# EXTRACTION AND PURIFICATION OF BETA-GALACTOSIDASE FROM LOCAL ALMOND AND ITS USE FOR LACTOSE INTOLERANCE TREATMENT

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**ABSTRACT**

**This study was aimed, extraction and purification of beta-Galactosidase from local almond(*Amygdalus communis*) for lactose intolerance treatment. The best one among 10 methods method of the extraction was using sodium phosphate buffer at 0.2 molar. Which was achieves the highest specific activity amounted to 3.66unit/mg protein. Then, partial purification of enzyme was done using five methods. The highest specific activity was obtained using the method of precipitation with ammonium sulphate at 30-70% since the specific activity was 15.85units/mg protein. Which represented the best way to precipitation the enzyme. Three iso enzymes were obtained. One of them was taken for its high specific activity(20.10units/mg protein) and ion exchange chromatography was used and followed by gel filtration technique using sephadex-100 column to increase purification. The specific activity was increased to 21.95units/mg protein. Lactose hydrolysis efficiency test was performed and the purified enzyme showed high efficiency in standard lactose hydrolysis test.**

**Key words: enzyme, lactose, lactase, specific activity, ion exchange, gel filtration**.

مجلة العلوم الزراعية العراقية -2020 :51 (3):767-776 العيساوي وآخرون

## استخلاص وتنقية انزيم بيتا–كالاكتوسايديز من اللوز المحلي لاستخدامه في معالجة ظاهرة عدم تحمل اللاكتوز

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**المستخلص**

أُجريت هذه الدراسة لأجل ايجاد مصدر طبيعي لإنزيم بيتا–كالاكتوسايديز من اللوز ***Amygdalus communis*** المحلي لغرض معالجة ظاهرة عدم تحمل اللاكتوز. اختيرت افضل طريقة من عشرة طرائق استخلاص فكانت طريقة استخدام دارئ فوسفات الصوديوم بتركيز 0.2 مولار حصلت هذه الطريقة على اعلى فعالية نوعية بلغت 3.66 وحدة/ملغم من البروتين. ثم اجريت تنقية جزيئية للأنزيم باستخدام خمسة طرائق وحصل اعلى فعالية نوعية عند استخدام طريقة الترسيب بكبريتات الامونيوم بنسبة 30–70% بلغت عندها الفعالية النوعية 15.85وحدة/ملغم، بعدها اكملت عملية التنقية باستخدام عمود التبادل الايوني (DEAE–cellulos)، وقد حصل على ثلاث متناظرات للإنزيم اخذ النظير ذو الفعالية النوعية الاعلى البالغة 20.10 وحدة/ملغم ثم تلت عملية التبادل الايوني استخدام تقنية الترشيح الهلامي باستخدام عمود(Sephadex–100 ) لزيادة النقاوة وعندها ارتفعت الفعالية النوعية الى 21.95وحدة/ واجري اختبار كفاءة تحليل اللاكتوز واظهر الانزيم كفاءة عالية في تحليل اللاكتوز القياسي.

**الكلمات المفتاحية: انزيم، الفعالية النوعية، التبادل الايوني، الترشيح الهلامي، اللاكتوز، اللاكتيز.**

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## INTRODUCTION

Beta- Galactosidase enzyme is widely found in nature, in microorganisms, plant and animal tissue. The enzyme has been isolated from various sources such as bacteria, molds, yeasts and vegetables, and is found in low percent in fruits such as tomatoes, apples, melons, avocado, kiwi, coffee, mango. the enzyme is also present in the animals during the Lactation period. So, many studies have been conducted to look for sources of beta-Galactosidase and its commercial production (28,43). This enzyme analyses the disaccharide lactose it two monosaccharides, galactose and glucose which enter into glycolysis. The enzyme also converts the lactose into allolactose, which decomposes into monosaccharides. Allolactose regulates the amount of beta- Galactosidase in the cell with positive feedback and people who have the lactose intolerance are lacking beta-Galactosidase in intestine (16). Francesconi *et al*(18) reported that people with enzyme-deficient consumed fermented dairy products do not contain lactose or contain very little lactose. Beta- is present in the mammals during lactation period. In most people, beta-Galactosidase activity decreases affter this period, which is characterized by the onset of lactose intolerance, which causes the symptoms of lactose intolerance. On the commercial scale , bacterial or fungi lactase is used in the treatment of milk products, and enzyme-containing drugs are available which can be taken before consumption of milk products (12,34). Almond seeds can be used to produce almond milk to treat enzyme deficiency conditions. Almonds have been found to be a very rich source of beta-Galactosidase, which is characterized by its biochemical properties, and it is best to study its properties for use to treat health and nutritional conditions(27)**.**

