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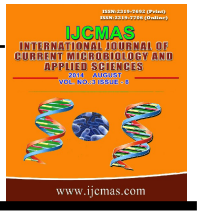

Original Research Article

Microbial Production and Characterization of Dextranase

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K e y w o r d s

Dextranase;
Dextran;
Microorganism;
Production;
Cultivation;
Characterization

A B S T R A C T

In sugar production, dextrans (undesirable compounds) synthesized by contaminant microorganism from sucrose. Formation of dextran increases the viscosity of the flow, reduces industrial recovery, and causes significant losses. The use of dextranase is the most efficient method for hydrolyzing dextrans at sugar mills. Some bacterial, fungal and yeast strains have been studied to produce dextranase. *Penicillium aculeatum NRRL-896* showed the highest production rate after 7 days of incubation. The highly purified enzyme was simply separated and purified by sequential DEAE-Sepharose and Sephadex G-100 columns, 42.8 fold with 14.78 yield.The enzyme exhibited an apparent molecular weight of 66,200 Da, a temperature optimum of 45 ˚C and a pH optimum of 4.5.The enzyme was extremely stable for 60 min at 35-45 ˚C. The K_m value of the purified dextranase was 52.13±0.02 mg ml^{-1}indicating that the enzyme had a very high specificity for dextran.

Introduction

It is well known that the presence of *Leuconostoc mesenteroides* and *Leuconostoc* species in sugarcane juice in sugar factories causes loss of sucrose and formation of jelly like material called dextran (a bacterial polysaccharide, which is polymer of glucose). The polysaccharide dextran interferes in sugar manufacturing process in addition to the loss of sucrose (Priyanka and Santosh, 2011).

The problems caused, the loss of sucrose, increase in viscosity of process syrups, and poor recovery of sucrose due to inhibition of crystallization.

Many investigations suggested a minimization of dextran levels in the sugar factory by different methods even with the chemical additions. More recent approaches propose the application of dextranase for removal of performed dextran. However, theses approaches were uneconomical due to high costs (Jimênez, 2005) or not practical because the effect of temperature on the activity of dextranases, (Eggleston and Monge, 2005 and Eggleston *et al*., 2006). It was found that enzymatic method does not interfere with the sugar manufacturing process (Tilbury and French 1974). This enzymatic method is quick, easy and can be practiced at commercial scale.

Enzymatic hydrolysis of dextran from sugar solutions could reduce the molecular mass and therefore the viscosity of juices (Cheetham *et al.,* 1991, Cuddihy *et al.,* 1999, Inkerman and Riddell, 1977, Madhu *et al.,* 1984, and Matheson, 1980).

Thus for improving the production performance, the removal of dextran is essential. Generally dextran levels present in mixed juice vary from 300 to 600 mg/l. The use of dextranase produced by microbial sources (i.e. bacteria, moulds like *Penicillium* and *Aspergillus* sp., and yeast) for removal of such polysaccharide is of great importance. Dextranase [(1-6) α-D-glucan, 6-glucanohydrolase or glucanase (E.C. 3.2.1.11) is specifically hydrolyze the α-(1-6) linkages in dextran. Since many dextrans contain a relatively high concentration of secondary linkages, other than α-(1-6), and enzyme that can break (1-2), (1-3) and (1-4) linkages in dextran, is also included together with true dextranase. The hydrolysis products of dextran by dextranase are glucose (with exodextranase), isomaltose and isomalto-oligosaccharides. Therefore, these enzymes are commonly called glucanases (E.C. 3.2.1.11) (Priyanka and Santosh, 2011).

One of the major industrial applications of dextranase is the reduction of slimes in sugar production processes. The growth of *Leuconostoc mesenteroides* and *Leuconostoc* species is the most important factor in the deterioration of cane sugar and frost-damaged beet sugar (Brown & Inkerman, 1992, Bucke *et al.,* 2001, Riffer, 1982, Singlenton *et al.,* 2001, and Steels *et al.,* 2001). The use of dextranase in sugar mills not only improves the factory performance but also the sugar quality (Fulcher and Inkerman 1974).

Tilbury (1971) removed about 68% of dextran in 20 minutes by using dextranase. The enzyme could be used without changing the pH of juice and at temperature of 50 ˚C. The excessive elongation of crystal and viscosity of syrups and molasses may be reduced by the enzymatic decomposition of dextran (Hidi, 1975).

The present investigation was carried out to produce dextranase by using different strains of Penicillium (*P. funiculosum NRRL-6014* and *P. aculeatum NRRL-896*), *Bacillus subtilus M-15*, *Leconostoc dextranicus B-512 FM*, *Saccharomyces cerevisiae YSF-5* and *Lipomyces starkyi ATCC-12659*. The characteristics of the produced dextranase were also determined.

Materials and Methods

Different microbial strains i.e. *Penicillium funiculosum* NRRL-6014, *Penicillium aculeatum* NRRL-896, *Bacillus subtilus* M-15, *Leconostoc dextranicus* B-512 FM, *Saccharomyces cerevisiae* YSF-5 and *Lipomyces starkyi* ATCC-12659 were obtained from the Microbiological Resource center "MIRCEN", Faculty of Agriculture, Ain-Shams University and the culture collections of the National Research Center were used to produce enzyme dextranase. Sephadex G-100 chromatography, DEAE-Sepharose chromatography, ammonium sulphate, dextran were purchased from Sigma.

Methods

Cultivation media

The basal medium used for the cultivation for the six different cultures (potato dextrose agar, peptone-yeast, Difco Bacto agar, sources of carbon, KH2PO4, $(NH_4)_2SO_4$, $MgSO_4.7H_2O$ and $FeSO_4$).

Production of Crude Dextranase from Different Microbial Strains

Yeast

The yeast strains *Saccharomyces cerevisiae YSF-5* and *Lipomyces starkyi ATCC-12659* were cultivated first on media (described as above), then obtained from the cultivation medium and submerged in enriched production medium composed of (g/l): KH_2PO_4 (2.5), $(NH_4)_2SO_4$ (5.0), $MgSO_4.7H_2O$ (0.1), $MgSO_4$ (0.1) and $NaCl_2$ (0.1); the pH of the medium was adjusted to 7.0 with KOH, and 1 % dextran (MW 40000) was added as described by David and Day (1988). Ten conical flasks (250 ml) each containing 25 ml medium were shaked and incubated at 30 ˚C for 7 days. After about a week a silky, yellow growth could just be observed in the flasks. Inoculate from the culture were spread on dextran agar medium (same as in the flasks plus 1.5 % w/v, Difco Bacto Agar). Yellow colonies developed within two days, and several transfers were performed with isolated the colonies and preserved as lyophilized ampoules stored at -20 ˚C.

The growth experiments were performed in conical flasks (250 ml) incubated at 30 ˚C for 12 days on rotator shakers. The volume of the medium was adjusted to a depth of a few millimeters to ensure proper aeration. The medium (submerged medium) described above was used, and the sources of carbon as dextran with other compounds were added as sterile solutions.

Growth was followed quantitatively by measuring the turbidity of culture samples in a spectrophotometer at 540 nm.

