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Extraction and purification of ßeta-galactosidase from a Local Almond seeds(*Prunusamygdalus*)

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Abstract: *ßeta-galactosidaseenzyme is greatly dispersed in plant tissues and it's known to be implicated in hydrolyzing terminal non reducingß.D.Galactosyl residues from polysaccharides . This study involved the selection of the best extraction of the enzyme among seven methods.The phosphate buffer (pH7) had given a highest activity of crude enzyme was 8.85 U. The aqueous crude of almond seeds was used to detect of some compounds by phytochemical screening tests and using Thin layer chromatography(TLC). The protein content was concentrated and precipitated by ammonium sulphate (0-40)% as apartial purification. The purification stages of crude enzyme were achieved by using gel filtrationchromatography (sephadex-75) at a yield of (136.4)% with activity was 4.4 u/ml . followed by ion-exchange column chromatography (DEAE-cellulose A52) and five iso-enzymes were obtained at a yield of 93% with 4.6 times of purification fold for iso-enzyme (I) while 49.6% and 4.2 times of iso- enzyme (V).*

Key words*: Almond seed ,ß.Galactosidase ,Purification methods*

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I. Introduction

Almonds Prunusamygdalus are one of the prevalent well known tree nuts on a world premise and rank number one in tree nut creation, they are a decent wellspring of fantastic weight premise [1] . Almonds have interesting organic properties for example narcotic ,hostile to tumor, anti-tumor,anti-inflammatory,anti-hyperlipidemic and against oxidant exercise [2]. B-Galactosidase (EC:3.2.1.23) enzyme could sever B-linkedGalactoseresidues from grouped mixes and it is commonly used to cut lactose into Galactose and Glucose [3]. It has been recognized in in awide scope of plant organs and tissues and it is portrayed by their capacity to hydrolyze terminal non-reducing B-D-Galactosyldeposit from B-D-Galactosidase [4]. B-Galactosidase was refined from different plant sources like chick pea [5] , almond[6] and apricot seeds [7]. It is found in nature and in various microorganisms , plant and creature tissues [8]. Almonds have been customarily overcome with drain in India , while taking a short at lactose narrow mindedness. It was accounted for that almonds are exceptionally rich wellspring of B-Galactosidase[9] . This chemical assume enter part in natural product maturing and it has movement amid organic product advancement and aging for rice ,B-Galactosidase from almond seeds was utilized for the planning of detactosed drain for those lactose narrow minded people [10] . In this investigation , B-Galactosidase from a neighborhood almond seeds has been removed , division and cleansed by Gel filtration, particle trade chromatography andcharacterized by phytochemical examination .

II. Materials and Methods

-Materials

Almond seeds were procured from the local market in Baghdad. Extraction and characterization of B-Galactosidase were conducted at the Biochemistry lab.

-Preparation of soaking almond seeds in water

Almond seeds were blending for 5 min to prepare the aqueous extract by soaking 10gm of it in 50ml of distilled water for 72 hour at room temperature . The extract was filtered through cotton sheet and it kept at 4 c^{o} until for use.

-Detection of active compounds in almond seeds

Soaking almond seeds in previous step was performed according to Neelapu*etal* [11]. Tests were Tannins , glycosides , Flavonoids , reducing sugars , Terpenoids , Saponins and Alkaloids.

-Separation of sugars by Thin layer Chromatography

Thin layer chromatography (TLC) was used notice lactose hydrolysis in to itsingredients : GlucoseandGalactose. This process performed according to Al-Hassnawi [12].

-Optimization of conditions for enzyme

The B-Galactosidase from almond seeds was extractedby buffers of different pH values and various solutions suchas distilled water , sodium Carbonate at the rate 1:5 , 0.2 M buffer phosphate (pH7) , sodium chloride (1:4) , 0.2 M buffer acetate (pH5) [13] , 0.2% potassium chloride and 0.2% Calcium chloride to determine the best solution of extraction.

-Assay of B-Galactosidase activity

The enzyme activity was estimate according to Sekimata*et al* [14] .

-Determination of Total protein

Total protein concentration was determined according to lowery *et al*. [15] by using Bovine serum albumin (BSA) as the standard .

-Partial Purification of B-Galactosidase from almond extract

Enzyme purification process was done by using the following steps .

