ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Isolation, Partial Purification and Physicochemical Characterization of *Pismussativum* pods invertase.

El-Sayed M El-Sayed[1], Sanaa T El-Sayed[2]*, Mohammed IY Elmallah[1], Abeer N Shehata[2], and Shimaa S Hanafy[2].

[1]Chemistry Department, Faculty of Science, Helwan University, Helwan, Egypt.
[2]Biochemistry Department, National Research Center, Giza, Egypt.

ABSTRACT

The aim of this study was to test the possible presence of potent invertase enzyme with promising industrial application in local low cost Egyptian plants. *Pismussativum* pods (pea pods) were chosen for further study as it shows highest invertse activity (82%U/g dry weight tissues) among the screened plants. Invertase enzyme was extracted with distilled water from pea pods and partially purified by precipitation with ammonium sulphate (0-60%). The partial purified enzyme was purified 15.88 times than the crude enzyme with 85.42% yield and specific activity 168.08 ± 0.02 U/mg. The biochemical properties of the prepared enzymes (crude and partially purified) were studied. They showed high invertase activity in a wide range of pH 5.0 -7.4 with optimum temperature at 40°C for the crude invertase and at 45°C for the purified one. The K_m values of crude and purified invertase were 33.33 and 20.83 mg (0.133 and 0.083 mM) at pH 5.0, respectively, using sucrose as substrate. Thus, the purified invertase has high affinity toward sucrose than the crude one. Both prepared enzymes reach to maximal activity after incubation without substrate for 30 mins at temperature up to 65°C. The crude invertase was found to be more stable when storage in distilled water at 4°C for 270 days (retained 97% of its initial activity) than the purified one (retained 88.6% of its initial activity). Invert syrup with 23.7% concentration was obtained by hydrolysis of sucrose using the prepared partial purified invertase.

Keywords: *Pismussativum (pea* pods), *Leguminoease*, invertase (EC 3.2.1.26), partial purification, characterization, invert syrup production.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Invertase (EC 3.2.1.26) is a sucrose hydrolyzing enzyme producing an equimolar mixture of glucose and fructose named invert sugar [1,2]**.** The enzyme occurs widely in many plants, microorganism and animal sources [3].Strong invertase activity has been detected in ripe grape berries, Japanese Pear fruit (*Pyruspyrifolia*), Pea (*Pisumsativum*), Oat (*Avena sativa*) and turnip leaves (*Brassica rapa*) [3-6].

Invert sugar can be produced by a chemical process (using acid as a catalyst) or biochemical process (using invertase as a catalyst). Invert sugar produced by acid hydrolysis of sucrose giving undesirable by-products, absence of sweet taste, low conversion efficiencies; high ash contents and so are highly uneconomical. On the other hand, the enzymatic hydrolysis gives more purity products and better in taste [7,8].One of the important applications of invertase lies in the production of non crystallizable invert syrup from sucrose. Invert syrup is sweeter than sucrose due to high degree of sweetness of fructose. Invert syrup is used as humectants in the manufacture of soft centered candies and fondants [9]. There are many industrial applications of invertase e.g. pharmaceutical formulations and in the production of lactic acid and ethanol [10,11]. It is also associated with inulinase in the production of fructooligosacchrides from inulin which are not degraded by human or animal digestible enzymes [3].

The lack of information in the properties of invertase from pod tissues promoted us to isolate and examine the enzyme from pea pods. The present article describes the extraction and partial purification of invertase enzyme from pea pods. Some of its physical and chemical properties (pH and temperature optima and kinetic, thermal stability, etc.) of the crude and partial purified invertase were studies. The potential use of the partial purified enzyme for the preparation of invert syrup from sucrose solution was tested.

MATRIALS AND METHODS

Healthy, fresh plant materials used in this study consisted of samples of different vegetative plant parts were buy from local market (Table 1). Sucrose was purchased from Merck chemical. CO. All the other chemicals were of analytical grade.

Table 1: Name of the different screened plant waste.

Latin name	English name	Family	Part used
Pisum sativum	**Pea**	***Leguminoseae***	**Pods**
Opuntia ficusnnnidica	**Cactus**	***Cactaceae***	**Pods**
Vicia faba	**Broad bean**	***Leguminoseae***	**Pods**
Phoenix dactylifera	**Data palm**	***Areceae***	**Seeds**
Cynara scolymus	**Artichoke**	***Asteraceae***	**Stem**
Brassica rapa	**Turnip**	***Brassicaceae***	**Roots**
Beta vulgaris	**Beetroot (Red Beet)**	***Chenopodiaceae***	**Roots**
Brassica olercea	**Cauliflower**	***Brassicaceae***	**Leaves**
Cynara scolymus	**Artichoke**	***Asteraceae***	**Leaves**
Beta vulgaris	**Beetroot (Red Beet)**	***Chenopodiaceae***	**Leaves**

Extraction of the crude enzyme

The healthy fresh plant parts and dried seeds were collected and cleaned thoroughly with water. They were sliced into small parts and homogenized in Braun multimix MX 32 with distilled water and left overnight at 11°C. The resulting homogenate was filtered through cheesecloth then centrifuged at 13,000 xg, at 5°C for 15 min. and the supernatant was used as the crude enzyme. The most active one was used as the crude enzyme (E_1).

Enzyme assay

The standard reaction mixture contained 1% (w/v) of the substrate (Sucrose) dissolved in 0.1M sodium acetate buffer, pH 5.0 and appreciable amount of an enzyme in a total final volume of 0.95 ml. The

reaction mixture was incubated at 40°C in water bath for 1.0 hr. The enzyme activity was accomplished by measurement of the liberated reducing sugar (fructose and glucose) by the procedure described by Somogyi, [12] and Nelson [13] method, using fructose as standard.

One unit of enzyme is defined as the amount of enzyme which will catalyze the formation of one µmole of reducing sugar (as fructose) per hr under the standard assay conditions. The specific activity is expressed as units per mg of proteins. Relative activity was expressed as a percentage of the maximum activity under the standard assay condition.

Protein determination

The protein concentration was determined by method of [14] using bovine serum albumin as standard.

Partial Purification of the crude invertase

The enzyme protein was precipitated from E1 enzyme preparation by ammonium sulphate according to the method of [15]. Adequate volume of the prepared E_1 enzyme was treated with different concentrations of ammonium sulphate to yield 0-10; 10-60; 60-70 and 70-100% saturation at 11°C. Each fraction was obtained by centrifugation at 13,000 r.p.m, 4°C for 15 mins. The resulting precipitates were dissolved in appropriate amount of distilled water and dialyzed exhaustively against distilled water for one day at 11°C to get rid of the excess of ammonium sulphate. Undissolved protein was removed by centrifugation before enzyme assay. Enzyme activity and protein content were determined in each fraction. The most active fraction was used as the partial purified enzyme (E_2).

