi. Optimal P^H of α amylase is -6.7 to 7.0. α- amylase breaks long chain of carbohydrates ultimately yielding maltotriose or maltose ,glucose and limit dextrin from amylopectin. α-amylase were named by alpha-amylase is present in the outer layer of root only.(5).

Beta- Amylase(EC.3.2.1.2)
Another form of amylase is β-Amylase alternative names :1,4-α-D-glocanmaltohydrolase,glycogenase; saccharogen amylase. β-amylase is an exoenzyme It is also synthesized by bacteria, fungi, and plants.(6). Animal tissue do not contain β- amylase. Optimum p^H of β-amylase is 5.2-5.4. β- amylase catalyzes the hydrolysis of the second Alpha –1,4 glycosidic bond, cleaving off two glucose units (maltose) at a time. Beta amylase was first purified in the seeds of higher plants and Sweet potatoes. It yields a single product maltose. Tuberous root of sweet potato is rich in the Beta amylase enzyme, accounting for approximately 37 % of the total soluble proteins. In contrast other tuberous roots only contain trace amount of β- amylase activity.
The gene that encodes for β-amylase is B.A1404 base pair open reading frame encodes for 499 a.a precursor to β-amylase subunit. Beta- amylase cut starch from non-reducing end. Beta amylase is distributed throught the root.Beta amylase is thermostable enzyme and can be employed for industrial purpose(7) .Most Beta amylases are monomeric enzyme. However that of Sweet potato is tetrameric consists of four identical subunit each subunit globular region is formed by long loops extending from Beta stands.

MATERIALS:
All chemicals used for this research work are from High Media and S.D.Fine, which are available in our laboratory.
High media –Ammonium sulphate,3,5,dinitrosalysilic acid sodium hydroxide, Starch, Maltose, Copper sulphate Distase, Zinc sulphate ,Gram's Iodine, Acyral amide ,Bis acryl amide.

S.D.Fine –Glycerol, Tris HCl, Isopropanol, Coomassie brilliant blue.

METHODOLOGY:

Extraction of crude enzyme (8).

Two hundred and fifty grams of clean Sweet potatoes were taken and peeled the skin off. Sweet potatoes were cut into small pieces and transferred into blender and grinded for ten minutes with 40ml of cold 20mM sodium phosphate buffer saline. Grinded material was filtered with muslin cloth. Filtered material was transferred into centrifuged tubes. Filtered material was centrifuged at 12000 rpm for 20 min at 4°C. After centrifugation supernatant was collected and pellets were discarded. This procedure was repeated for two times.

Partial purification of crude enzyme:- (9)

Ammonium sulphate (0.47g/ml) was added in supernatant and kept it for overnight. Next day again solution was centrifuged at 12000rpm. for 20 min. Supernatant was discarded and pellets were suspended in 20 mM phosphate buffer. This collected solution was kept in an activated dialysis membrane for one day. Dialysis bag was kept in sucrose solution for one hour. Enzyme was collected from dialysis bag.Test and assays were performed to check enzyme activity.

Starch hydrolysis confirmatory test:

Partially purified enzyme was confirmed by the presence of Beta amylase after overnight incubation on starch agar plate a clear zone of hydrolysis was observed on addition of iodine solution..

Optimization of enzyme parameter

Effect of pH on Beta amylase:

Beta amylase activity from the dialysed samples were determined using 1% soluble starch as substrate at pH range from 5.2 to 6.2 using 20mM phosphate buffer, the optium pH for enzyme activity was determined using DNSA reagent.

Effect of temperature on Beta amylase:

A temperature gradient was employed in order to determine the Beta amylase activity. Substrate solution, buffer and dialyzed samples were incubated at different temperature between 30ºc to 80ºc.The optimum temperature was determined using DNSA reagent for enzyme activity.