-Precipitation by using $(NH_4)_2SO_4$Sulphate

The crude enzyme was saturated with inorganic salt like ammonium sulfate (0-40)% and gradually was added to 5ml of crude extract in a beaker with magnetic stirring at about $4C^o$ for one hour until the solution became turbid , and then the precipitated proteins were centrifuged at speed (3500rpm) for 10 min to split the precipitate. The precipitate was dissolved in 3 ml of buffer solution (pH=7) then The B-galactosidase activity and total protein were estimated.

-Dialysis

It was one of important steps used in enzyme purification. To make it free from sulphateions , the ammonium sulphate fraction was dialyzed by using cellulose tubing 3ml of crude extract that prepared in the previous step was put into atightly wrapped Cellophane bag from bottom and from top , then the pipe was put into a container which contains 1L of buffer solution (pH=7) inside refrigerator for 24 hour with repeated changes of buffer . The enzyme activity and total protein were performed .

-Gel filtration

The dialysate solution was concentrated against solid sucrose . 2ml of almond extract was passed through Column of sephadex gel (G-75) column with dimension of (40x 1.5 cm) . Twenty fractions were collected by passing buffer solution (PH7) through the column . with flow rate of 2ml/min .Enzyme activity and total protein concentration was also determined in each fraction .

-Ion Exchange chromatography

2ml of the fresh filtered extract was passed through a column of diethyl amino ethyl cellulose A-52 (DEAE) with dimensions of (15x 3cm) equilibrated with phosphate buffer pH7. Collection forty fractions for each one contain 2ml and complete isolation by phosphate buffer . Entire operation were carried out inside a refrigerator at flow rate of (2ml/4min) . B-Galactosidase activity and total protein for each fraction were determined.

III. Results and Discussion

-Phytochemical screening

It involves testing of almond extract for various phytochemical by qualitative chemical test to give general idea regarding the nature of components present in crude seeds. Table (1) shows the active compounds that tested in soaking of almond seeds such as Gycosides , Trepenoids , Alkaloids , Reducing sugar and Saponins . The positive result were attributed the role of almonds as antibacterial , antifungals , antivirals and anti- carcinogenic [16].

Table (1) : Phytochemical screening of almond aqueous crude

	Active compounds	Results
1	Tannins	-
2	Terpenoids	+
3	Flavonoids	-
4	Reducing sugar	+
5	Alkaloids	+
6	Saponins	+
7	Glycosides	+

(+) positive result (-) Negative result

A number of different extraction process have been reported using a different buffering systems , but all of them use of precipitation and Centrifugation to isolate the active protein . seven Solutions for enzyme extraction from almond seeds have been used . The results mentioned in Table (2) indicate that the extraction by 0.2M buffer Phosphate (pH7) is more efficiently compared with other extraction solutions. The specific activity and total activity of the enzyme extracted by above solution were 1.9×10^{-1} U/mg and 8.85U respectively. In the same table , the highest level of specific activity along with total activity may be attributed to the increase in the

ionic power which is the great effect of dismantling the ionic linkage connecting the enzyme and other components ,a factor that increases the enzyme dissolution [17].

Table(2): Methods of enzyme extraction from almond seeds

Extraction solution	Volume (ml)	Activity (U/ml)	Protein (mg/ml)	Specificactivity U/mg	Total activity (u)
Distilled water	44	1.0	35.71	2.8×10^{-2}	1.23
Na_2Co_3 (0.5%)	42	0.893	37.47	2.38×10^{-2}	1.0
0.2M buffer phosphate (PH7)	46	2.29	11.90	1.9×10^{-1}	8.85
NaCl (10%)	45	1.16	41.369	2.8×10^{-2}	1.26
0.2M sodium acetate (pH5)	44	0.638	12.66	5.03×10^{-2}	2.21
KCL (0.2%)	42	0.96	25.59	3.7×10^{-2}	1.57
$CaCL_2$ (0.2%)	46	0.96	15.431	6.22×10^{-2}	2.86

-Partial Purification of ß-Galactosidase

Table (3) illustrates the purification steps of ß-Galactosidase enzyme for obtaining a Pure enzyme from extract. The results derived from enzyme concentration by Salting out with ammonium sulfate. Ammonium sulfate solution which is a widely used because it is highly dissolved in water and not damage the protein [18]. The ß -Galactosidase activity reached to 1.83U/ml by using ammonium sulfate salt.With saturated percent (0-40%). This activity is slightly high than the activity of the crude extract was 1.29U/ml .Javevi*et al* [19] indicated a partial purification method by (30-60)% of Ammonium sulphate. In the present studythe purity degree ofß -Galactosidase was reached to 3.6 fold with yield 85.1% by dialysis while it was 2.9 fold with yield 136% by Gel filtration.