Physicochemical properties of the crude and partial purified enzymes

Effect of the different pH's on the enzyme activity

E_1 and E_2 enzymes were assayed with different three buffering solution, namely acetate buffer, pH 3.0 -5.5, phosphate buffer, pH 6.0 – 7.5 and tris- HCL buffer, pH 8.0 – 9.2 at 0.1 M for each one for recording pH profile under the standard assay conditions.

Activity of the enzymes at different incubation temperatures

The activities of E_1 and E_2 enzymes were determined at different incubation temperatures ranged from 30°C to 60°C.

Thermostability

Small aliquots of E_1 and E_2 enzymes were pre-heated at different temperatures from 30°C to 90°C for varying time intervals from 15 to 120 mins. The remaining enzymes activities were then estimated using the standard assay conditions. Residual activity was expressed as a percentage of the initial activity under the standard assay condition.

Effect of the different Substrate concentrations on the enzyme activities

The effect of different substrate concentrations ranging from 2.5 to 25 mg per reaction mixture on the E_1 and E_2 enzyme activity were estimated with the same amount of the enzyme concentration. Then, the enzymatic activity was plotted against substrate concentration. Apparent Michaelis constant (K_m) values of E_1 and E_2 enzymes toward sucrose were determined according to the method of [16].

Determination of substrate specificity

The E_1 and E_2 enzymes activities were estimated using various disaccharides (sucrose, lactose and maltose) and polysaccharide (inulin and cellulose). The substrates were incorporated in the reaction mixture at

different concentration varying from 0.25 to 20 mg/reaction mixture. Relative activity was calculated as a percentage of enzyme activity compared to the corresponding assay condition using sucrose as substrate.

Invert syrup production

The production of invert syrup was accomplished through enzymatic hydrolysis of sucrose using the prepared E_2 invertase.

Digestion

The reactions were performed in a mixture containing sucrose in 0.1 M acetate buffer, pH, 5.0 with an appreciable amount of E_2 invertase. The reactions were incubated at 40°C for 1.0 hr. The supernatant (hydrolysate) were separated by centrifugation at 13,000 r.p.m, at 4°C and was used as invert syrup solution.

Determination of fructose and glucose in the digested sample (invert syrup)

The digested solution (invert syrup) was quantitatively estimated by Somogyi, [12] and [13] method and calculated as µg fructose.

Hydrolysis measurment

Degree of hydrolysis and concentration of prepared invert syrup were calculated. Degree of hydrolysis percentage was calculated using the following formula:

$$\text{Hydrolysis (\%)} = \text{TRS}/S\circ \times 100,$$

where TRS is the total reducing sugar (glucose and fructose) in mM, $S\circ$ is the initial sucrose concentration in mM.

Concentration of invert syrup = concentration of hydrolysate as fructose (mg) per 100 ml solution.

Statistical analysis

Data are expressed as the mean ± standard error (± SE) from at least three experiments.

RESULTS AND DISCUSSION

Screening study focused firstly on sources that have high invertase activity. The screening for invertase activity was applied by using sucrose in 0.1 M acetate buffer, pH 5.0. We found that invertase activities were estimated in pea, cactus and broad bean pods and data seeds extract. The highest value of invertase activity was found in pea and broad bean pods (82 and 11.7 U/g dry wt. tissues, respectively). They are members of the family *leguminoseae.*We also found that stems and leaves are poor in invertase activity, where the seed of date palm have appreciable amounts of invertase activity. Thus, the enzymatic activities were varied not only with the species of the tested plants, but also according to the morphology of the examined parts and family type. Pea pods were chosen for further study as it shows highest invertase activity.The aqueous extract of pea pods was taken as a crude enzyme source (E_1).

Precipitation of the invertase enzyme

The ammonium sulphate fraction at 0 to 60% concentration give high invertase activity with specific activity 168.08 U/g and high yield 85.42 % (table 2). It was taken as purified invertase, E_2. Further increase of ammonium sulfate concentration resulted in a proportional decrease or loose of invertase activity. The E_2 had a high specific activity about 16 times more than of the E_1.Inorganic salts can be utilized for the precipitation of proteins, with ammonium sulfate being the most common. The advantages of ammonium sulfate are: (1) at saturation, it is of sufficiently high molarity that it causes the precipitation of most proteins. (2) Its concentrated solutions are generally bacteriostatic. (3) In solution it protects most proteins from denaturation [17]. Previously, enzyme invertase was detected in healthy tomato juice and partially purified with 87.62 U/mg

ISSN: 0975-8585

and 23.19% yield [18]. It was also detected and purified from wheat, ripe grape berries and turnip leaves [19-21].

Table 2: Partial purification of invertase from 50 gm fresh pea pods

Steps	Volume (ml)	Enzyme activity (U/ml)	Total activity (U)	Protein (mg/ml)	Total protein (mg)	Specific activity (U/mg)	Yield (%)	Purification fold
Crude (E1)	370	1.35	499.96	0.127	47.24	10.5 ± 0.08	100	1.0
Partial purified invertase (E2)	52	8.24	428.58	0.049	2.55	168.08± 0.02	85.42	15.88

Physicochemical propeties

The properties shown by an individual enzyme depend upon its source.

Efect of pH on invertase activity

The invertase activities of E_1 and E_2were monitored at different pHs (Fig. 1). High invertase activity was at wide range of pH from 5.0 to 7.4 with optimum at 6.5 for both E_1 and E_2 invertase. While at pH 8.5 and 9.2, the invertase activity completely lost for E_1 and E_2 invertase, respectively. The results are agree with that reported by Rashad*et al.,* [22] for *Saccharomyces cerevisiae*invertase (optimum pH 6.5) and lower optimum pH values (5.0) for invertase isolated from *Oryza sativa*, cherry, grape berries and tomato were reported [18,23-25].

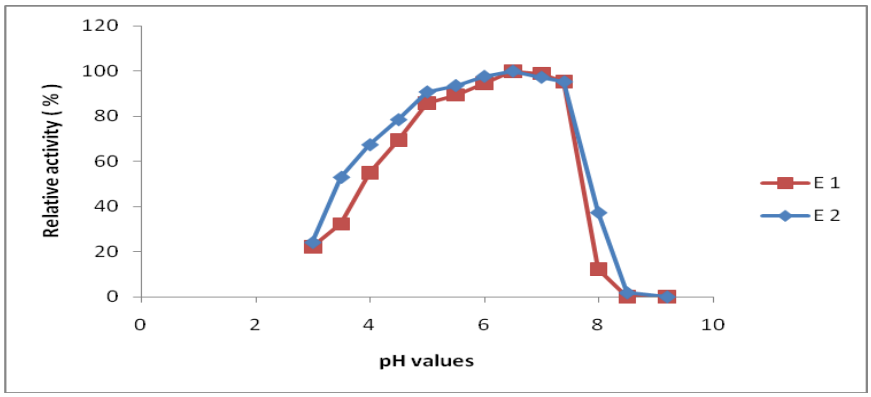

Figure 1: Effect of pH on the activity of E_1 and E_2 invertase.