Effect of metal ions on Beta amylase activity:

The effect of metal ions on the activity of Beta amylase were determined by adding known concentration of metal ion such as $CaCl_2$,$HgCl_2$,$CuCl_2$,$CuSO_4$ $MgCl_2$, $MnCl_2$, $CoCl_2$ $ZnCl_2$ $AlCl_2$ and KCl. Each of these metal ions were added to the enzyme substrate reaction and incubated for 10 min and its activation and inhibition property of each metal ion on enzyme activity was determined using DNSA reagent.

Effect of Activator on Beta Amylase Activity:

The effect of Activator on the activity of Beta amylase was determined by adding known concentration of activator $ZnSO_4$. This metal ions were added to the enzyme substrate reaction and incubated for 10 minutes. And its activation property of metal ions on enzyme activity was determined by using DNSA reagent.

Effect of Inhibitor on Beta Amylase Activity:

The effect of inhibitor on the activity of Beta amylase was determined by adding known concentration of inhibitor $CuSO_4$. This metal ion was added to the enzyme substrate reaction and incubated for 10 minutes. And its inhibition property of metal ions on enzyme activity was determined by using DNSA reagent.

Effect of substrate concentration on Beta amylase activity:
The enzyme activity of Beta amylase was determined by taking different concentration of soluble starch. The kinetic constant Km and Vmax also determined.

Optical rotation method
An optical rotation method developed by Chang(10) allow a quantitative evolution of Beta amylase activity. The method makes use of the change in optical rotation during enzymatic reaction and the activity unit of Beta-amylase is defined in terms of change of optical rotation versus time. The method can be used for Alpha- and Beta amylase. When plane polarized light is passed through a solution the rotation of the light in degree is found to be directly proportional to the number of asymmetric molecules of the compound through which the light passes. Therefore the rotation observed will be dependent on the nature of the asymmetric compound , the concentration of the compound in **Optical Rotation Method** solution ,and the length of the light path through the solution, The variation are related as in the following formula:

$$[\alpha]^{T}_{D} = \frac{[\alpha]_{obs}}{1 \times c}$$

Here [α] is the observed rotation , c is the concentration in g/ml, and 1 is the length of the light path in decimetres. The quantity is called the specific rotation at the temperature T When the D line of the sodium spectrum is used as the light source. Frequently this formula is written

$$[\alpha]^{T}_{D} = \frac{[\alpha]_{obs} \times 100}{1 \times C}$$

Where the unit of c are g/100 ml instead of g/ml
During mutarotation only alpha and beta forms exists and the reaction follows first order kinetics

Determination of Beta-amylase activity by Polarimetry:(13)
Polarimetry measurement was made with an ETL-NPL Automatic polarimeter.
The concentration of enzyme was chosen so that when 1 ml enzyme was mixed with 9 ml substrate at 30˚C, the achroic point would be observed within 15-20 minutes. An aliquot of enzyme and substrate was withdrawn and transferred to the polarimeter cell and the optical rotation measured at 30˚C. The optical rotation was observed over a time span past the achroic point. When starch was hydrolyzed it yield Beta-maltose and limit dextrin. Starch and limit dextrin have specific rotation.

Thin layer chromatography of beta amylase:
Thin layer chromatography of Beta amylase was performed by taking samples spot glucose, maltose ,alpha amylase and Beta amylase .Enzymes are spotted after degration of starch and Rf values were calculated.

Electrophoresis of beta amylase:
Partially purified Beta amylase was subjected to 12% polyacrylamide gel electrophoresis by using Biolab protein marker (2-212 kDa).