Table(3): Steps of ß-Galactosidase enzyme purification from almond seeds extrac t

Steps	Elute (ml)	Activity U/ml	Total Activity (U)	Protein (mg/ml)	Total Protein (mg)	Specific Activity (U/mg)	Fold purification	Yield %
Crude extract pH7	5	1.29	6.45	11.9	59.5	0.10	1	100
Ammonium Sulphate (40-40%)	3	1.83	5.49	8.5	25.5	0.21	2.1	85.1
Dialysis	2	2.31	4.62	6.4	12.8	0.36	3.6	71.6
Sephadex G-75	2	4.4	8.8	15	30	0.29	2.9	136.4
Ion Exchange								
IsoI	2	3	6.0	6.5	13	0.46	4.6	93.0
IsoII	2	2.3	4.6	3.05	6.1	0.75	7.54	71.3
IsoIII	2	1.8	3.6	6.15	12.3	0.29	2.9	55.8
Iso IV	2	2.44	4.88	5.1	10.2	0.47	4.7	75.65
IsoV	2	1.6	3.2	3.8	7.6	0.42	4.2	49.61

Gel filtration is known as size exclusion or molecular sieve chromatography. ß-Galactosidase gave one peak of protein with one peak of enzyme activity were 15mg/ml and 4.4 u/m respectively as shown in Fig(1).

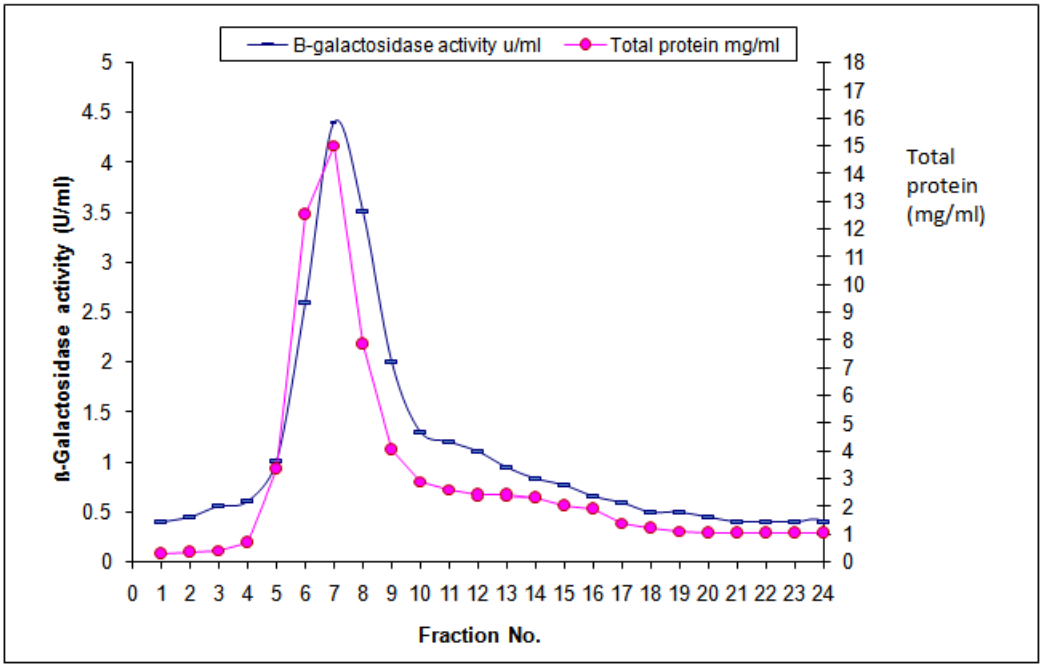


Figure (1): Elution of Gel filtration Chromatography (Sephadex -75) fractions plot of protein content values and ß-Galactosidase activity from a local Almond seeds extract.