Fungal Strains

The strains of *Penicillium funiculosum NRRL-6014* and *Penicillium aculeatum NRRL-896* were obtained from the cultivation medium and transferred to production medium containing (g/l): Glucose (30); peptone (5); K_2HPO_4 (1); $Mg_2SO_4.7H_20$ (0.5), and yeast extract (0.5). The pH was adjusted to 6.0 and this was considered as a complete medium (CM) but the minimal medium (MM) had the following ingredient (g/l): $NaNO_3$ (3); KCL (0.5); $MgSO_4.7H_2O$ (0.5); $FeSO_4.7H_2O$ (0.01); KH_2PO_4 (1); glucose (40); agar (20) and the pH was adjusted to 6.8.

Spore suspension was prepared in saline solution (0.85 % NaCl) from slant of 10 days old. These plates were incubated for three days at 29 ˚C and then single colonies were separated to complete media (CM) as described by Mohamed *et al.,* (2007).

Bacterial Strains

The *Bacillus subtilus* M-15 strain was cultivated on a Petri dishes culture medium composed of (w/v): corn steep liquor (0.3%); peptone (0.3%); yeast extract (0.3%); agar (20); 0.2% K_2HPO_4 and 0.2% KH_2PO_4, to increased the culture cells, the pH of the medium was then adjusted to 7.5 and incubated for 7 days at 37˚C. The growth white halo cells (colonies) on surface medium were obtained, according to **Elvira *et al.*, (2003)**, and then submerged in a production medium composed of (w/v): dextran (0.5%), K_2HPO_4 (0.1%) and KH_2PO_4 (0.15%), the pH of the medium was adjusted to 4.5, and 0.3 g soil sample was added, and then 5 ml of the submerged culture was transfered to 20 ml test tube and incubated for 12 days at 37 ˚C.

The dextranase production medium for *Leconostoc dextranicus B-512 FM* strain was composed of (g/l): $NaNO_3$ (2), KH_2PO_4 (2), $CaCl_2.H_2O$ (0.3), $MgSO_4.H_2O$ (0.3), then supplemented with locust bean gum,

corn steep solids, yeast extract, potato dextrose agar, and the pH was adjusted to 5.5 (Alberto and Owen, 1991).

Dextranase Activity

Dextranase activity was assayed by a modification method of Webb and Spencer-Martin (1983) as follows: reaction mixture containing 2 ml of 2.5 % dextran in acetate buffer (0.05 M, pH 5.0) and 10 µl of enzyme in a total volume of 3 ml was incubated at 50 ˚C for 10 min. Reaction was stopped by adding 2 ml of 3,5-dinitrosalicylic acid color reagent and read at A_{540}. One unit of dextranase is defined as the amount of enzyme which liberates one µmole of isomaltose (measurement as maltose) in 1 min under the described conditions.

Protein Determination

Protein was determined according to the method described by Lowry *et al.* (1951).

Dextranase Extraction & Purification

The enzyme in the cell-free culture was extracted by ammonium sulphate, then purified using gel filtration on DEAE-sepharose and Sephadex G-100 columns according to the method described by Elvira *et al.*, (2003). The fractions containing substantial dextranase activity were collected (Fig. 1).

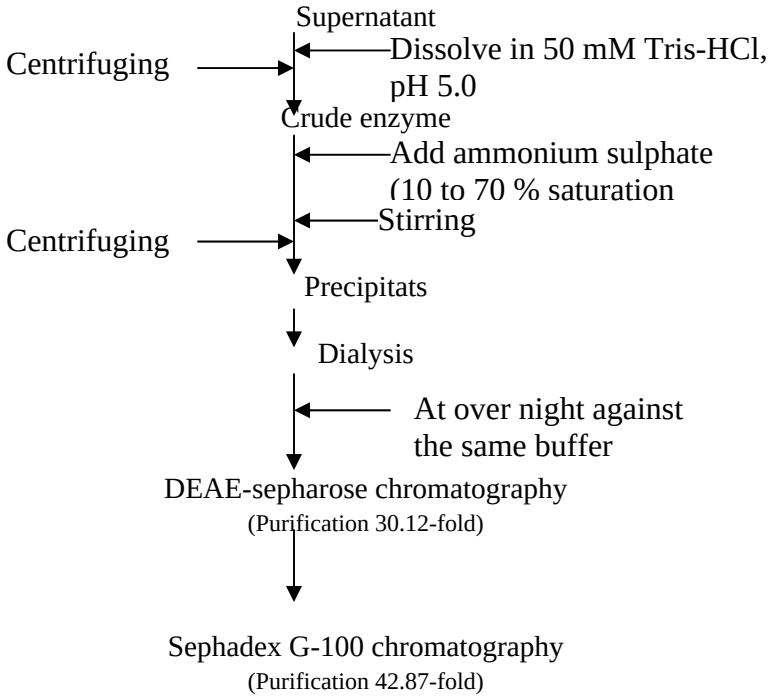


Fig.1 Extraction and purification steps of dextranase from *Penicillium aculeatum NRRL-896*

Characteristics of the Purified Dextranase:

Optimum pH & Temperature of dextranase:

Optimum temperature and pH were determined by changing separately the conditions of activity assays: pH from 3 to 8; temperature from 5 to 70 ˚C; and dextran concentrations 2.0 % (w/v). Buffer solutions of potassium phosphate pH from 3.5 to 5.5 were used according to the method described by Yakup and Aziz (2007).

Determination of thermostability of dextranase:

Dextranase (159.31 units) was incubated in calcium acetate buffer (pH 4.5) at temperature from 5 to 70 ˚C for 60 min and the remaining activity was determined, using standard activity assay method described by Yakup and Aziz (2007).

Determination of pH stability of dextranase:

Dextranase (159.31 units) was incubated in various buffers pH (3.0 – 8.0) at optimum temperature (37 ˚C) for 60 min and the remaining activity was determined, using standard activity assay method described by Yakup and Aziz (2007).

Determination of (K_m & V_{max}) of dextranase:

The enzyme reaction velocities against different substrate concentration were carried out according to Rogalski *et al.,* (1997). Both K_m and V_{max} were calculated by using the saturation curve, Lineweaver-Burk plots, and Hans-Plots.

$$\frac{1}{V} = \frac{K_m \times 1}{V \quad S} + \frac{1}{V}$$

Where: K_m: Michales constant; V_{max}: Maximum velocity; V: Initial rate of reaction; S: substrate concetration

Molecular Weight determine of Dextranase:

The purified dextranase was separated by using Sodium Dodecyle Sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method described by Hames and Richwood (1990). The mixture of molecular-weight standards (mol. Wt) were used as Myosin (200 KDa), β-galactosidase (116 KDa), phosphorylase *b* (97.4 KDa), Serum albumin (66.0 KDa), Ov-albumin (45.0 KDa), Carbonic anhydrase (31.0 KDa), Trypsin inhibitor (21.5 KDa), Lysozyme (14.4 KDa) and Ribo-nuclease (13.3 KDa).