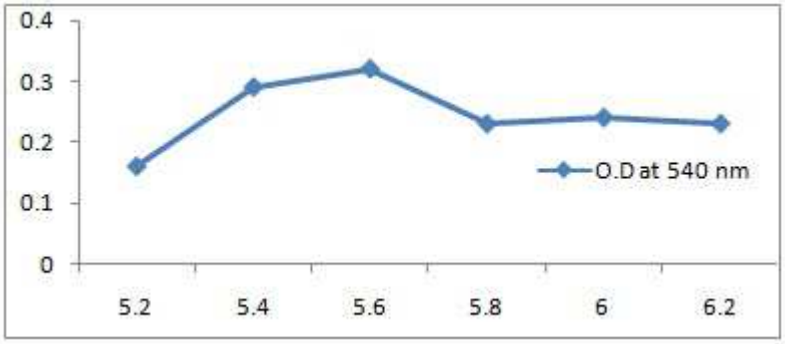
RESULTS
Extraction and partial purification of enzyme
Extraction and partial purification of enzyme was done by ammonium sulphate precipitation followed by dialysis.
Starch hydrolysis test-
Partially purified enzyme was confirmed by the presence of Beta amylase overnight incubation on starch agar plate a clear zone of hydrolysis was observed on addition of iodine solution a zone clearance was observed as shown in fig. 1.

Figure: 1(starch agar plate zone of hydrolysis)

Optimization of enzyme parameter

Effect of pH on Beta amylase activity:

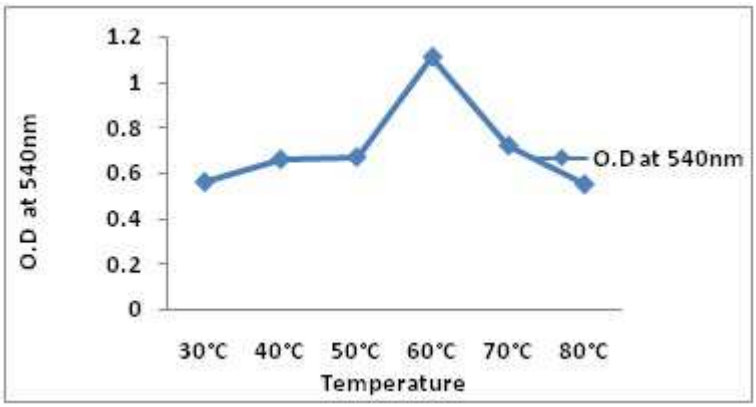
Different pH range were taken from 5.2 to 6.2 using 20mM phosphate buffer to find out optimum pH of Beta amylase ,for this assay was performed by taking 1%(2ml) starch, buffer solution(2.5ml) of different range and dialyzed enzyme (0.5ml).Incubated for 5 min. at room temperature and then reaction was inhibited by adding sodium hydroxide then from these tubes 0.5ml reaction mixture was taken and DNSA reagent was added and enzyme activity was determined by taking O.D. Beta amylase activity was found to be maximum at pH 5.6 so optimum pH of Beta amylase is 5.6.see graph no. 1.



Graph 1:-Effect of pH on Beta amylase activity

Effect of temperature on Beta amylase activity:

A temperature gradient was performed from 30^0c to 80^0 order to determine optimum temperature of Beta amylase assay was performed as mention in effect of pH. The activity of Beta amylase was found to be maximum at 60^0so the optimum temperature of Beta amylase is 60^0 see graph no. 2.



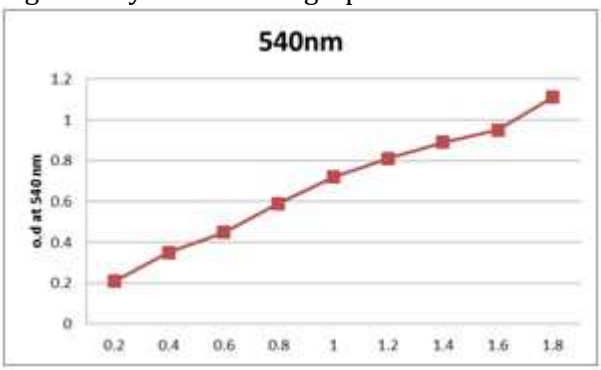
Graph 2:-Effect of temperature on Beta amylase activity

Effect of metal ion on Beta amylase activity(Activator and inhibitor):-

The effect of metal ions on the activity of Beta amylase were determined by adding known concentration of metal ions such as $Cacl_2$, Hgc_{l2}, Cuc_{l2}, $Cuso_4$, $Mgcl_2$, $Mncl_2$ $Cocl_2$, $Zncl_2$, $Alcl_2$, Kcl. Each of these metal ions were added to the enzyme subastrate reaction and incubated for 10min. and it's activation and inhibition of each metal ion on Beta amylase enzyme was determined using DNSA reagent.