Emadelin and Konozy [20] were used gel filtration (G100) to purify ß-galactosidase From *Erythrinaindica* seeds and Ajay *et al* [21] were extracted and purified of B-galactosidase from almond seeds (*AmygdalusCommunis*) by using the same Sephadex . The purification by ion exchange chromatography to separate of ions and polar molecules according to the charge . This enzyme was purified by using Diethylaminoethyl (DEAE-Cellulose A50) for binding to net negatively charged proteins [18] . In this study five isoenzymes were obtained as mentioned in Fig (2) and the purity degree of isoenzyme (I) was 4.6 fold with yield 93% , while it was of isoenzyme (V) was 4.2 fold with yield 49.6 with yield 49.6% of iso-enzyme (V) .

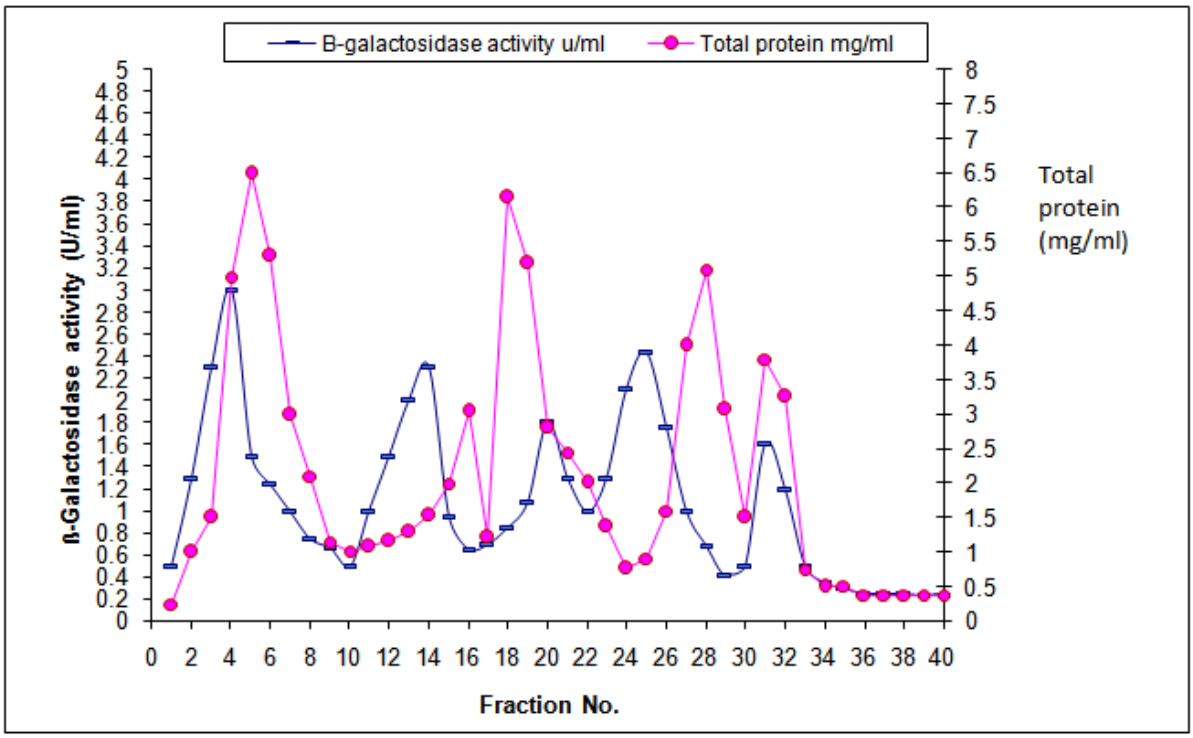
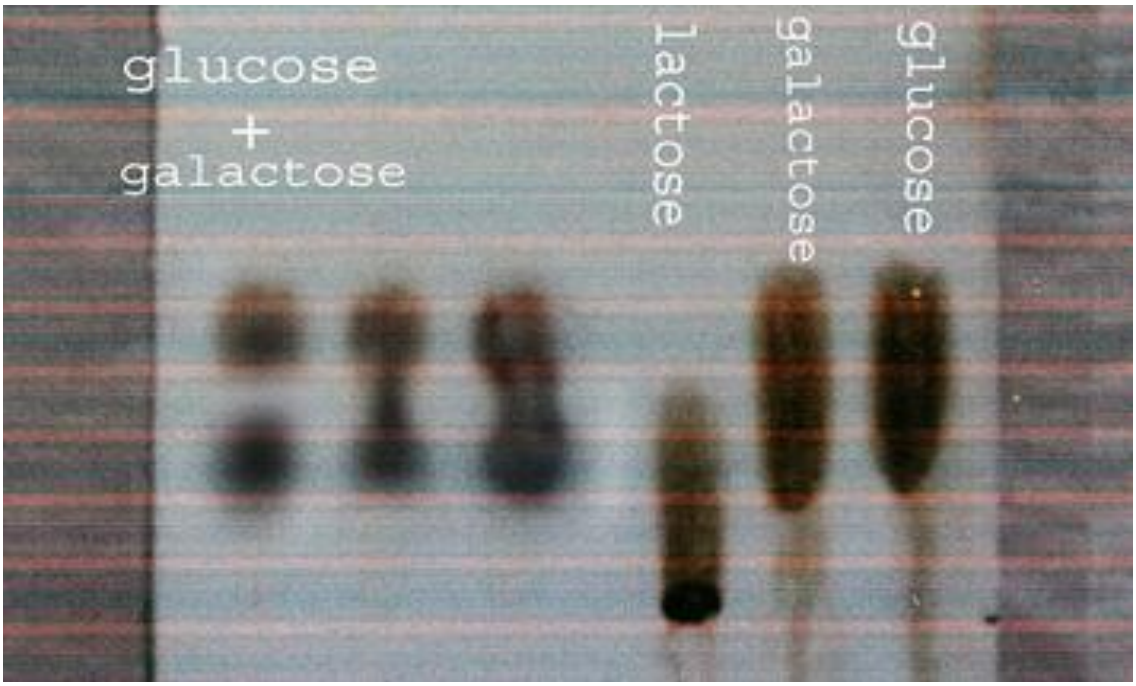