Results and Discussion

Extraction and purification of dextranase from different microbial strains:

Extraction of dextranase:

Among 6 microbial cultures investigated for the production of dextranase with potential industrial applications, only *P. aculeatum* was selected as the best dextranase producer. The six cultures were grown on the basal medium for different incubation periods i.e. 2, 4, 7, 10 and 12 days (Table 1). The NRRL 896 fungal strain *P. aculeatum* showed the highest enzyme yield (101250.00 unit100ml^{-1} culture filtrate) after 7 days. It was therefore selected for further investigation.

Incubation time

The time course of biomass and dextranase production by microbial strains in basal medium is shown in (Fig. 2). Maximum dextranase activity was reached after 10, 10 and 7 days of incubation period for *S. cerevisiae*, *L. dextranicus* and *P. aculeatum* strains, respectively; thereafter the enzyme activity declined. Accordingly, the highest fungal biomass production was at 7^{th} days (Fig 2) for *P. aculeatum*, after which, there was no more increase in the biomass.

These results show that reaching the maximum cell mass is important for the dextranase production from *P. aculeatum* (101250.00 units) compared to either yeast or bacterial strains.

The *Penicillium aculeatum* dextranase showed 1981.22 unit/mg protein as specific activity.

Effect of different carbon and nitrogen sources on the production of dextranase

Effect of different carbon sources

The effect of different carbon sources that substituted dextrans (M.W. 40,000 Dalton) on dextranase activity in the basal medium was investigated (Table 2). Results showed that dextrin, starch, glucose and lactose caused a reduction in the enzyme activity.

Maximal yield of dextranase (99758.24 unit/100ml) was obtained with dextran at 3.5% as a source of carbon. This value is the highest among all tested carbon sources.

These results are in agreement with that obtained by Priyanka and Santosh (2011) & Mohamed *et al.,* (1999).

Effect of different nitrogen sources

Effect of nitrogen sources on the production of dextranase from *P. aculeatum* was shown in Table (3).

Results indicated that using a combination of sodium nitrate and yeast extract as a source of nitrogen produced the highest activity (98751.65 units / 100ml) compared to other nitrogen sources.

On the other hand using ammonium sulphate gave the lowest level of enzyme activity (721.08 units/100ml). These results are similar to those reported by Mohamed *et al.,* (1999).

Extraction and Purification of Dextranase from *Penicillium aculeatum NRRL-896*

The outline of the purification procedures of *P. aculeatum* dextranase is presented in Table (4).

After, removal of the insoluble materials by centrifugation, the clear supernatant was loaded onto a DEAE-Sepharose column (Elvira *et al.,* 2003). The dextranase adsorbed was eluted by a linear gradient system of NaCl (0.21 M). The dextranase fraction at (fraction number 43) gave a single active peak (Fig. 3) that had the maximum enzyme.

The dextranase active peaks (D&E) were collected, saturated and applied to Sephadex G-100 column and also one active dextranase peak was formed and collected symmetrical at fraction number 43, Fig. (4).

The dextranase [1-6-α-D-glucan, 6-glucanohydrolase (E.C. 3.2.1.11)], synthesized by some molds and bacteria, is great important in the sugar industry. It is capable of degrading the biopolymer dextran

formed in the cane juice during the production of sugar.

It could be concluded that from the six examined microbial strains, *P. aculeatum* was chosen to produce the highest activity of dextranase with satisfactory level of 14.78, high purity 42.87 fold, and for subsequent studies.

Characterization of Dextranase Purified from *Penicillium aculeatum NRRL-896*

Determination of Optimum Temperature: Results in (Fig. 5) showed that the highest activity of dextranase occurred at 45 ˚C, and a sharp decrease in activity was observed between 45 and 70 ˚C.

Determination of Optimum pH

Quantitative dextranase assays were performed for each pH value (Fig. 6), and the greatest activity was found at pH 4.5. A sharp drop in activity was recorded between pH 5 to 8.

These results are in agreement with Shamsolahrar *et al.,* (2002) who indicated that the optimum pH of *Penicillium lilacinum* dextranase was 4.5.

Determination of Thermostability of Dextranase

Thermal stability is perhaps the most frequently encountered and most thoroughly investigated mode of enzyme inactivation. Thermal stability was examined by incubation of the enzyme at different temperatures.

As shown in Fig. (7), the enzyme retains 96.3 % of its activity at temperatures up to 45 ˚C during 60 min incubation. The optimum temperature for the enzyme activity was in the range of 35-45 ˚C.

These results are in agreement with that reported by Elvira *et al.,* (2003) and Madhu and Prabhu (1984). It is obvious that the enzyme is stable within its active range and this determines the optimal conditions of the reaction. These results offer optimum conditions for industrial usage.

Determination of pH stability of dextranase

Inactivation and modification of enzymes due to extremes of pH is a common well documented phenomena in protein studies. The degree of inactivation can range from minor conformational changes to irreversible inactivation depending on the incubation conditions (Yakup and Aziz, 2007).

Therefore, for investigation of pH stability, the enzyme was incubated at various pH values in the range of 3.0 to 8.0 at 45 ˚C for 60 min and the activity was monitored. Figure (8), shows that the enzyme is stable at pH range of 4.5 to 6.5 which is close to isoelectric point of the enzyme found to be equal to 4.5. The optimum pH activity was found to be 4.5. This result is in agreement with that obtained by Mohamed *et al.,* (2007).

The effect of pH and temperature on the enzyme activity and stability was studied and the enzyme was stable at a pH range of 4.5 to 8.0, and at pH value of 4.5. Optimum temperature for the enzyme activity was also obtained in the range of 35–45 ˚C and the enzyme retains 96.3 % of its activity under temperature up to 45 ˚C in 60 min.

Determination of dextranase K_m and V_{max}

Michaelis constant and the maximum

velocity of the enzyme were calculated from Lineweaver-Burk plot by determining the initial velocities of enzymatic reactions at different concentrations of the substrate.

Results in (Fig. 9) showed that K_m and V_{max} were 52.13±0.02 mg ml^{-1} and 2.97±1.02µmole maltose, respectively, indicating highest affinity of dextranase to daxtran. The results were higher than these obtained by Yakup and Aziz (2007) and El-Tanash *et al.,* (2011).

Molecular-weight Estimations by SDS-PAGE

The purified fraction produced from *P. aculeatum* was electrophoresed on a Sodium Dodecyl Sulphate (SDS)-polyacrylpmide gel (10.0 % w/v), together with protein molecular weight markers, Fig. (11). A linear relationship of the logarithm of the protein molecular weight and the dextranase enzyme has been observed.

The produced dextranase fraction showed high specific activity and molecular weight of 66,200 Da (Fig. 10). This result is in agreement with that found by Abdel-Aziz *et al.,* (2007) for *P. funiculosum* being 67,000 Da.

Effect of Inhibitors on Dextranase Activity

Inhibition of enzyme within the juice could be one of the most important problems that might arise in industrial application. To deal with this problem, the effect of some inhibitors on enzyme activity was investigated (Shamsolahrar *et al.,* 2002).