Optimum incubation temperature

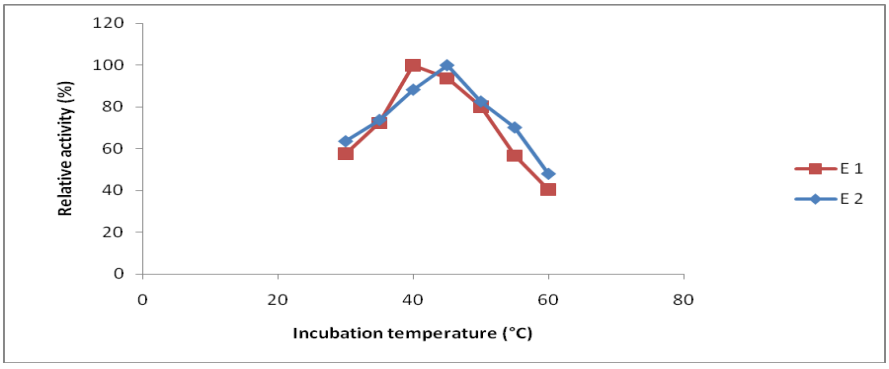

Figure 2: Effect of temperature on the activity of E_1 and E_2 invertase.

E_1 and E_2 exhibit optimum invertase activity at temperature 40°C to 45°C, respectively (Fig. 2). The results were similar to that reported for *Saccharomyces cerevisiae*invertase [26], on the other hand these results was higher than optimum temperature (35°C) for invertase enzyme isolated from beta *vulgaris* roots, turnip leaves and tomato juice [18,21,27].

Effect of the reaction times on enzymes activities

E_1 and E_2 enzymes were incubated with sucrose for different time intervals from 10 to 190 mins for recording time profile under the standard assay conditions. The results are summarized and graphically illustrated in figure (3). The enzymes activity were increased proportionally in a linear manner with the increase of the time up to 120 and 60 mins for E_1 and E_2, respectively, beyond this time the inhibition occurred. We concluded that increasing time may be lead to excess products, which may be inhibiting the enzyme activity. Based on these results, all tested were conducted for 60 mins to place them in the linear portion of the curve.

Effect of the different enzyme concentrations

The invertase activities were estimated at different concentrations of E_1 enzyme at range from 0.085 to 0.2554 µg per reaction mixture, while E_2enzyme at range from 0.043 to 0.098 µg per R.M., with sucrose as substrate at standard assay conditions. Then, the products were estimated and its amount of hydrolysate was calculated as µmole fructose /reaction mixture. Relation between enzyme concentration and reaction products was plotted. Figure (4) illustrate that the enzymes activities was increased proportionally in a linear relationship with the increase of enzyme concentration up to 0.145 µg per R.M. for E_1 and 0.057 µg per R.M. for E_2. From the above results, as a linear function was found with enzyme concentration in reaction mixture, all tests conducted to place them in the linear portion of the curve.

Effect of different substrate concentration

A direct linear relationship is observed between sucrose concentration and the invertase activities of $E_1$and E_2 reach to the maximum activities at 15 and 10 mg/R.M, respectively (Fig. 5).

Determination of Michaelis' constant (K_m) and Maximum Velocity (V_{max})

The K_m values of E_1 and E_2 values were 33.33 mg (0.133 mM) and 20.83 mg (0.083 mM) toward sucrose, respectively with V_{max} values of 40 and 222.2 U/mg, respectively (Fig. 6 a&b). Invertase E_2 has higher affinity toward sucrose than E_1 invertase. Km values were lower than that reported previously [1,28,29] with range from 25 to 90 mM and similar to that reported for *Saccharomyces cerevisiae*invertase with 0.11 mM [22].

Substrate specificity

The substrate specificity of E_1 and E_2 invertase are represented in table (4). It illustrates that E1 and E2 hydrolysis sucrose than lactose, maltose and inulin and cannot hydrolysis cellulose. E_2 invertase is probably specific for sucrose. Thus, purified invertase is fructofuranoside and specific for hydrolyzing α 1, 2-glycosidic linkage similar to that reported for turnip leaves and yeast invertase [21].