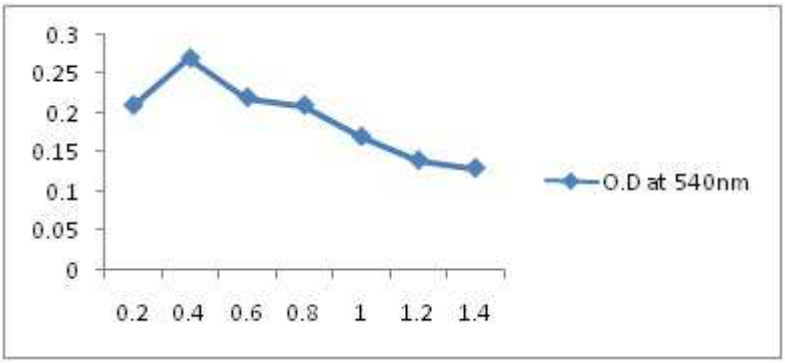
Effect of activator on Beta amylase enzyme(11)

The activator for Beta amylase was found to be $Znso_4$.This metal ions were added to the enzyme substrate reaction incubated for 10min.DNSA reagent was added and it was observed that enzyme activity increases gradually as shown in graph no.3.



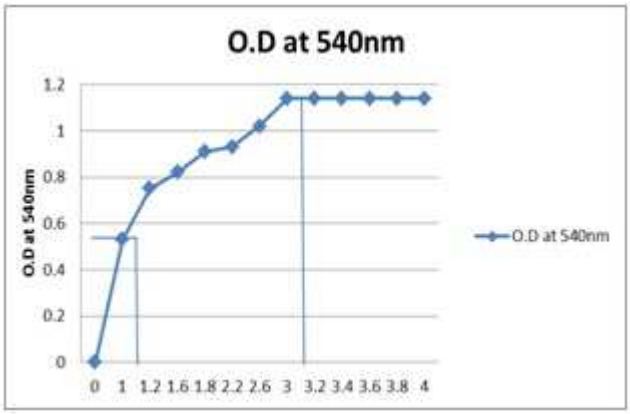
Effect of inhibitor on Beta amylase activity:-

The inhibitor for Beta amylase was found $Cuso_4$.This metal ions were added to the enzyme substrate reaction incubated for 10min. DNSA reagent was added and it was observed that enzyme activity decreases gradually as shown in graph no 4.



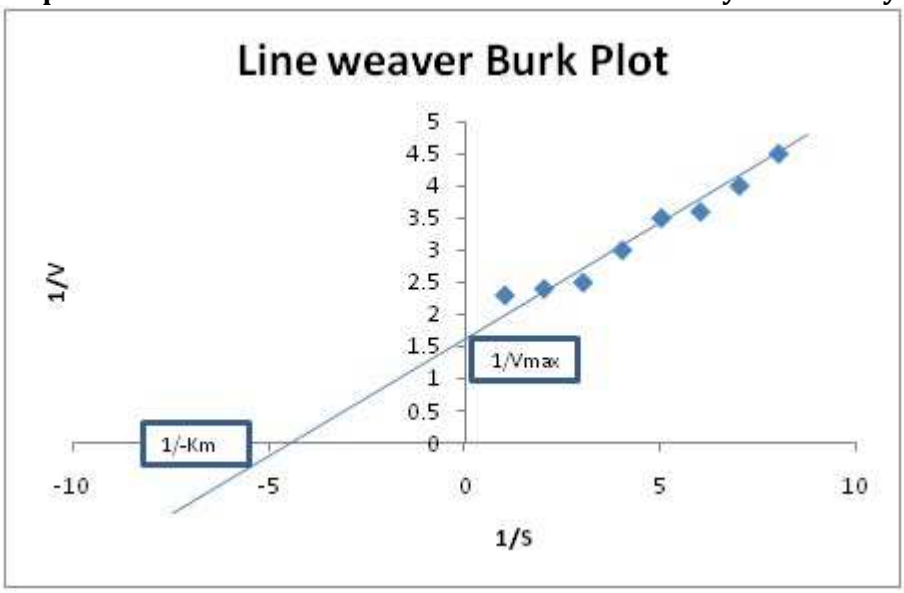
Effect of substrate concentration on Beta amylase activity and determination of Km and Vmax(12).