## MATERIALS AND METHODS

A sample of unpeeled almonds was purchased from the Sulaymaniyah market and then peeled and crushed well using a domestic blender mill and kept in the nylon bags under freezing until use.

**Determination of the basic components**

The basic components of almond powder were estimated according to the methods in AOAC (1).

**Extraction**

Methods were adopted using different solutions for the purpose of determining the optimal method for extracting beta-Galactosidase from almonds. The best method was to extract the enzyme by Al-Arrji(6) method by mixing 10 g of almonds with 100 ml of 0.2 molar phosphate solution buffer pH number 7 in the electric mixer for 15 minutes, and then filtered through a damp cloth after centrifugal rejection at 6000 cycles / min at 4 Cº for 20 minutes, the supernatant was collect in tightly-closed plastic tubes and keep in freezing temperature at - 20 Cº until use in estimating enzymatic activity and protein concentration to calculate specific Activity.

**Enzyme activity assay**

The method described in Food Chemical Codex (17) was followed to estimate the activity of beta- Galactosidase

**Protein concentration assay**

The Lowry method indicated by Everett *et al*(15) was used to measure protein concentration.

**Purification of the beta- Galactosidase enzyme**

**Concentration the crude enzyme (partial purification):** The crude enzyme extract was concentrated using several different methods to determine the optimal method of concentration for specific activity and the highest percentage of the yield of the enzyme as follows:

**Precipitation by cold acetone**

The method which was mentioned by Al-hassnawi(8) was used**.** The enzyme extract was mixed with cold Acetone in ratios of 0.5: 1, 1: 1, 1: 2, 1: 3 and 1: 4 (V / V) respectively with continuous blending in a ice bath using magnetic stirrer. Then, the mixture was centrifuge at 5000 rpm for 15 min. the supernatant was discarded and the precipitate was dissolved in a least amount of 0.2 molar Phosphate buffer solution ( pH 7). The volume of the enzyme solution was measured and the activity and protein concentration were estimated.

**Precipitation by ethyl alcohol:** The volume of 30, 50 and 60% ethyl alcohol was gradually

added to obtain gradual precipitation rates with continuous mixing and the process was complete as the previous step1 **(**32).

**Precipitation by ultrafiltration**

The ultrafiltration process of the raw enzymatic extract was carried out using 50ml capacity ultrafiltration cell(type Amicon-Model K-52-PS, MAX Nether land) equipped with Diaflo Ultra filter( type XM-100) after one soaking process. Enzymatic extract was added to the cuvette and closed. Nitrogen gas was used to push the extract through the filter at pressure 3.5 kg / cm with the cell placed on a magnetic stirrer until the ultrafiltration is finished. The volume of the extract was then measured and the enzyme concentration and activity was estimated(24)

**Precipitation by ammonium sulfate**

The crude enzyme concentration was precipitated using different saturation rates of ammonium sulfate ranging from 30-70% according to the table by Segel(36) and precipitation was completed as in the previous step.

**Enzyme concentration by dialysis**

The method of Hussein (23) was developed by placing the raw enzymatic extract in dialysis tube, then placed in beaker? surrounded by the sucrose, and stored in the refrigerator until the next day. then enzyme size, protein concentration and enzyme activity was measured.