ISSN: 0975-8585

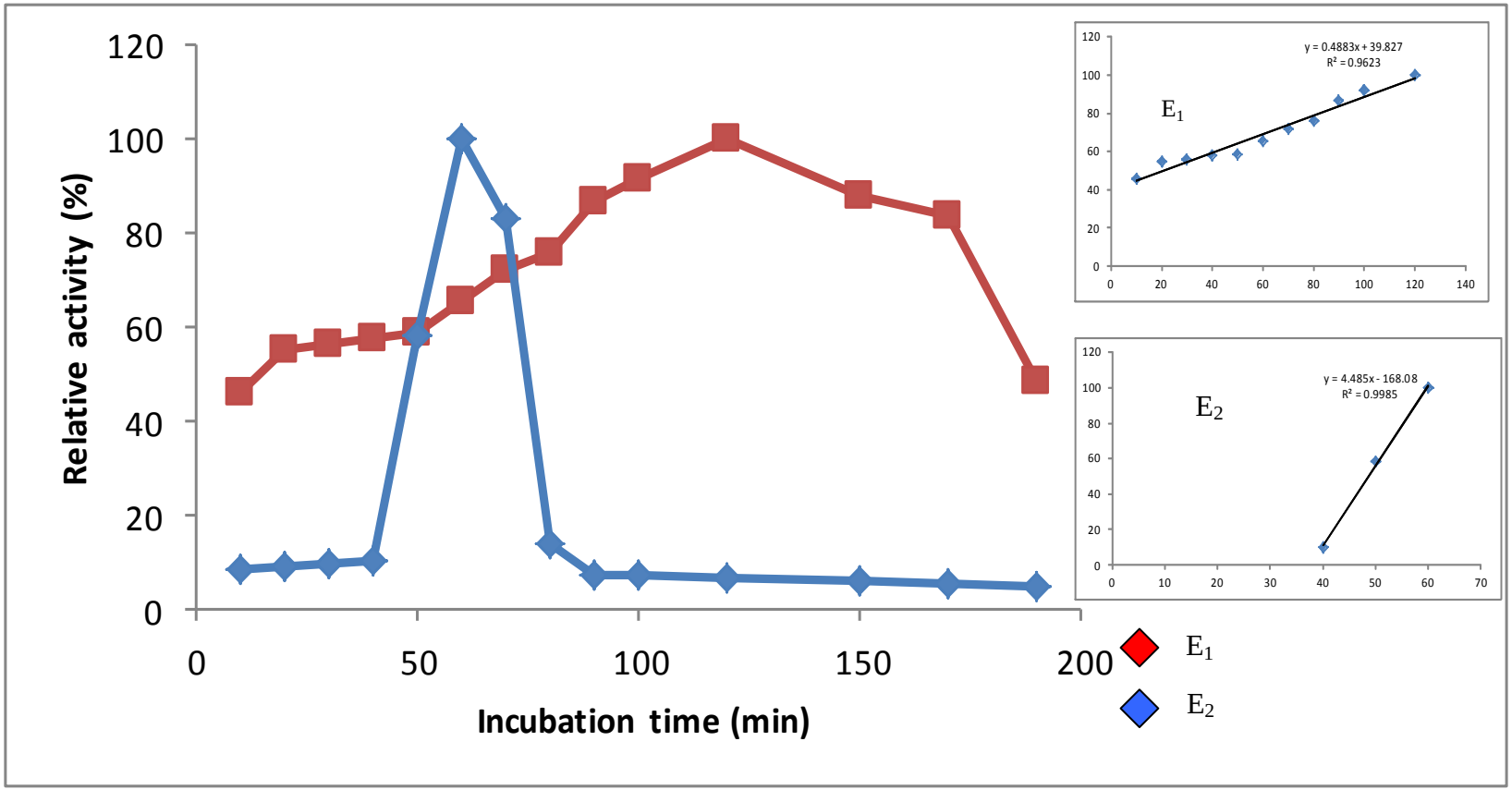

Figure (3) : Effect of incubation time on the activity of E_1 and E_2 invertase.

ISSN: 0975-8585

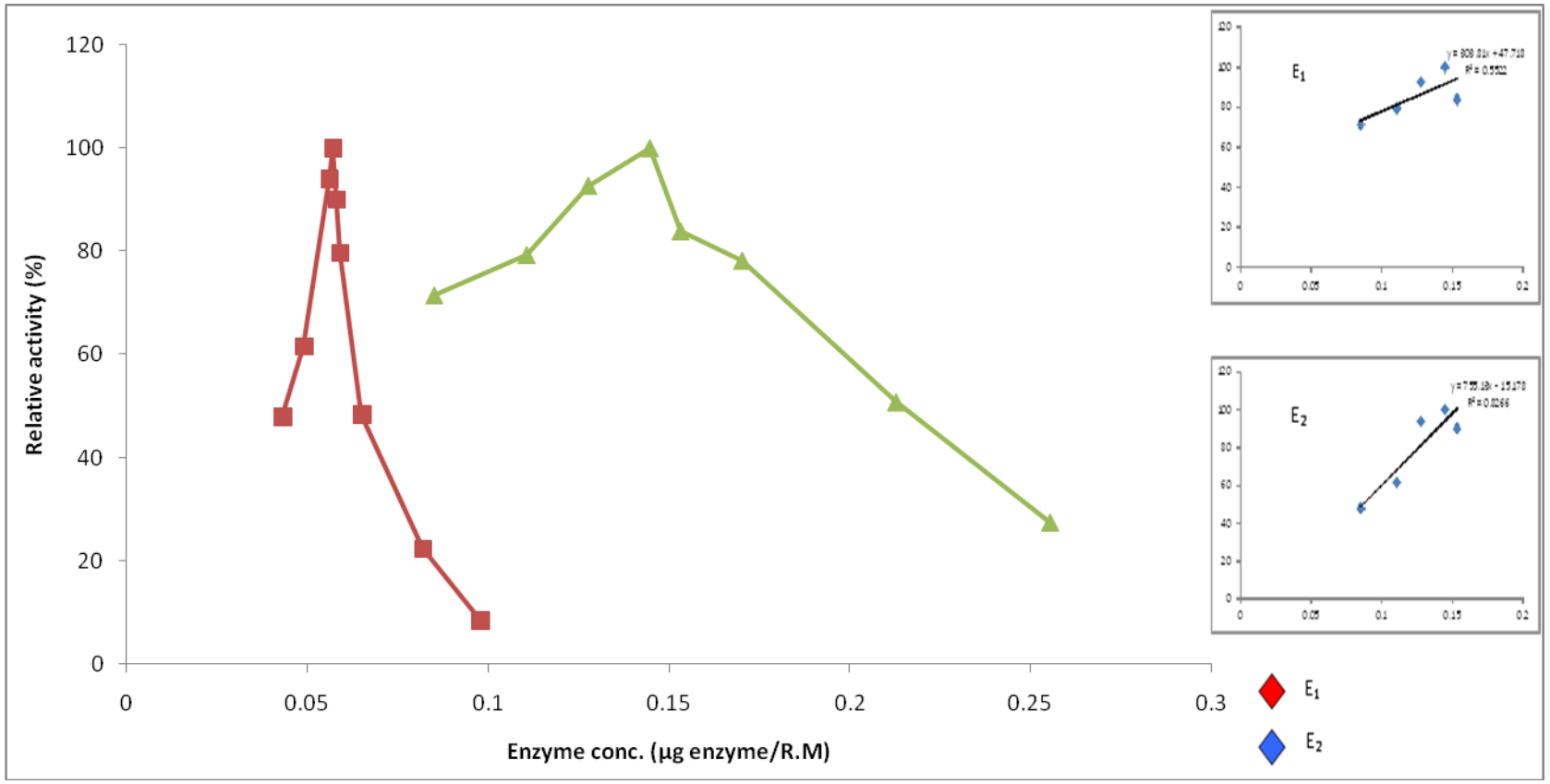

Figure (4) : Effect of E_1 and E_2 enzyme concentration on invertase activity.