The enzyme activity of Beta amylase was determined by using different concentration of soluble starch as substrate. The kinetic constant Km and Vmax was determined using Line weaver Burk plot the value of Km was found 5mg/ml and value of Vmax was 1.5mg/ml as shown in graph no.5 and 6.



Concentration of substrate (starch)

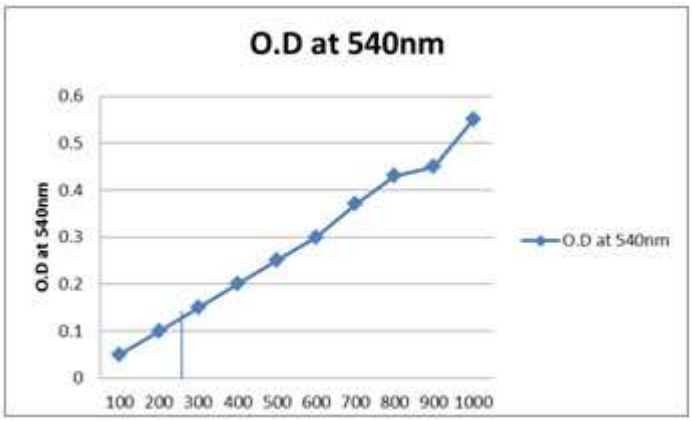
Graph 5:- Effect of substrate concentration on Beta amylase activity.



Graph 6:- Line weaver Burk Plot

Enzyme activity of Beta amylase was also determined by Maltose assay.

Enzyme activity of partially purified Beta amylase from sweet potato was determined using Maltose assay. It was found to be 0.28 IU.(112µg/ml/0.5ml.).