**The step of enzyme purification (total purification)**

**1-Ion Exchange chromatography:** The method used by Whitaker (40) to prepare the ion exchange (DEAE-Cellulose) was applied in a column of 58 x 1.6 cm and was balanced with the same solution until the following day with a flow rate of 5 ml / min. After the equilibrium of the ion exchange column, using the pipette, 2 ml of the enzymatic extract solution which was partially purified with ammonium sulphate (30-70%) was applied. The enzymatic extract was then passed through the column. The fractions were collected in 3ml volume and flow rate 0.5ml/min. absorbance of the fractions was red at a wavelength 280 nm until absorbance fades. A volume of sodium chloride solution in concentrations ranging from 0.2-1 molar was then passed through the column to gradually break the protein bonds from the ion exchanger and to separate the enzyme with the recovery solution. The fractions were collected in tubes with 3 ml / tube and flow rate 0.5 ml /min. The absorbance was measured at a 280 nm wavelength. The absorbance readings function of the wash and elution fractions versus the fraction number was plotted and the tops of the wash and elution fraction were determined and the enzymatic activity of the fraction was estimated. The fractions that gave the highest activity were combined together. The volume protein concentration, enzymatic activity were measured, and kepts in closed plastic tubes and put in the freezing.

**2-Gel filtration chromatography by Sephadex G-100**

The Sephadex gel G-100 was suspended in Sodium Phosphate( pH 7) buffer solution of 0.01M. After degassing by a vacuum pump it was filled in a glass column with a dimension of 60x1.6cm. After filling and balancing the column, The concentration enzymatic extract which was obtained from the ion exchange process and used the sephadex G-25 for it concentration was added to the gel surface. The elution processes were carried out using the phosphate solution (0.01 M and pH 7) and with a flow rate of 0.5 ml/min and 3 mL per fraction. The absorbance was red at a 280 nm wavelength of the elution fractions. The fractions containing the activity were collected, Protein concentration and preservation of the purified enzyme in the freezing (32).

**Ability of enzyme to hydrolysis lactose:**

**Lactose analysis by thin layer Chromatography (TLC):**

A chromatographic method (TLC) was used to follow lactose hydrolysis to galactose and glucose as indicated by al-Hasnawi (8).

**RESULTS AND DISCUSSION**

**Estimation of the basic components of local almond**: The results showed that the percents of them were: Fats 42.83%, protein 18.68%, carbohydrates 19.63%, moisture 7.8% and fiber 9.75%. These compounds gives a large calories content of up to 542 kcal /100g. The results show that the high fat content increased the calories of the food. It was also increased the essential fatty acids of the mono and

polyunsaturated, natural antioxidants (xanthophylls) and phytosterols of the food.

**Extraction:** The results in Table 1 shows that the best way to extract the enzyme from almond is the 0.2molar sodium phosphate solution at pH 7, which gave the highest specific activity up to 3.66units/mg protein, and total activity of 1015.12units. The results were consistent with both Al-Arrji (6) and Pal(27) who extracted beta-Galactosidase from sweet almonds and deferent from Salah(8) who extracted the same enzyme from Apricot and Jassim(24) extracted it from the sheep liver. The difference in the efficiency of methods of extraction is due to several factors including the source of the enzyme, whether a plant or animal or a microorganism and the location of the enzyme in the source (inside the intra-or intercellular walls of cells or between cells), the size of the enzyme, molecular weight type extraction solutions, pH, ionic strength, ability of the enzyme to decompose the bond between the enzyme and the source, such as pectin and carbohydrate content, the potential of the enzyme to dissolve and spread through the extraction solution (2,7). The difference in activity may be mainly dueto the decline of these varieties of different genetic origins. Most plant products are good sources of polyphenols, which are known to be insoluble compounds with enzymes, which in turn inhibit the process of extracting enzymes and reducing their activity(21). The ability of phosphate buffer (pH7) to dissolve the bond between the enzyme and the components of the almond bean and its ionic strength play a role in separating the enzyme from the tissue containing it and increasing its specific activity (40,41).