ISSN: 0975-8585

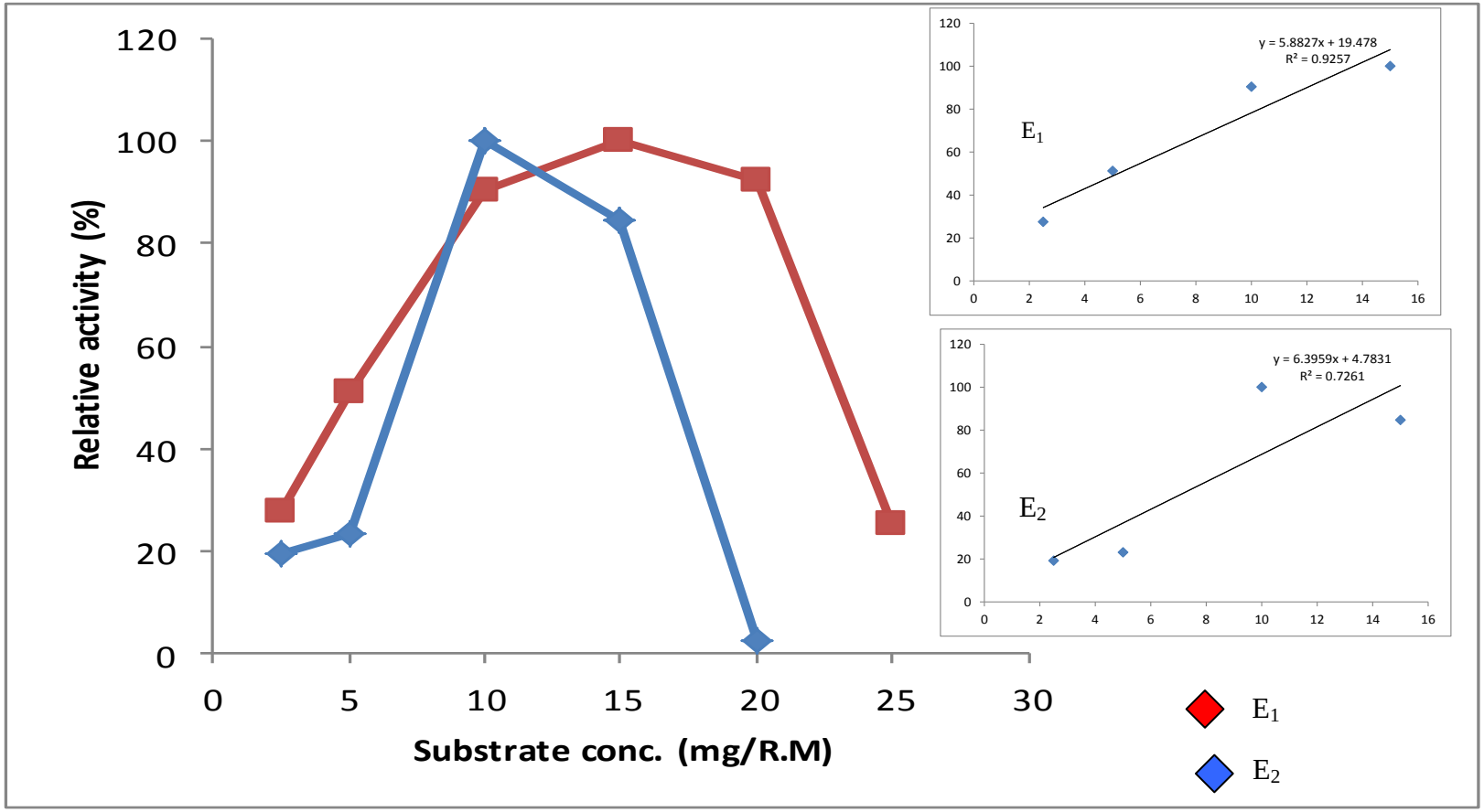

Figure (5): Effect of different sucrose concentration on the activity of E_1 and E_2 invertase.

ISSN: 0975-8585

Table (3): Summarized of substrate specificity of E_1 and E_2invertase.

Substrate	Linkage type	Relative activity (%)	
		E_1	E_2
Sucrose (glucose - fructose)	α 1,2 - glucosidic linkage	100	100
Lactose (glucose – galactose)	β- 1,4 – glucosidic linkage	0.21	3.1
Maltose (glucose- glucose)	α- 1,4- glucosidic linkage	0.01	0.68
Inulin (polymer of fructose)	β- 1,2 - glucosidic linkage	0.07	3.9
Cellulose (polymer of glucose)	β- 1,4 - glucosidic linkage	0.0	0.0

Thermostability

The E_1 and E_2 invertase reach their maximum activities after incubation without substrate for 30 mins at temperatures up to 65°C , respectively. While after incubation E_1 and E_2 at 55°C and 60°C for 60 mins retained only 54 and 49 % of their activities, respectively (Fig. 7 a&b). Further increase in temperature lead to lowered activity as a result of unfolding of enzyme protein. Thus, the purified invertase E_2 is less heat stability than E_1 one. Thermostability of our prepared enzymes was highly higher than that exhibited by the invertase from *Azotobacterchroococcum*, whose half life at 60°C was 3 min [30]. Lower heat stability was reported by Finger and Koltz [27] and Alam*et al*., [18] where rapid inactivation of invertase enzymes occurs at 40°C and complete inactivation occurs at 55°C. El-Sayed and Jwanny [21] also reported that invertase enzyme from turnip leaves was heat stable at 30°C for 30 min with only 10% loss of its activity.

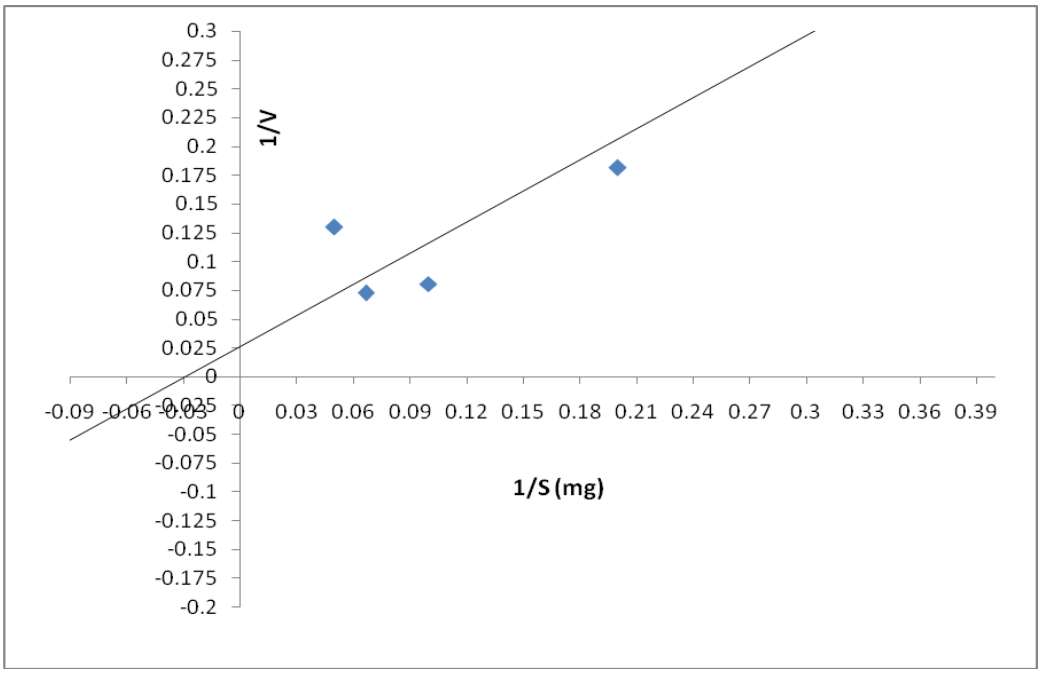

Figure (6 a) : Lineweaver - Burk plot's of E_1 invertase.