According to the composition of sugarcane juice, most likely existing inhibitors, e.g., glucose, dextrin, sucrose, galactoronic acid, galactose, maltose, raffinose and pectin were considered in this study. Figures $(12)_a$ to $(12)_h$ present Lineweaver-Burk plot of different concentrations of inhibitors. It was deduced that all inhibitors have a competitive inhibition on the enzyme activity. Among the inhibitors studied, pectin and raffinose had a strong effect on the enzyme activity; they inhibited the enzyme completely over the concentration of 0.1 %. Sugiura and Ito (1974) and Shamsolahrar *et a*l., (2002), have reported the same results for dextranase produced by *Penicillium funiculosum*.

As shown in Figures $(12)_a$ to $(12)_h$, comparing the amount of k_m for substrate with the amount of k_i for each inhibitor, it was demonstrated that k_i of each inhibitor was much higher than k_m of substrate. Therefore, it was deduced that the inhibition was greatly increased at high concentration of substrate. Consequently, there would be no problem in applying the enzyme to the contaminated juices with a high concentration of dextran.

Inhibition studies demonstrated that glucose, sucrose, raffinose, galactose maltose, pectin and galactoronic acid and dextrin had a competitive inhibition on the enzyme activity. Comparing the amount of k_m for substrate with the amount of k_i for each inhibitor, it was shown that k_i of each inhibitor was much higher than the k_m of the substrate. Therefore, this indicated that the enzyme could greatly bind with the substrate (dextran) more than that of inhibitors.

Effect of different concentrations of stabilizing agents on the enzyme activity

Stabilizing agents are other factor that may severely affect the yield of the enzymatic reaction at low activity. Since the enzyme usually lose its activity at dilute conditions, the effect of stabilizing agents were

investigated using polyolic, proteinic and polymeric compounds.

These reagents may stabilize the enzyme at specific concentrations and inactivate at other ranges. Therefore, the effects of several concentrations of egg albumen, PVP, sorbitol, glycerol, BSA, PEG and gelatin on the enzyme activity were determined. As shown in Fig. (13_a, 13_b, 13_c and 13_d), egg albumen, PVP, sorbitol and glycerol improved the activity of the enzyme to 76.5, 12.74, 64.0 and 24.26 % at a factor of 0.7, 0.1, 0.7 and 0.3 %, respectively. BSA (Fig. 13_e) has no significant effect on the activity over a broad range of the concentrations. PEG and gelatin (Fig. 12_f and 12_g) decreased the activity in the range of 9.13–15.38 % and 11.46–16.76 %, respectively. According to the results, it was declared that sorbitol, glycerol and egg albumen slightly inhibit the enzyme to 50%. These compounds probably preserve the active conformation of the enzyme. Substances such as glycerol or sorbitol have a high content of hydroxyl groups.

The effects of several stabilizing agents were investigated to improve the enzyme activity and it was shown that egg albumen, PVP, sorbitol, glycerol and BSA had no effect on the enzyme activity at concentrations of 0.7, 0.1, 0.7, 0.3 and 1.5, respectively.

Effect of Different Metals on Dextranase Activity

The effect of different metals on dextranase activity was shown in Table (5). Results indicated that $FeCl_2$ and $FeCl_3$ strongly inhibited the dextranase activity, while $MnCl_2$ and $MgCl_2$ had no effect on dextranase activity. Theses results are in agreement with that reported by Mamoru *et al.*, (1973).

Table.1 Dextranase activity (unit $100ml^{-1}$) of the culture filtrates of the microbial isolates at different incubation periods

Microbial culture	Strains	Dextranase activity (Units /100ml), at different incubation periods				
		2 days	4 days	7 days	10 days	12 days
Yeast	*S. cerevisiae YSF-5*	197.86	306.11	497.35	1078.18	39.11
	L. starkyi ATCC-12659	168.27	736.52	744.61	312.50	--
Bacterial	*B. subtilus M-15*	476.31	2985.03	1306.27	843.06	--
	L. dextranicus B-512 FM	234.60	562.88	1964.07	27591.49	161.02
Fungal	*P. funiculosum NRRL-6014*	396.54	579.07	34790.45	68048.20	178.99
	P. aculeatum NRRL-896	1002.5	23653.1	101250.00	54320.48	247.60

Fig.2 Effect of incubation period on dextranase production by different microbial strains

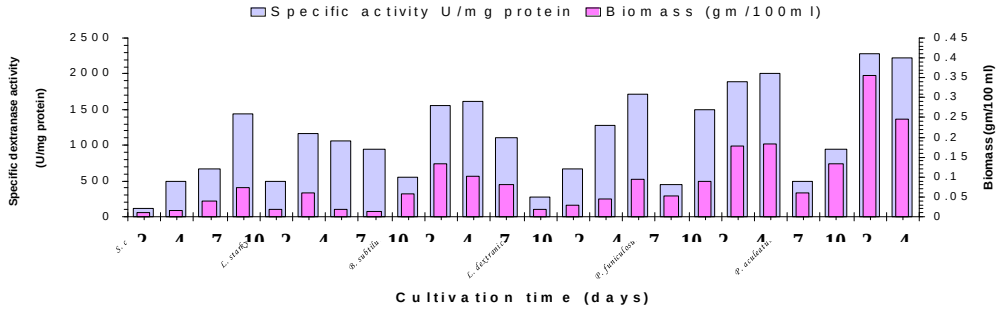


Table.2 Effect of different carbon sources on the production of dextranase by *Penicillium aculeatum NRRL-896*.

Carbon sources	Dry weight of mycelium (mg /100 ml culture)	Protein content of culture filtrate (mg/100ml)	Dextranase activity (U/100ml)
***Dextran (%w/v)**			
dextran (0.5 %)	175.39	14.35	5880.64
dextran (1.0 %)	364.02	17.99	30870.84
dextran (1.5 %)	398.52	21.11	31850.71
dextran (2.0 %)	502.64	22.00	34300.52
dextran (2.5 %)	731.87	25.28	66152.13
dextran (3.0 %)	1267.06	42.60	73533.29
dextran (3.5 %)	1288.51	48.70	99758.24
dextran (4.0 %)	1401.63	51.59	88212.34
Glucose (1.0 %)	394.07	17.92	197.39
Lactose (1.0 %)	285.89	20.29	171.86
Dextrin (1.0 %)	362.11	7.21	348.61
Starch (1.0 %)	457.36	5.58	343.70

*M.W. Dextran = 40.000 Dalton

Maximal yield of dextranase (99758.24 unit/100ml) was obtained with dextran at 3.5% as a source of carbon. This value is the highest among all tested carbon sources.

These results are in agreement with that obtained by Priyanka and Santosh (2011) & Mohamed *et al.,* (1999).

Table.3 Effect of different nitrogen sources on the production of dextranase by *Penicillium aculeatum NRRL-896*.