**Table 1. Methods of extraction using different solutions**

| No | Solutions extract | volume (ml) | Activity unit/ml | Total activity U | Protein conc.mg/ml | Total protein mg/ml | Specific activity U/mg |
|---|---|---|---|---|---|---|---|
| 1 | Distilled water | 92 | 8.478 | 779.97 | 3.870 | 356.04 | 2.190 |
| 2 | sodium chloride%10 | 90 | 8.442 | 759.78 | 5.390 | 485.10 | 1.566 |
| 3 | Sodium carbonate 0.5 molar | 88 | 8.339 | 733.83 | 2.912 | 80.256 | 2.863 |
| 4 | Sodium phosphate0.2molar | 92 | 11.04 | 1015.1 | 3.012 | 277.11 | 3.664 |
| 5 | Buffer acetate 0.2molar | 90 | 8.187 | 736.83 | 3.123 | 281.07 | 2.622 |
| 6 | Ascorbic acid %0.06 | 95 | 7.991 | 759.14 | 2.34 | 222.30 | 3.415 |
| 7 | Potassium chloride 0.2molar | 90 | 2.113 | 190.17 | 1.923 | 173.07 | 1.098 |
| 8 | %2 Calcium chloride 2% | 95 | 9.380 | 891.10 | 5.042 | 478.99 | 1.860 |
| 9 | Glycerol solution 20% | 95 | 3.941 | 374.39 | 3.428 | 326.61 | 1.146 |
| 10 | Citric acid 0.01molar | 94 | 2.913 | 273.82 | 3.990 | 375.06 | 0.730 |

**Purification of Beta-Galactosidase**

Various methods were used to purify the crude enzyme after extraction for the purpose of its concentration, increase its activity and eliminate many of the overlapping substances and its associated proteins. These impurities may reach 65% of the extract components.

**Partial purification of beta-Galactosidase**

**Precipitation by cold acetone:** The results showed the highest activity 12.42 units / mg protein after it was in the original extract 3.66 units / mg. The ratio of acetone to the crude enzyme which gave the highest specific activity was 3-1 volume / volume. some researchers found that this ratio is the best(42). So, it was to precipitate the enzyme in the partial purification carried out by Al-Hasnawi who extract lactase from hin liver. Yahia *et al.* extracted the same enzyme from the brain of new born goat. Also, Prasad(29) used it to extract the enzyme from the soybean. It was also used to extract the enzyme from the soybean. The acetone has an effect on positive and negative charges of protein, as the addition of acetone leads to a change in the polarity of the solution through the new Line up of the charges. It leads to increase negative or positive charges and change the state of stability between the charges, which makes protein particles aggregate and gather in heavy units, and hence are easily precipitate according to the relationship mentioned by Whitaker (40) which states that the gravitational force is equal to the sum of the charges divided by the electric separation constant multiplied by the distance between the protein molecules as follows: $F = Z + * Z- / Dr^2$ where: F = the gravitational force and Z and Z- The positive and negative charge, electrostatic separation constant, and r distance

between two molecules of protein. Many researchers referred to that the role of acetone, ethanol and methanol in the process of enzymatic protein deposition is the reduction of the dielectric constant, which increases the attractiveness. Because of the constant separation of organic solvents, ethanol, methanol, in the process of enzyme protein precipitation is lowering the dielectric constant which leads to increase attractive force, and because the value of dielectric constant of organic solvents ethanol, methanol and acetone is 30 and for water is 80, so it increases the attractiveness and then the deposition of the protein. In order to avoid breaking the bonds in the protein synthesis and preventing the occurrence of protein mutagen when using organic solvents, cold acetone is used(11,37).

**Precipitation by ethyl alcohol:** Table 2 shows the results of beta-Galactosidase deposition by 60% cold ethanol, a specific activity of 21.31 units / mg obtained protein. Number of times of purification was 5.82 times and the yield was 44.8%. Ethanol, methanol and acetone have the ability to be mixed with water which is dependent on dielectric ability, which is much lower than water, which leads to a decrease in the value of the constant and increase the force that attracts the particles and hence aggregation and then precipitation. Many researchers have confirmed the activity of concentration of these organic solvents and preferred to concentrate using organic solvents in the precipitation of enzymes of its extracts without the use of salts in precipitation(20).