ISSN: 0975-8585

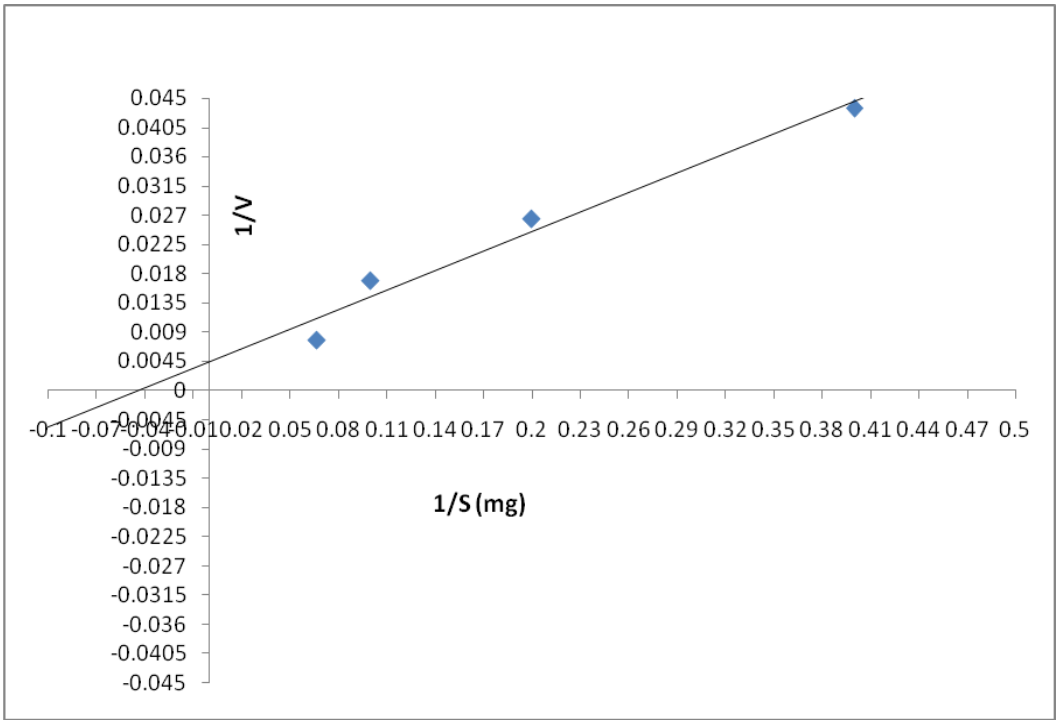

Figure (6b) : Lineweaver - Burk plot's of E_2 invertase.

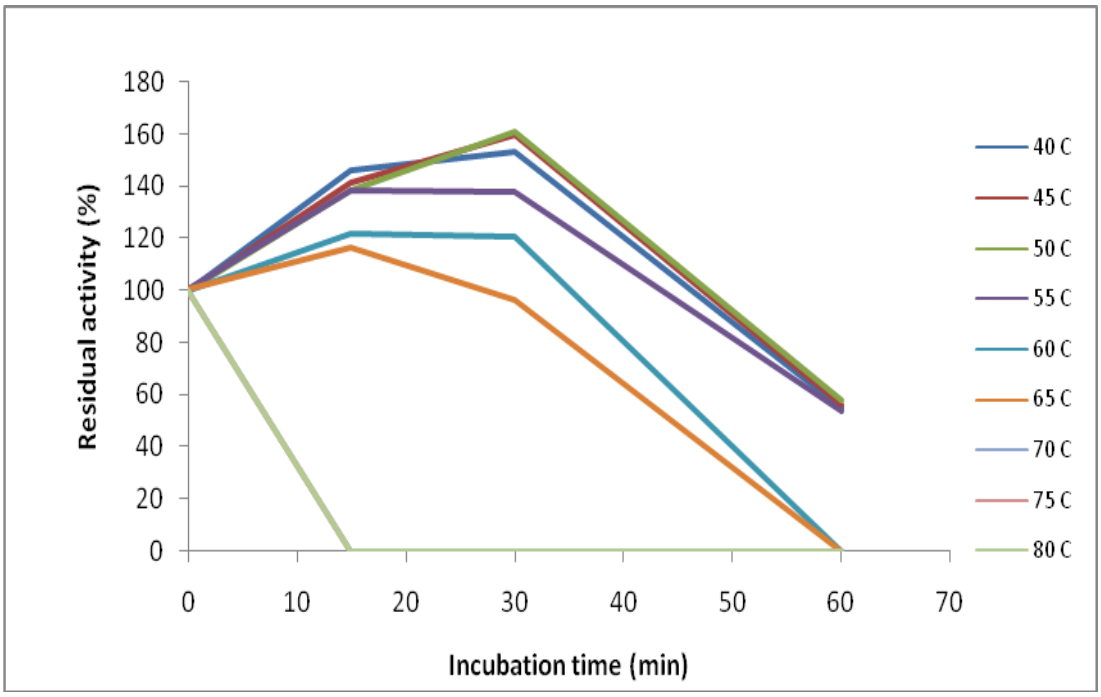

Figure (7a):Thermostability of E_1 invertase.