Figure (2): Elution of the Ion-Exchange Chromatography(DEAE cellulose -52)fractions versus protein content values of ß-Galactosidase from a local Almond seedsextract.

Although numerous of ß-Galactosidase have been purified from micro organisms . only Few reports are available from plant sources . Twoisoenzymes of ß-Galactosidase were purified from cowpea cotyledons [22], and from barley *HordeumVulgare* [23]. Also , ß-Galactosidase was purified from almond seeds and these findings indicated that the almond seeds can be successfully employed for the production of low-lactose/ delactosed milk from lactose-intolerant people [24].

-Thin layer Chromatography (TLC)

Fig. (3) shows the B-Galactosidase power to degrade the lactose to ingredients of monosaccharaides , Glucose and Galactose these spots were clearly appeared especially of glucose and they identical to spots of standard saccharides . These findings may be attributed to genetic and ecology factors such as Components of soil , pH, temperature , intensity and duration of light that effected in types and quantity of compounds [25]. The carbohydrates have extremely hydrophilic Compounds that have strongly attach to absorbents such as silica gel , alumina and cellulose. Thus highly polar solvents were necessary in the mobile phase of TLC development [26]. Similar results were reported by Shahad [27] who studied the hydrolyzed of lactose to their monosaccharaides by B-Galactosidase that extracted by apricot . Also , these results were agreement with pervious study who illustrated the purified of this enzyme from *Psychrotolerant Bacillus* and treated with lactose at 50C^{o} for 4 hours [28].



Figure(3): Hydrolysis of Lactose by ß-Galactodidase from Almond seeds by Thin Layer Chromatography(TLC)

IV. Conclusion

The Crude extract of seeds of local almond was used as a source for enzyme ß-Galactosidase . The phytochemical screening of almond aqueous crude, separation by TLC, enzyme activities by natural salt fractionation (0-40)%, Gel filtration and ion exchange were investigated . ß-Galactosidase have a maximum activity at pH7 was 4.4 u/ml by gel filtration .Five isoenzymes were obtained by using Ion-Exchange Chromatography with a highest yield was 93% and 4.6 fold for isoenzme (I),TLC indicated the degradation of lactose by this enzyme.

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