**Precipitation by Ultra-filtration:** The ultra-filtration method is used extensively in the purification of enzymes at the industrial and laboratory levels because they are carried out in cold conditions and do not affect the protein phase , do not become from soluble to precipitate and do not need many chemicals. This technique was used by a number of researchers, it was used by Saaed(31) in the concentration of enzyme and protease enzyme produced and extracted from the yeast *Candida albicans* and Dubey *et al*(14) in the purification of some enzymes extracted from different types of bacteria.

**Table 2. partial purification results**

| extract | volume (ml) | Activity unit/ml | Protein conc.mg/ml | Specific activity U/mg | Total activity U | Purification number | yield |
|---|---|---|---|---|---|---|---|
| **Crude extract** | **82** | **11.03** | **3.012** | **3.66** | **904.46** | **1** | **100** |
| **Acetone** | **20** | **17.5** | **1.41** | **12.41** | **350.0** | **3.39** | **38.7** |
| **Ethyl alcohol** | **25** | **16.2** | **0.76** | **21.31** | **405.0** | **5.82** | **44.8** |
| **ultrafiltration** | **25** | **10.02** | **2.03** | **4.93** | **250.5** | **1.38** | **27.7** |
| **Multiethyl glycol** | **15** | **13.7** | **1.4** | **9.78** | **205.5** | **2.67** | **22.72** |
| **Ammonium %30** | **15** | **12.6** | **2.00** | **6.29** | **189.0** | **1.71** | **20.9** |
| **sulfate %70** | **15** | **37.17** | **2.34** | **15.88** | **557.55** | **4.34** | **61.64** |

The results in Table 2 show that the specific activity obtained was 4.93 units / mg and the total activity was 250.5 units and the number of times was 1.38 times and the yield was 27.7%. In this case, the result is less than when using cold acetone, ethylene and sulfate.

**Precipitation by ammonium sulfate:** Ammonium sulfate does not affect pH and it precipitate the highest proportion of proteins present with the enzyme and does not affect the enzymes as it does not cause the denaturation of many proteins when deposition(25,26). As a result of neutralization on the protein particles, a decrease in protein solubility and exude of water layer coated the protein particles may occur and hence particles is deposited. A relationship is found between the concentration of sulfate used in precipitation and the quantity and distribution of loads and the number of non-ionic and water-damaging aggregates on the surface of the protein molecule. An enzyme deposition occurs according to salting out. The salts pull the molecules of water around the enzyme molecule and reduce the power of the electrical separation between the particles of the protein, which increases the strength of the graft and then the aggregation and sedimentation. The saturation rate was used by most researchers from 15-90% (8,11,22). The results showed that beta-Galactosidase precipitation processes were taken place by adding ammonium sulfate at saturation rates from 30-70 table 2. The highest specific activity in 70% saturation was 15.88 mg / mg, 557.55 units total activity, 4.34 times of purification and 61.64 % yield compared with

ammonium sulfate addition by 30% saturation with a specific activity of 6.29 units / mg and a total activity of 189.0 units and a fold of purification of 1.71 and a yield of 20.9%. Results varied with most of the researchers, but the specific activity and total activity were in agreement with the results of Pal *et al*(27),Hashim and Nema(19),Abood and Hakeem(4) and the results of Al-Arraji(6) , who extracted the enzyme from a plant source while the specific activity and total activity is much less than that of Zwaini(10) who extracted the enzyme from a microbial and animal sources. Upon reviewing many other studies, it was found the superiority of microbial and animal sources in the abundance of the enzyme on plant sources.

**Concentration by dialysis:** Membrane separation bag was used for reducing the volume and increase purity. The specific activity reached 22.42 units / mg and a slight change in the total activity of 442 units, the number of times up to 6.13 and a yield of up to 48.86% were noticed. This change is due to the ability of the sucrose grains to withdraw water from the enzymatic solution with some low molecular weight proteins dissolved in water. This method was used to concentrate the enzymatic extract for the purification process in the ion exchange column and gel filtration. These granules are used in purification only for their limited ability to separate small and medium particles without large molecules**(**5) and it was used as an alternative for the non-available polyethylene glycol material(15), Sangeetha (33) also used this technique to remove non-enzymatic proteins in the lactase enzyme from the crud extract.