Nitrogen sources	Dry weight of mycelium (mg /100 ml culture)	Protein content of culture filtrate (mg/100ml)	Dextranase activity (U/100ml)
$NaNo_3$ + Yeast (control)	585.32	3.74	**98751.65**
$NaNo_3$ (1.0%, w:v)	1395.74	2.69	2878.27
Yeast extract (0.2%, w:v)	1815.66	11.35	65014.43
Ammonium sulphate	512.49	2.41	721.08
Peptone	793.61	10.03	62431.62
Tryptone	1034.08	9.87	48011.05
Casein hydrolyzate	1282.10	9.95	33572.81
Urea	1224.63	3.72	2174.64

Results indicated that using a combination of sodium nitrate and yeast extract as a source of nitrogen produced the highest activity (98751.65 units / 100ml) compared to other nitrogen sources.

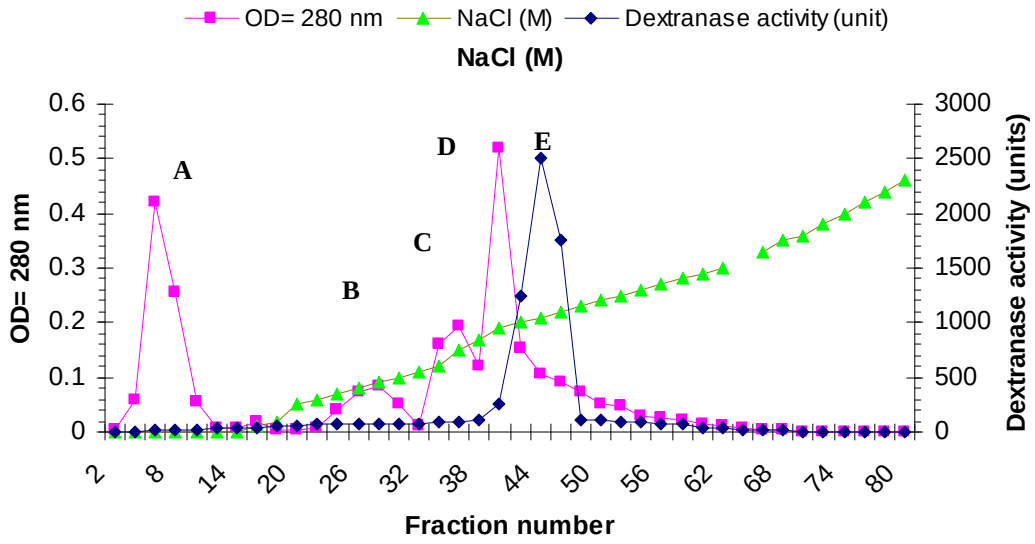
On the other hand using ammonium sulphate gave the lowest level of enzyme activity (721.08 units/100ml). These results are similar to those reported by **Mohamed *et al.,* (1999).**

Table.4 Purification scheme for dextranase from *Penicillium aculeatum NRRL-896*

Purification step	Total activity (Units/100ml)	Total protein (mg)	Specific activity Units/mg protein	Yield %	Purification fold
Culture filtrate	101140.00	51.00	1983.14	100.00	1.00
Ammonium sulfate	67280.17	4.62	14562.81	66.52	7.34
Dialysis	26819.41	1.39	19294.54	26.51	9.73
DEAE-Sepharose	17322.91	0.29	59734.18	17.13	30.12
Sephadex G-100	14945.63	0.18	83031.28	14.78	42.87

After, removal of the insoluble materials by centrifugation, the clear supernatant was loaded onto a DEAE-Sepharose column **(Elvira *et al.*, 2003)**. The dextranase adsorbed was eluted by a linear gradient system of NaCl (0.21 M). The dextranase fraction at (fraction number 43) gave a single active peak (Fig. 3) that had the maximum enzyme.

Fig.3 Chromatogram of crude *Penicillium aculeatum NRRL-896* dextranase on DEAE-sepharose fast flow



The dextranase active peaks (D&E) were collected, saturated and applied to Sephadex G-100 column and also one active dextranase peak was formed and collected symmetrical at fraction number 43, Fig. (4).

Fig.4 Elution profiles for protein and dextranase activity on Sephadex G-100. The absorbance values at 280 nm (protein) were redrawn from the recorder chart. Fractions of 2 ml were collected and measured for activity.

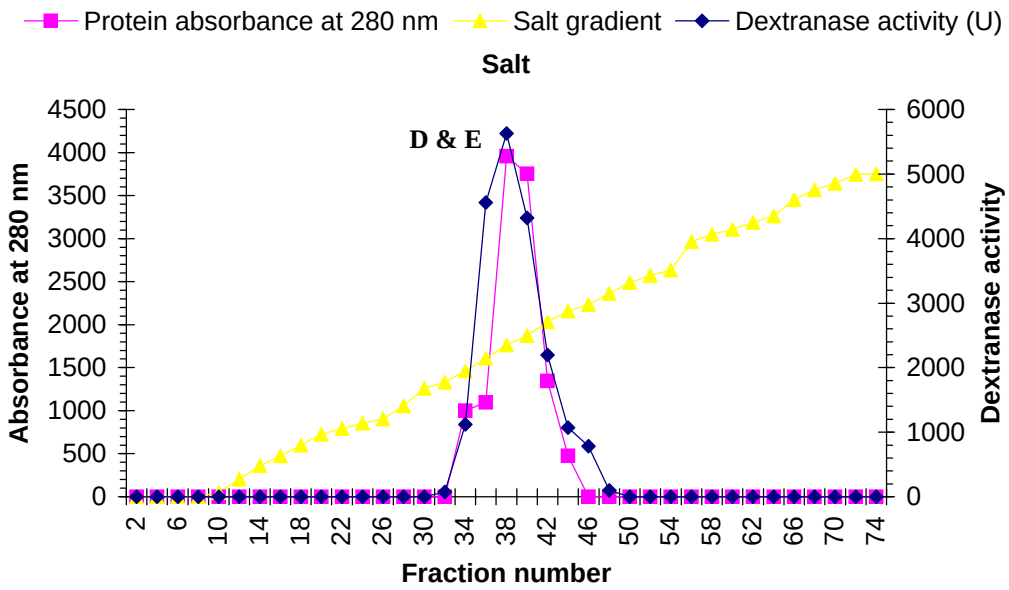


Fig.5 Effect of temperature on soluble dextranase, enzyme concentration 0.28 u/ml, dextran as substrate 0.625 % (w/v), with 20 % sucrose, (0.025 M acetate buffer pH 4.0) and reaction time 30 min