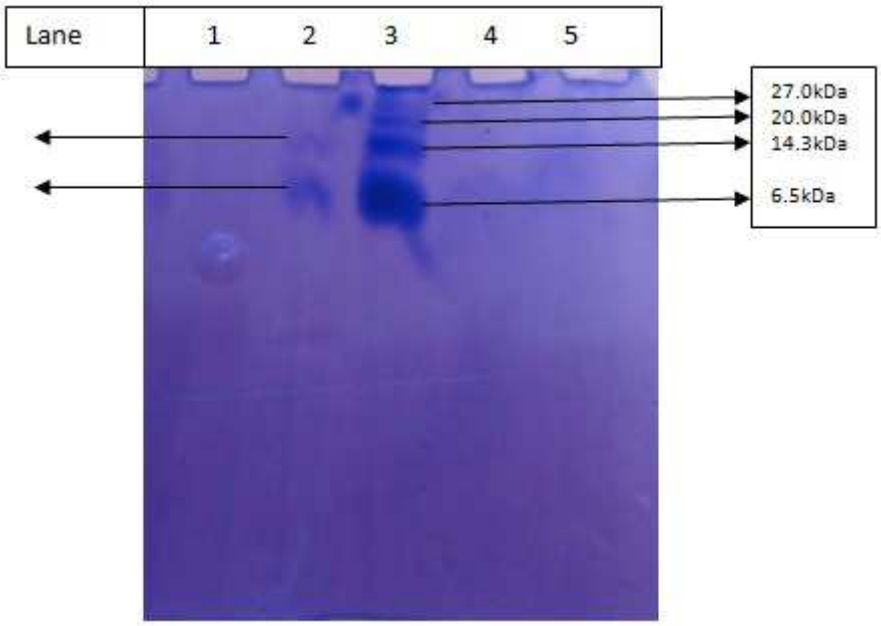


Determination of Beta amylase activity by polarimetry (10):
The observed optical rotation for Beta amylase was 0.045◦ This rotation is different for alpha amylase because it degrades starch into glucose. Whereas Beta amylase degrades starch into maltose. Therefore it indicates the difference between alpha amylase and Beta amylase.

Thin layer chromatography of Beta amylase:-
Thin layer chromatography was performed by using sample spots glucose, alpha amylase, maltose and Beta amylase .Enzymes are spotted after degradation of starch. It is observed that glucose spots matches to alpha amylase and maltose spot matches to Beta amylase. Rf values for separated samples spots are 0.19, 0.20, 0.11, 0.12 which is approximately similar to standard values as shown in fig.2.

Fig.2: TLC of beta amylase

Determination of molecular weight of Beta amylase:-
The partially purified Beta amylase was subjected to determination of molecular weight by using 12% PAGE and compared with standard protein marker. It shows 6.5kDa as shown in fig.3.



Lane 2: partially purified β amylase; Lane3: Molecular Protein Marker

DISCUSSION

The amylase, which is an important enzyme employed in the starch processing industries for the hydrolysis of polysaccharide(17).Amylase has vast industrial applications. The enzymes in Sweet potato are affected by various factors such as pH, temperature and metal ions. The present investigation was carried on to check pH affect the activity of the β-amylase, whose activity is mostly high in acidic which support the previous studies.,(3). The most important factor is the temperature, which is essential and also affects the enzyme activity.

Our studies revealed that β-amylase are highly active at 60˚C which justifies from the work by Caraban A,(15).It has also been reported that Beta amylase is active at 70˚C this agrees with the results obtained in the present study. Beyond this range there is decline in the activity because of the Structural unfolding trasition at high temperature(4).

Various metal ions play a major role either as activator or an inhibitor for enzyme activity. $ZnSO_4$ enhanced enzyme activity. This is in line with the findings of Kimura,A, (12) and V.A.Prassana (3). Most of the findings have shown that $CuSO_4$ is a potent inhibitor for enzyme activity and, reports the inhibitory activity by Cu^{+2} in microbial amylase which coincides with our present finding. As per the studies on Beta amylase in plants.M.Boldon,et,al.(6).

And the reports by J.Beckord (16)closely relates with our present findings but doesn't supports the findings of, enzyme activity is also based on the substrate concentration (Km) and its maximum velocity (Vmax), the result obtained in the present research closely relates with the work on kinetic study by A.Pandey(21) .And this also supports with the work by G.Sani(17). Low Km activity indicates high affinity of the enzyme for the substrate(14).Based on the present work, it can be speculated that β-amylase can be employed for industrial purpose.

CONCLUSION

In this present study Beta-amylase was extracted from sweet potato and partially purified, kinetic properties of Beta- amylase also studied. Beta amylase activity was also determined by polarimeter. It was also visualised by PAGE and the molecular weight of Beta amylase was found 6.5KDa. This Beta-amylase enzyme was applied to hydrolyzed corn starch.

Sweet potato a extract could be used of a low cost for the estimation anddetermination of starch in food stuffes in place of important amylolytic enzyme. More research and development is still required to refine the extract and homogeneity of Sweet potato amylolytic extract.

ACKNOWLEDGEMENTS:

We thank Mrs. Savita A. Kate, Dr. Preetinanda M. Dash, Dr. Vaishali U. Dange, Ms. Farha Quadri for their kind support during preparation of manuscript. We are grateful to Principal, MSP Mandals' Shivchhatrapati College for providing necessary facilities for the said work.