ISSN: 0975-8585

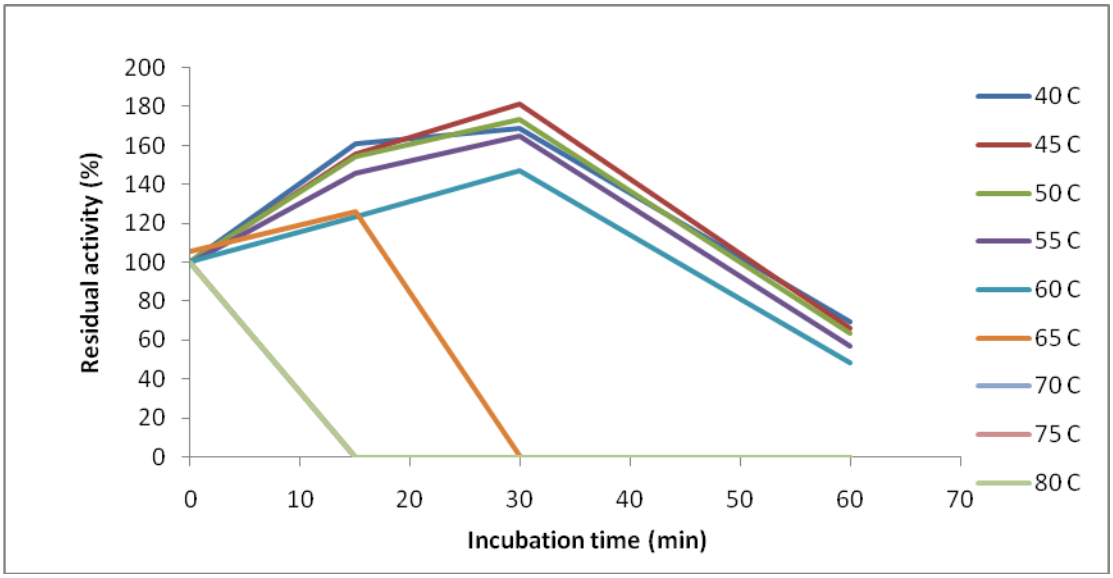

Figure (7b) :Thermostability of E_2 invertase.

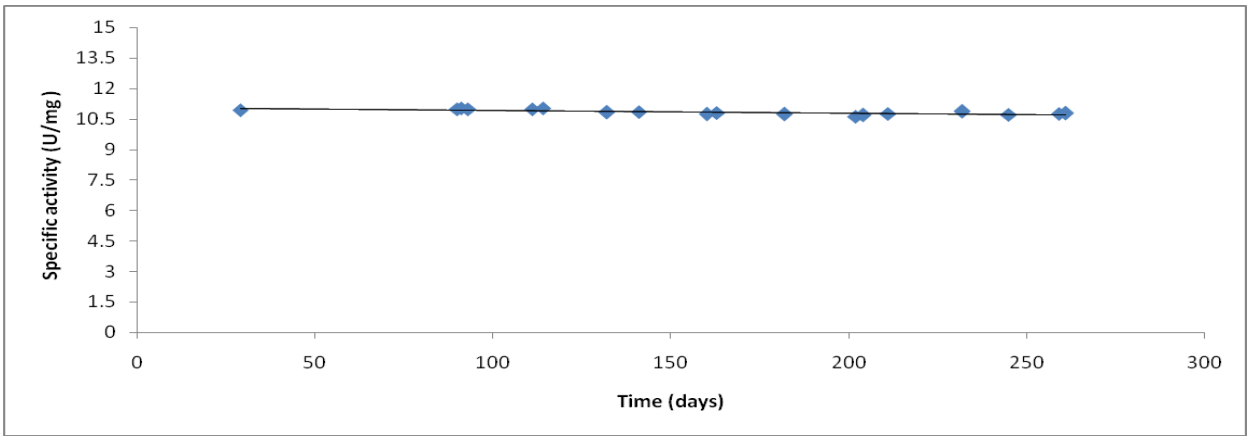

Figure (8a): Storage stability of E_1 invertase stored in distilled water at 4°C.

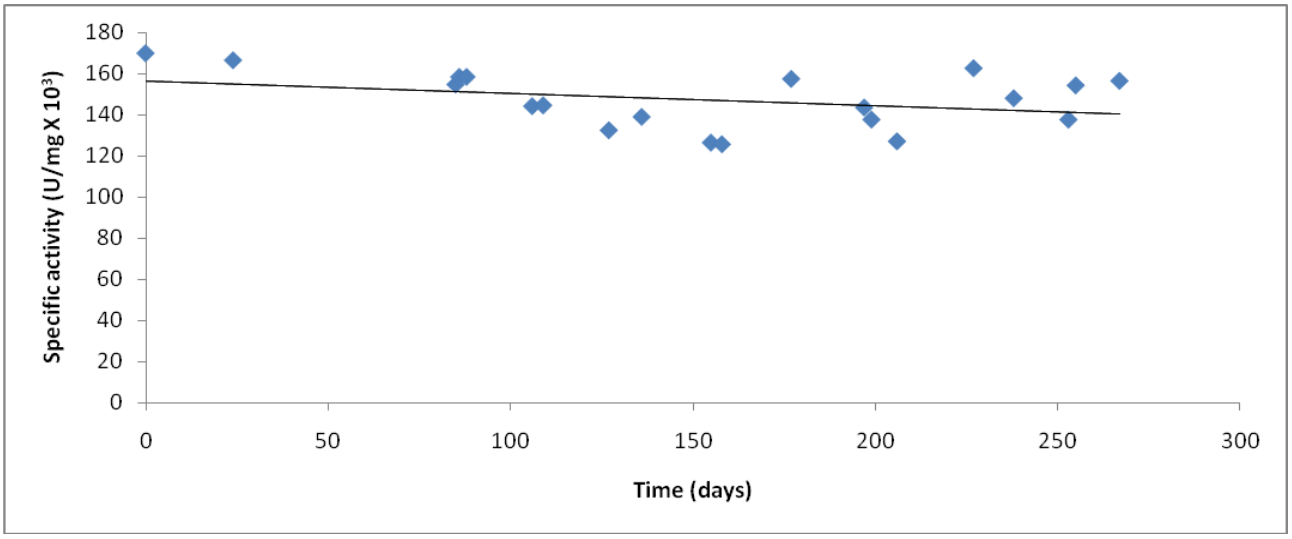

Figure (8b) Storage stability of E_2 invertase stored in distilled water at 4°C.

Storage stability

This experiment was conducted to determine the stability of E_1 and E_2 enzymes in distilled water during the storage for about 270 days at 4°C (fig 8a&b). The residual activities were measured by the standard assay procedure described earlier. The activity of the enzymes was expressed as a percentage of its residual activity compared to the initial activity. After 270 days of storage in water at 4°C, the prepared E_1 and E_2 enzymes have lost about 3 and 11.4 % only of their initial activities, respectively. Thus, E_1 is more stable than E_2.

The satisfactory heat and storage stability of our E_1 and E_2 enzymes suggests that they could be employed constructively in an industrial.Since there was not found in literature any work for storage of crude and partial purified invertase with the similar days or relative activity, the result obtained in this search represents the highest operational stability so far described.

Invert syrup production

Hydrolysis of sucrose using E_2 invertase (prepared from 100 gm fresh pea pods) under the digestion condition mention before, we can obtain 156 mg invert sugar. The concentration of the invert solution was 23.7 % (mg/100 ml). The degree of hydrolysis value is 3.9%.