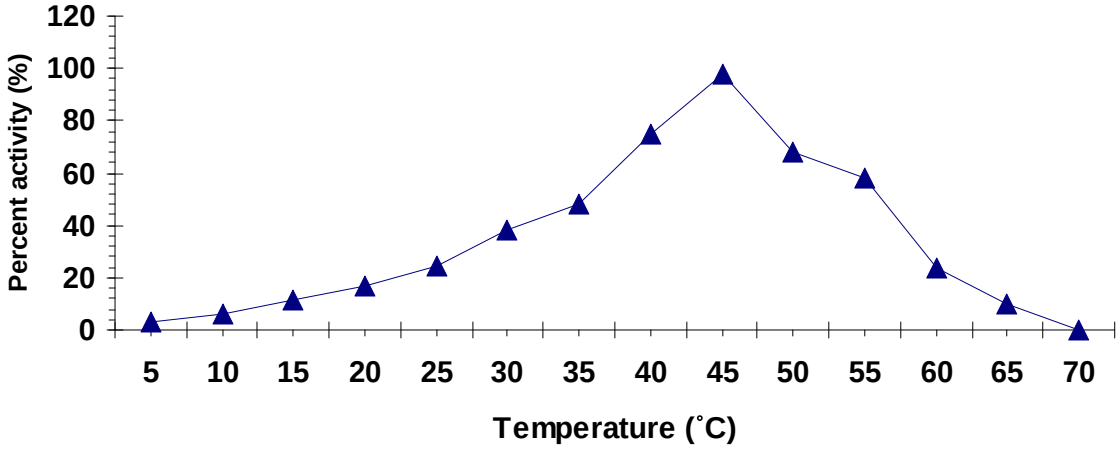


Fig.6 Effect of pH on soluble dextranase, enzyme concentration 0.18 u/ml, dextran as substrate 0.625 % (w/v), reaction temperature 45 ˚C and reaction time 30 min.

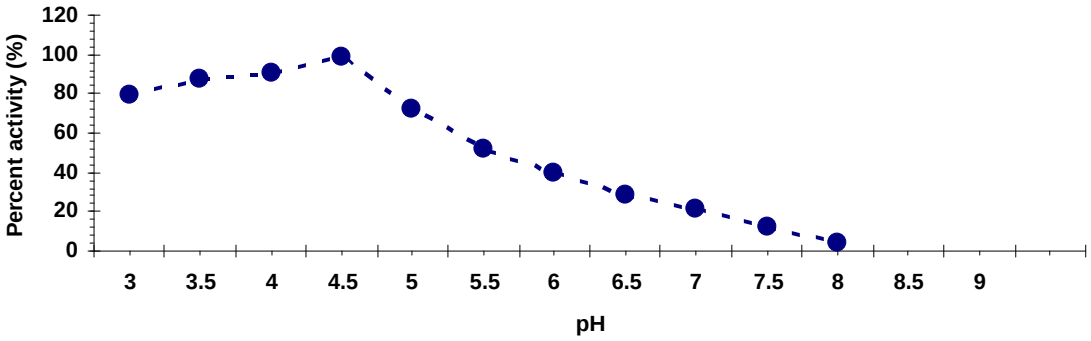


Fig.7 Effect of temperature on stability of soluble dextranase.

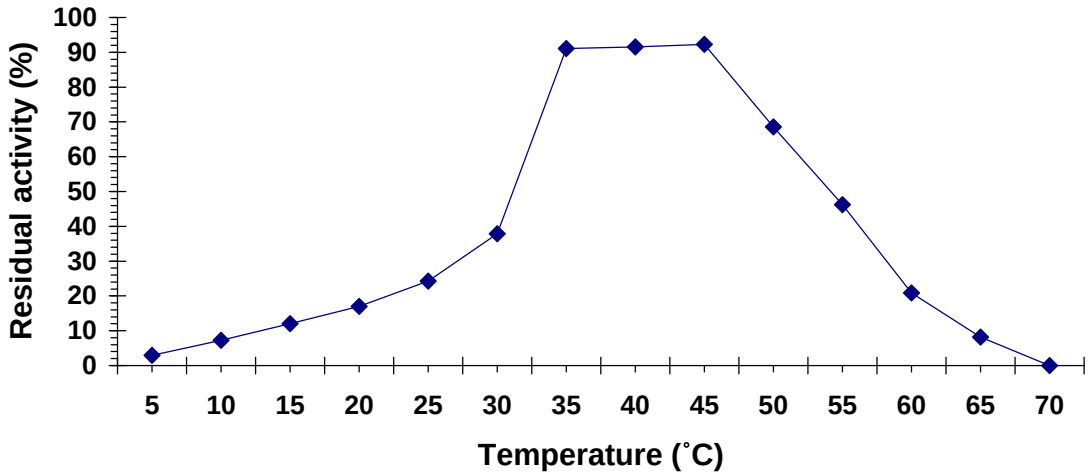


Fig.8 Effect of pH on stability of dextranase.

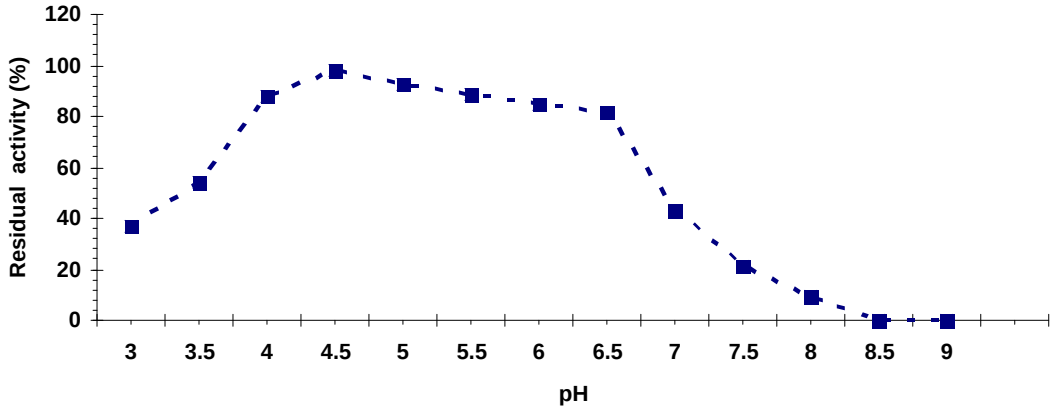


Fig.9 Lineweaver- Burkplot of free dextranase purified from *Penicillium aculeatum NRRL-896*