REFERENCES :

[1] Linnaeus,Exploration,Maitenance and utilization of Sweet potato(1753) The Sweet potato was described as convolvulus batatas by Linnaeus.
[2] Lamarck,Exploration Maintance and cllsification of Sweet potato(1791) the botanist Lamarck described it as *lpomoea*
[3] V. A. Prassana, Amylases and their applications. *Journal of Biotechnology 4(13)*, 2005, 1525-152
[4] J.F.Carlos,purification and characterization of an extracellular amylase from *Lactobacillus plantarum* strain AbJournal of Applied Bacteriology 7,2000,276-287.
[5]. Hill, G.A., Macdonald, D.G. and Land, X., (1977) Alfa amylase inhibition and inactivation in barley malt during cold starch hydrolysis, Biotechologlogy Letters, 19 (11), pp. 1139-1141
[6] M.Boldon, and p.Effront,Microbial amylolytic enzymes.Critical Riview Biochemistry Molecular Biology 24,2000,329-418.
[7]. Hill, G.A., Macdonald, D.G. and Land, X., (1977) Alfa amylase inhibition and inactivation in barley malt during cold starch hydrolysis, Biotechologlogy Letters, 19 (11), pp. 1139-1141
[8] O. H. Lowry , N.J. Rosebrough, A.L. Farr and R.L. Randall, Protein measurement with folin phenol reagent. *Journal of Biology and Chemistry 193,* 1951, 265-273.
[9] O.O.Olaniyi,B.J. Akinyele and D.J.Arotupin, purification and characterization of Beta amylase from *Volvariella volvacea.*Nigerian Journal of Microbilogy 24(1),2010,1976-1982.
[10] Chang A. ,optical rotation of alpha and Beta amylase from barly plant (1923)
[11] T. Rani, T. Takagi, and H. Toda, Bacterial *and mold amylases*. *In* Boyer, P. (ed.). The enzymes. Academic Press Inc., New York, 2007, 235-271
[12] Kimura,A.Robyt,J.F.(1995) Reaction of enzymes with starch granules kinetics and products of the reaction with glucoamylase.CarbohydrateRes.277,87-107.
[13] W.D.Mariae Hain, assay of beta amylase in malt by optical rotation, kansar state university(1970).
[14] Cărăban A, Stănăsel O, Gavriş G, Badea G, (2008), Studies about the influence of some food lipids over the starch hydrolysis reaction using laser interferometry technics, Proceedings of Indian Conference, Impending approaches to Environmental Menace (IMAEM) 2008 TBML College, Tamil Nadu, 25-26th September, South-INDIA, pp.191-193.
[15] Cărăban A., Bungău S, Fodor A, Stănăşel O., (2006), Influenţa acidului ascorbic, tiaminei şi riboflavinei asupra reacţiei de hidroliză a amidonului cu amilaze endogene, utilizând interferometria laser, Revista de Chimie, 57, pp 607-609
[16] J.Beckord,Selection of microorganism which produce raw starch degrading amylase. Applied Microbiology and Biotechnology 27,2005,443-446.
[17] G. Sanni, Thermostable amylolytic enzymes from a new *Clostridium* isolate. Applied and Environmental Microbiology 53, 2003
[18] R. R. Ray, Purification and characterization of extracellular β – amylase of *Bacillus megaterium* B (6). *Acta Microbiology and Immunology 47*, 2000, 29-40.
[19] P. Bernfield, Amylases and methods. *Enzymology 1,* 1955, 147-154.
[20] A. T. Martinez, Molecular biology and structural function of lignin degrading heme peroxidases. *Enzyme and Microbial*
[21] A. Pandey, P. Nigam, C.R. Soccol, V. T. Soccol, D. Singh, and R. Moham, Advances in microbial amylases. *Biotechnology and Applied Biochemistry 31,* 2000, 135-152.