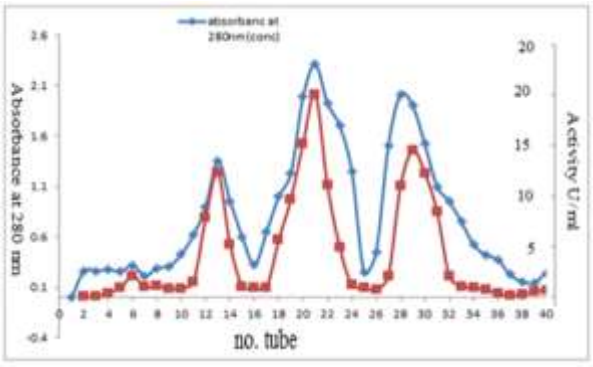



**Figure 1. Ion exchange chromatography column(DEAE-Cellulose), washing elute 0.01M sodium phosphate buffer, Mobil phase 0.2-1M sodium chloride**

**Final purificati**

**Ion exchange chromatography:** The sample of the concentrated enzymatic solution was passed through the negative ion exchanger column using the phosphate buffer solution and the separated fractions were received at a flow velocity of 5 ml / min. The absorbance was measured at a wavelength of 280 nm for the separated fraction of the exchanger during the wash phase. At this stage the positive proteins were eliminated with the wash solution and the relationship between the protein concentration and absorbance was determined as in Fig 1. Closely adjacent peaks for proteins that did not be alined with ion exchanger. These proteins were eliminated. It was observed that there was no or little activity in the separated fractions because of enzyme binding with exchanger. The beta-Galactosidase enzyme carries an opposite negative charge of the DEAE-cellulose and for the purpose of gradation in the separation of the weakly charged proteins first and then the stronger proteins are then separated and therefore the gradient is used to concentrate the saline solution. The results in Table3 shows that there were three isoenzymes separated from the ion exchange column by measuring the activity of the separated fractions as shown in the table. The highest specific activity was observed for isoenzyme 3, which was 20.10 units / mg, 5.5 number of times, yield 46.74%. As shown in figure 4 there were multiple peaks of concurrent activity with protein concentration indicating the beta-Galactosidase as isoenzymes. The results differed with most of the authors, but they were close to Pal *et al*(27) and Al-Arrji (6) who extracted the enzyme from almonds and less than that of Salah(32) who extracted the enzyme from peach and Jasim(24) who extracted the enzyme from sheep liver. It is noted that the results in general vary with most researchers, as each research has its special circumstances and the source of extraction of the enzyme, and the difference of food type such as almonds as well as plant nutrition, genetic status and environmental conditions. All these factors have a direct impact on the results obtained.

**Gel filtration chromatography:** After the completion of the filtration step using the ion

exchange column fractions with activity were collected and they were three. The highest activity peak was taken (isoenzyme 3). They were concentrated using the sephadex G-25 granules to eliminate water and ammonium sulfate and then transferred to the enzyme purification phase using the Sephadex-100 gel filter column which was equilibrated with 0.1M phosphate buffer pH 7. One protein peak and the peak of the activity were obtained at close numbers of the separated fractions as shown in Fig 2.

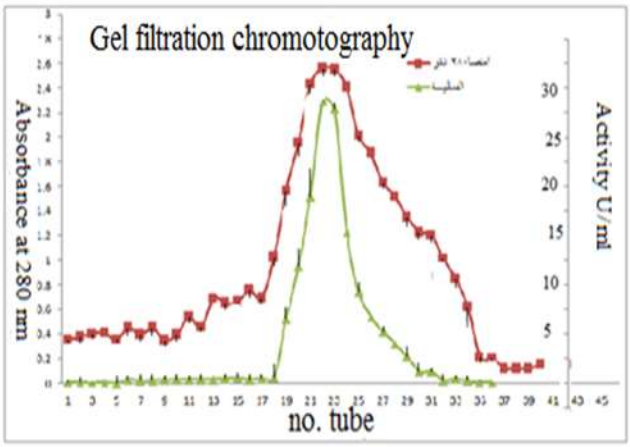



**Figure 2. The gel filtration chromatography (1.6x60cm sephadex G-100 column, 0.01M sodium phosphate buffer solution of Mobil phase. 0.5ml.min flow speed).**