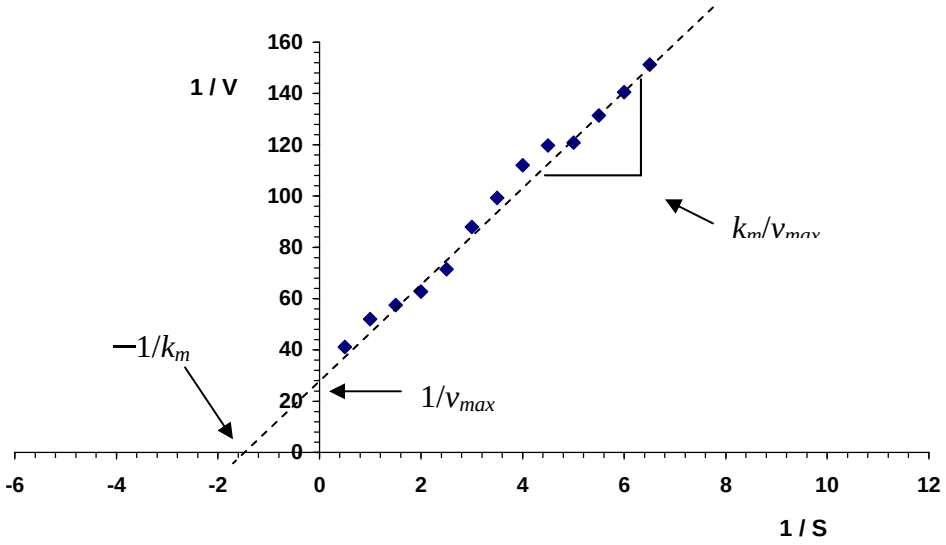


Fig.10 Molecular-weight estimations by SDS-PAGE

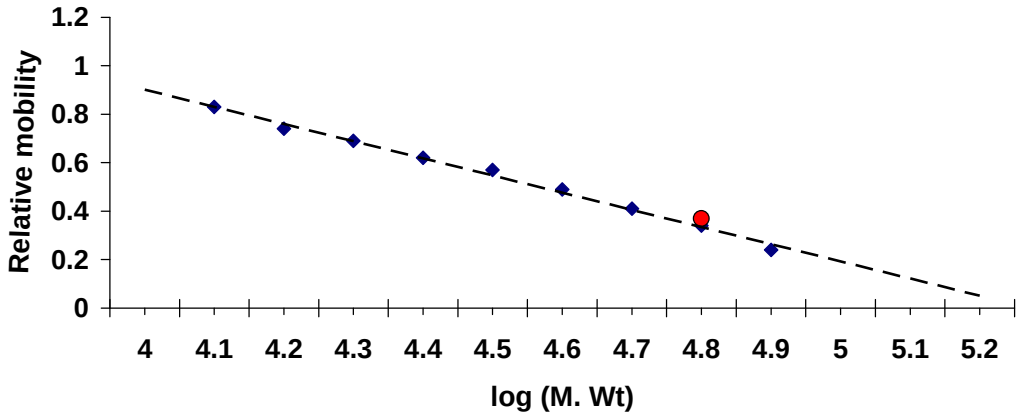
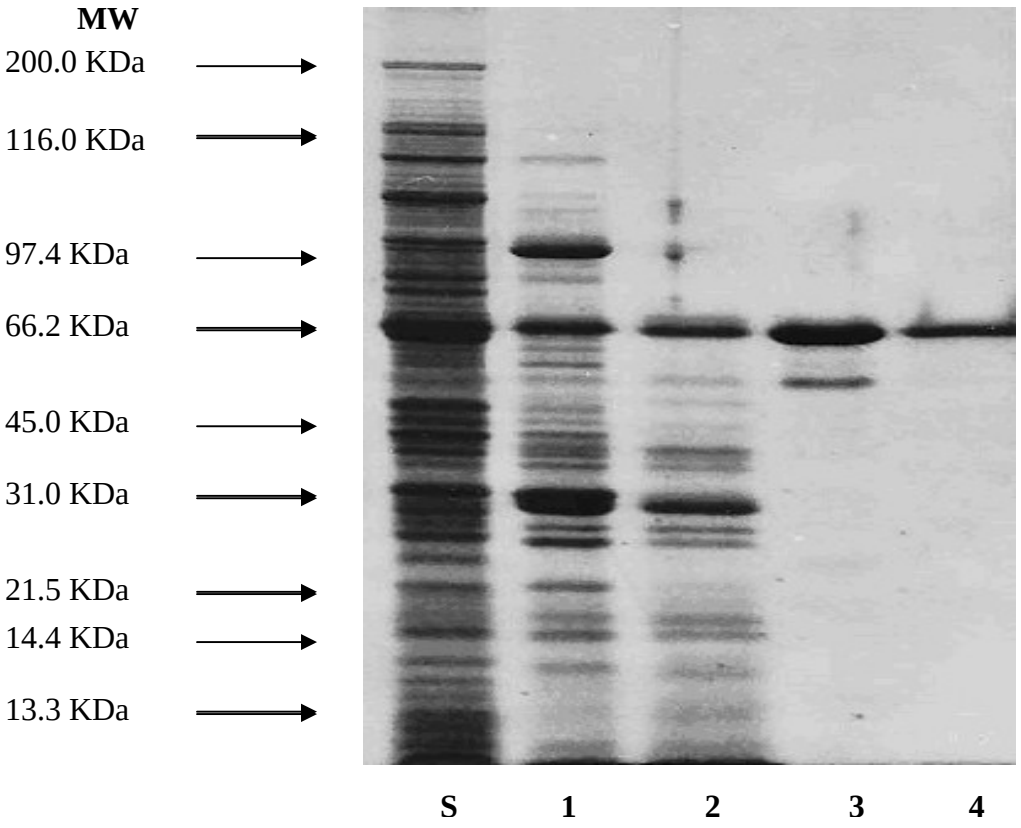


Fig.11 Electrophoresis analysis of purified dextranase from *Penicillium aculeatum NRRL-896*



Where: S: Strand proteins; 1: Ammonium sulfate; 2- Dialysis; 3: DEAE-Sepharose; 4: Sephadex G-100

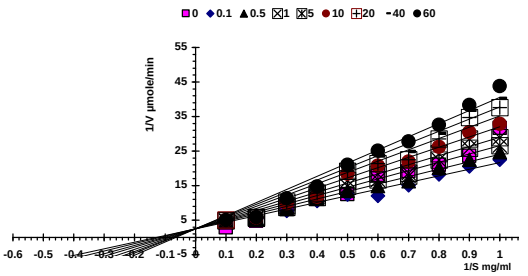
Table.5 Effect of different metals on dextranase activity:

Metals (1mM)	Free dextranase (Residual activity) %
Control	100
Ferrous chloride ($FeCl_2$)	6.2
Ferric chloride ($FeCl_3$)	9.6
Cobalt chloride ($CoCl_3$)	78.5
Manganese Chloride ($MnCl_2$)	100.0
Lead dichloride ($PbCl_2$)	37.6
Copper chloride ($CuCl_2$)	48.4
Aluminum chloride ($AlCl_3$)	73.4
Magnesium chloride ($MgCl_2$)	100.0
Mercuric chloride ($HgCl_2$)	31.2

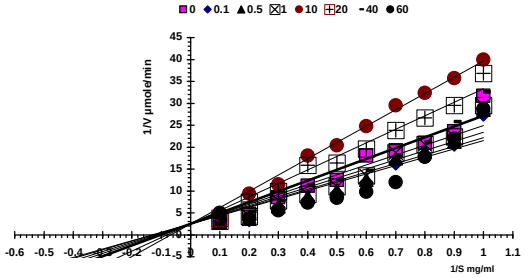
Results indicated that $FeCl_2$ and $FeCl_3$ strongly inhibited the dextranase activity, while $MnCl_2$ and $MgCl_2$ had no effect on dextranase activity. Theses results are in agreement with that reported by Mamoru *et al.*, (1973).