CONCLUSIONS

This study indicated that pea pods are rich in invertase enzyme. It was chosen to prepare invertase enzyme. Pea pods are considered as a very economic source for invertase production because of its availability as an agricultural waste. It is inexpensive raw plant source. The prepared invertase E_2 with high invertase activity (168.08 U/mg) hydrolysis sucrose at wide range pH (5.0-7.4) at 45°C. It is specific for hydrolyzing α 1,2-glycosidic linkage. E_2 is stable at temperature up to 65°C for 30 mins. E_2 is also highly stable when storage in water at 4°C for 270 days. The prepared enzyme may be suitable for industrial application in sucrose hydrolysis for fructose syrup production.

REFERENCES

[1] Vu TKH and Le VVM. Biochemical Studies on the Immobilization of the Enzyme Invertase (EC.3.2.1.26) in Alginate Gel and its Kinetics. ASEAN Food Journal, 2008; 15: 73- 78.

[2] Mahmood WA. Hydrolysis of sucrose by immobilized Saccharomyces cerevisiaeinvertase. Mesopotamia Journal of Agriculture, 2010; 38-44.

[3] Yadavilli SK, Sindhi V, Tyagi P and Kulshrestha S. Invertase and its Applications – A Brief Review. Journal of Pharmaceutical Research, 2013; 7:792-797.

[4] Arnold WN. β- fructofuranoside from grape berries. Biochemistry and Biophysics Acta, 1965; 110:134-147.

[5] Peter B and Stephanie D. Sugar Accumulation in tomato and partial purification of the buffer insoluble invertase. Phytochemistry, 1994; 36: 837- 84.

[6] Jwanny EW and El-Sayed ST. Glycosidases of Turnip Leaf Tissues. II. Isolation, Purification, and Some Physicochemical Characterization. Applied Biochemistry and Biotechnology, 1994; 49: 23-33.

[7] Aranda C, Robledo A, Loera, O, Contreras-Esquivel JC, Rodrigues R and Aguilar CN. Fungal Invertase Expression in Solid - State Fermentation. Food Technology and Biotechnology, 2006; 44: 229-233.

[8] Oztop HN, Hepokur C and Saraydin D. Hydrogels for Immobilization of Invertase. Journal Food Science, 2009; 74: 45-49.

[9] Kotwal SM and Shankar V. Immobilized invertase. Biotechnology Advanced, 2009; 27: 311- 322.

[10] Acosta N, Beldarrain A, Rodriguez L and Alonso Y. Characterization of Recombinant Invertase Expressed in Methylotrophic Yeasts. Biotechnology Application of Biochemistry, 2000; 32: 178 -179.

[11] Sanchez MP, Huidobro JF, Mato I, Munigategui S and Sancho MT. Evolution of Invertase Activity in Honey over Two Years. Journal of Agriculture Food Chemistry, 2001; 49: 416- 422.

[12] Somogyi M. Notes on Sugar Determination. Journal of Biological Chemistry, 1952; 195: 19 - 23.

[13] Nelson W. A Photometric Adaptation of the Somogyi Method for Determination of Glucose. Journal of Biological Chemisrty, 1944; 153:375- 380.

[14] Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. Protein Measurement with Folin Phenol Reagent. Journal of Biological Chemistry, 1951; 193:265-275.
[15] Green AA and Hughes WL. Protein fractionation on the basis of solubility in aqueous solution of salts and organic solvents. In: Methods in Enzymology, 1955. (Ed by Colowick, S.P. and Kaplan, and N.O.); 1: 67-90. Academic press, New York.
[16] Lineweaver H and Burk D. The Determination of Enzyme Dissociation Constant, Journal of American Chemistry Society, 1934; 56:658-666.
[17] Deutscher MP. Methods in Enzymology. Guide to Protein Purification, New York: Academic Press, 1990; 182: 285-306.
[18] Alam JM, Rahman HM and Abu Sayem MS. Extraction, Partial Purification and Characterization of Tomato Invertase. Process Pakistan Academic Science, 2007; 44: 98- 103.
[19] Krishnan HB, Blanchette JT and Okita T. Wheat Invertase; Characterization of Cell Wall Bound and Soluble Forms. Plant Physilology, 1985; 78: 241-245.
[20] Nakanishi K and Yokotsuka K. Purification and Some Properties of thermostableInvertase from Wine. Fermentation Bioengeneering, 69; 16-22.
[21] El-Sayed ST and Jwanny EW. Glycosidases of Turnip Leaf Tissues. I. Physicochemical Properties of Myrosinase and Disaccharase Enzymes. Applied Biochemistry and Biotechnology, 1994; 49: 11-21.
[22] Rashad MM, Nooman MU, Abdou HM, Mahmoud AE. Production and Immobilization of Extracellular *Saccharomyces cerevisiae*Invertase using Soybean Residue as a substrate. Advanced Food Science, 2011; 33: 79-88.
[23] Ishikawa NH, Nakagaw D and Ogura K. Isoforms of invertase in grape berries. Agriculture Biological Chemistry 1989; 53: 837-838.
[24] Hari BK, Krishan J and Steven GP. Cherry fruit Invertase: Partial Purification, Characterization and Activity during fruit development. Journal of Plant Physilogy, 1990; 135: 662-666.
[25] Isla MI, Salemo G, Pontis H, Vattuone MA and Sampietro AR. Purification and Properties of the Soluble Acid Invertase from *Oryza sativa*. Phytochemistry, 1995; 38:321-325.
[26] Talekar S, Ghodake V, Kate A, Samant N, Kumar C and Gadagkar S. Preparation and Characterization of Cross- linked Enzyme Aggregates of *Saccharomyces cerevisiae*Invertase. Australian Journal of Basic and Applied Science, 2010; 4: 4760 – 4765.
[27] Finger FL and Klotz KL. Properties of soluble acid invertase from Beta *vulgaris L*. Roots. Plant biology, 2000; 597-560.
[28] Emregul E, Sungur S and Akbulut U. Polyacrylamide- Gelatin Carrier System Used for Invertase Immobilization. Food Chemistry, 2006; 97: 591- 597.
[29] Hertz PF, Rodrigues RC, Klein MP, Alves JS and Valerio SG. High Operational Stability of Invertase from *Saccharomyces cerevisiae* Immobilized on Chitosan nanoparticles. Carbohydrate Polymers, 2013; 92: 462-468.
[30] De la Vega M, Cejudo F and Panwque. Purification and Properties of an extracellular invertase from Azotobacterchroococcum Enzyme. Microbial technology, 1991; 13: 267-71.