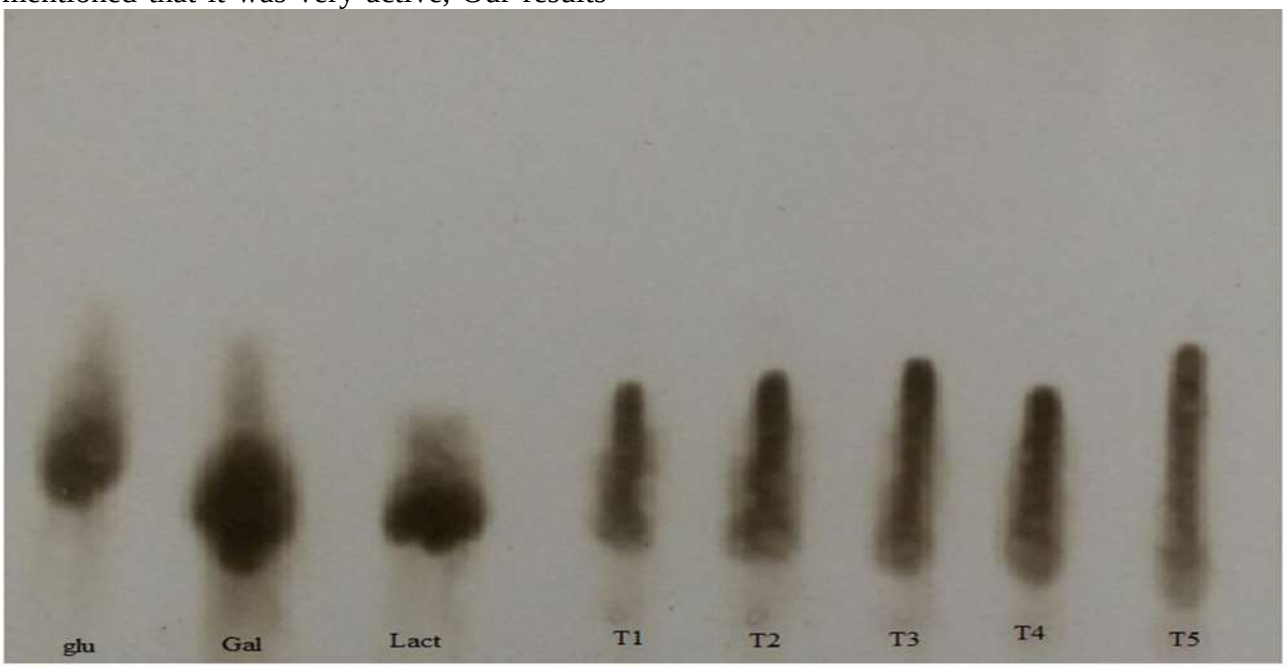
The separation mechanism takes place in this part of the purification process, depending on the molecular weight and shape. The molecules of large molecular weight pass initially through the spaces available in the gel column, followed by the least weight and then the smaller and the small particles are delayed to the end of the separation for penetration into the gel molecules after the passage of molecules that exceed the molecular weight. On this basis molecules of the enzyme and molecules that are equal in molecular weight are passed at the same time. The separation of the enzyme alone is taken place with a proportion of proteins similar in the shape and molecular weight. They are separated together with the enzyme. Sephadex G-100 had the possibility of separation of protein molecules with molecular weight ranging from 5-300 kDa(39). The results showed that the total yield was 39.93%, the number of purification times was The results showed that the total yield was 39.93%, the number of purification times was 5.87 times, the specific activity was 21.50 units / mg, and the total activity was 361.2 units when the final purity by sephadex G-100 was used to purify the beta-Galactosidase extract from local almonds. This result of the yield is in agreement 33.72% with Pal(27) who extracted the enzyme from almonds and differ in the specific activity ( 4.58 units / mg) and for the times of purification (50.9) it is different. These results are also different from Jasim(24) who extracted the enzyme from sheep liver. The differences are in yield (41.48%), purification times (8.55) and specific activity (3328.8units/mg).