Fig.12 Lineweaver-Burk plots for different concentrations of inhibitors

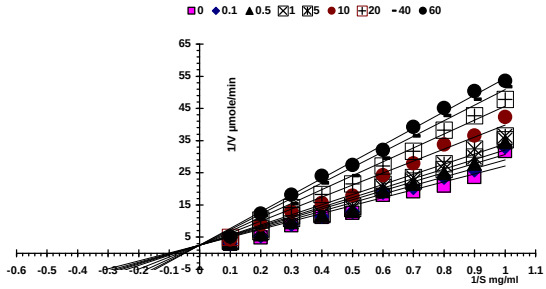
a) Glucose concentrate (%):



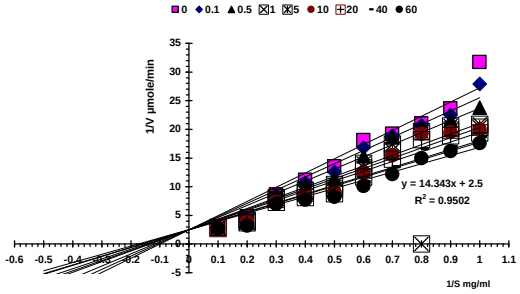
b) Dextrin concentrate (%):



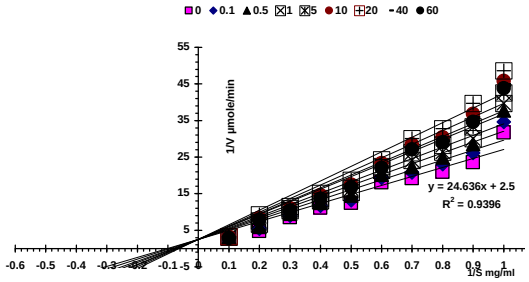
c) Sucrose concentrate (%):



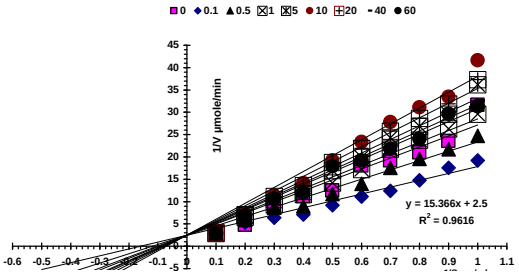
d) Galactoronic acid:



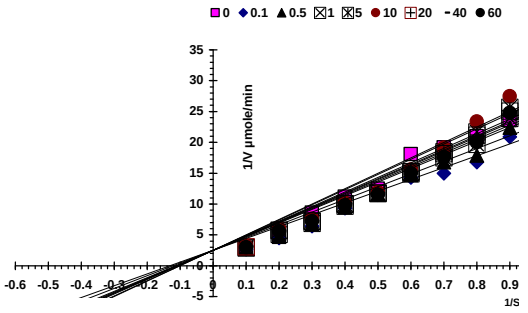
e) Galactose concentrate (%):



f) Maltose concentrate (%):



j) Raffinose concentrate (%):



ıı) Pectin concentrate (%):

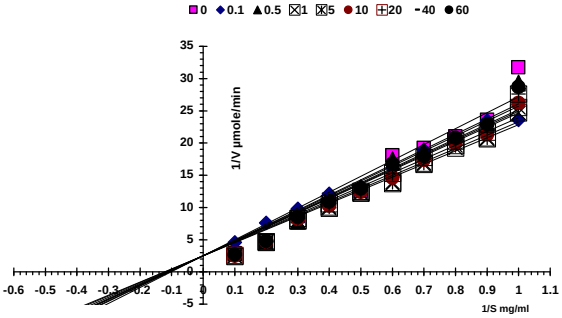
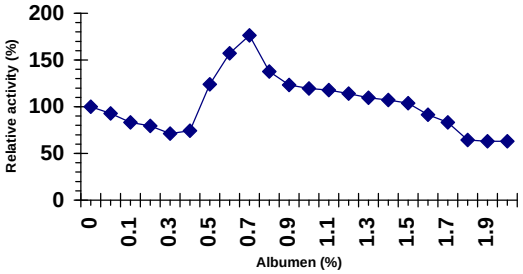
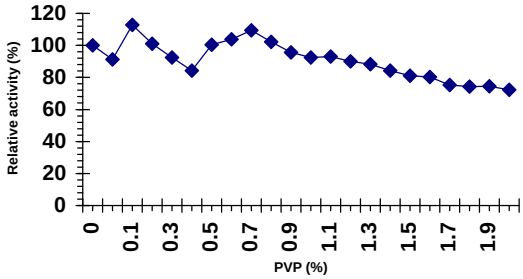


Fig.13 Effect of different concentrations of stabilizing agents on the enzyme activity

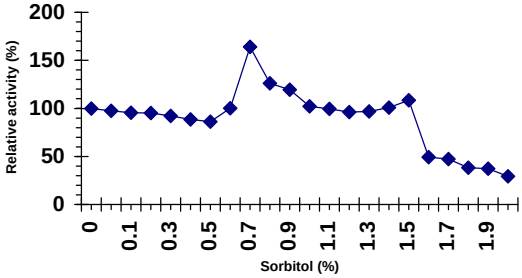
a) Egg albumen:



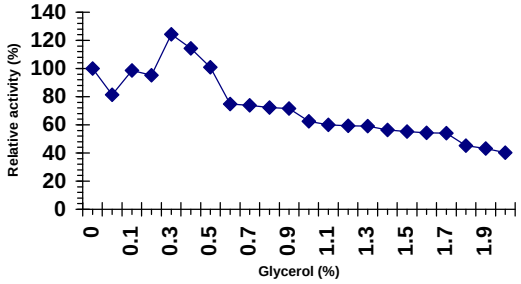
b) PVP:



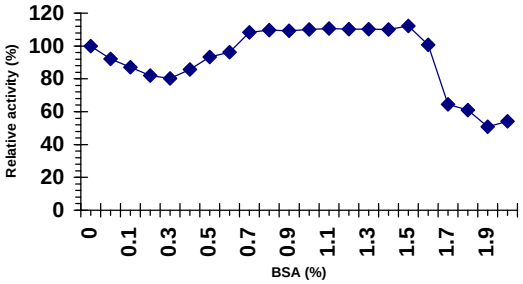
c) Sorbitol:



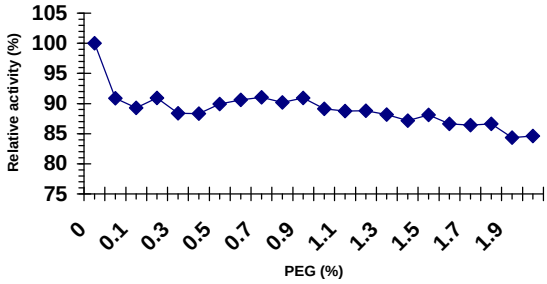
d) Glycerol:



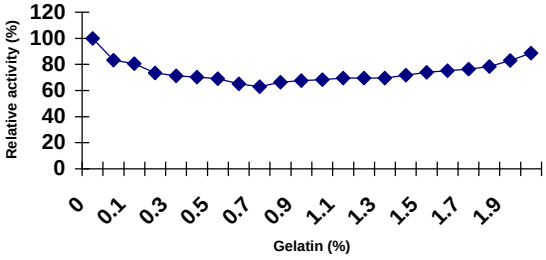
e) BSA:



f) PEG:



g) Gelatin:



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