**Table 3. Results of the steps of the final purification processes**

| Steps of purification | vol (ml) | Activity U/ml | Protein conc.mg/ml | Sp. activity U/mg | Total activity U | Purification number | Yield % |
|---|---|---|---|---|---|---|---|
| Crude extract | 82 | 11.03 | 3.012 | 3.66 | 904.46 | 1 | 100 |
| (30-70%)$(NH_4)_2SO_4$ | 15 | 37.17 | 2.34 | 15.88 | 557.55 | 4.34 | 61.64 |
| Dialysis | 9 | 48.00 | 2.14 | 22.42 | 442.00 | 6.13 | 48.86 |
| Ion exchange isomer 1 | 16 | 38.00 | 2.00 | 19.00 | 608.00 | 5.19 | 67.2 |
| Ion exchange isomer 2 | 14 | 33.20 | 1.7 | 19.53 | 464.80 | 5.3 | 51.38 |
| Ion exchange isomer 3 | 14 | 30.20 | 1.5 | 20.10 | 422.80 | 5.5 | 46.74 |
| Gel filtration | 14 | 25.80 | 1.2 | 21.5 | 361.20 | 5.87 | 39.93 |

**Lactose degradation (hydrolysis):** Thin layer chromotography (TLC), described Salah(32) was used to follow standard lactose hydrolysis by the enzyme beta-Galactosidase extracted from almonds to galactose and glucose. Standard solution of lactose, glucose and galactose were prepared at concentration of 5% in distilled water for each solution. They were incubated for various duration from one to eight hours. They were placed on the base line by microtubes and let to dry. The results showed that beta-Galactosidase was effective in hydrolysis of lactose to glucose and galactose as shown in fig1. by calculating Rf for each of the standard solutions of lactose, galactose and glucose (0.36, 0.49 and 0.50 respectively) and compared with Rf for lactose It was found that the Rf for lactose, which is

hydrolysis by the enzyme, is 0.40 during the first hour of addition of the enzyme and incubation. After 2 hours it was 0.42 and rose after 4 hours to 0.47 and after 6 hours it was 0.45 and the highest Rf after 8 hrs reached 0.50 so it corresponds to the Rf for standard solutions. As glucose and galactose have the same molecular weight and Rf are close to each other, they cannot be separated from spots to get some overlap between them. These results were inagreement with Jasim(24) who used lactase enzyme from sheep liver for hydrolysis of lactose and Salah (32) who used apricot beta-Galactosidase to hydrolysis lactose sugar. A number of authors have studies Beta-Galactosidase activity from different resources to hydrolyze lactose. Some mentioned that it was very active, Our results are in qualitative and quantitative agreement with Al-Hasnawi (8) which hydrolyzed lactose by Beta-Galactosidase produced from chiken liver and with Song(38) which used the yeast lactase at 50c° for 4hrs and Al-Manhal(9) who hydrolysis lactose by beta-Galactosidase enzyme produced from the fungus *Aspergillus oryza*. They stated that monosaccharides are separated upon cleavage of the glycosidic bond beta-Galactosidase(1-4). The products have less molecular. Weight and so it moves far than the large-molecular.Weight disaccharide (lactose). Small weights are affected by many factors like enzyme source, substrate, pH, temperature, reaction time and inorganic ions.




**Imag no 1 analysis of standard lactose by the enzyme beta-Galactosidase (from lift: glucose, galactose, lactose and T1-T5 time)**

The local almonds are a very rich source of beta-Galactosidase, which is an available, cheap and safe source. The sodium phosphate buffer of concentration of 0.2 molar is the best solution to extract the enzyme from the local almond. The enzyme has proved highly effective against ONPG(ortho nitro phenyl beta-Galactoside) and also very effective in the hydrolysis of lactose to galactose and glucose. Among the five methods used for partial purification using ammonium sulphate saturation of 70% was the best.

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