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Extraction, Purification and Characterization of B-Galactosidase from Tomato (Lycopersicom Esculentum) and It's Application in Milk

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Abstract: β-galactosidase (EC 3.2.1.23) is important in the formation of a medicinal plant tomato (Lycopersicom esculentum). The plant extracts of tomato (Lycopersicom esculentum) were used to characterize the enzyme in the term of pH, temperature, enzyme kinetic and effects of some heavy metals on its activity. The enzyme activity was measured by its ability to hydrolyze the substrate 2-nitrophenyl β-D-galactopyranoside (ONPG). the
isoelectric point for enzyme was 4.4. Carbohydrate concentration of enzyme was found to be 19.5 % by employing phenol-sulfuric acid method. The Km and Vmax values of the enzyme were 3.65 mM and 0.18μmol/min, respectively. The activities of β-galactosidase from tomato (Lycopersicom esculentum)is completely inhibited by HgCl2 and KCN. After 4h incubation, lactose in milk was reduced by 38.5 and 70% by β-galactosidase from tomato (Lycopersicom esculentum), respectively. The study showed that Hg+2 was the most potent inhibitor while Cu+2 exhibited the least inhibition degree on β-galactosidase activity in the tomato (Lycopersicom esculentum). These finding indicated that the enzyme β-galactosidase in the leaves extract of tomato (Lycopersicom esculentum) can be used in industrial and medical applications.
Keywords: tomato (Lycopersicom esculentum) β-galactosidase, Enzymatic Kinetics, Heavy Metals

Introduction

β-galactosidase (also β-D-galactohydrolase) called lactase and transglycosylases [1] are group of enzymes able to cleave β linked galactose residues from various compounds and is commonly used to cleave lactose into galactose and glucose [2], it was widely distributed in nature and found in many microorganisms, plant and animal tissues [3, 4].β-galactosidases have many biological roles include degradation of structural polysaccharides in plant cell walls; thereby can promote their loosening and th consequent elongating of the cell [5]. They have many medical and industrial applications include treatment of lactose malabsorption and production of lactose hydrolyzed milk [6, 7, 8]. These enzymes have two important applications: the removal of lactose from milk products for lactose intolerant people and production of galactosylated products [12, 13].β-galactosidases have been detected in a wide range ofplant organs and tissues and are described by their ability to hydrolyze terminal non-reducing β-D-galactosyl residues from β-D-galactosides [9]. It has been purified from various plant sources, like chick pea [10], almond [11], apricots [12], Vigna unguiculata [13], apricot seed [14]. β-galactosidase play key roles in fruit ripening. β-galactosidase activity was reported during fruit development and ripening for rice [15], pepper [16] and Arabidopsis [17]. Many studies have indicated remarkable increase in expression level of mRNA β-galactosidase during fruit ripening in many fruits [18]. It was reported that β-galactosidases are widely distributed in many plant tissues, like seeds [11,

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19], stems [19], and meristem zones of roots, trichomes, cotyledons, vascular tissues, and pollens [20, 21]. On the other hand, it also participates in the cell wall modification during elongation and differentiation of plant cells [22, 23]. Plant β- galactosidase would be best suited for industrial applications because of its easy availability, cost effectiveness and easy adaptability [24]. β-galactosidase from almond seeds was used for the preparation of delactosed milk for those lactose intolerants individuals [11].Heavy metals are essential and important for plants growth, and play a great role in many vital compounds [25]. Some of these metals are micronutrients necessary for plant growth, such as Zn2+, Cu2+, Mn2+, Ni2+, and Co2+, while others have unknown biological function, such as Cd2+, Pb2+ and Hg2+ [32]. At high concentrations, all heavy metals have strong toxic effects and are regarded as environmental pollutants [26]. They may alter the reaction rates and influence the kinetics properties of enzymes which cause changes in metabolism of plant, or any excessive amount of heavy metals may drive the oxidative stress [27]. (Lycopersicom esculentum) tomato in Arabic, is a perennial fragrant shrub, bushy herbs, grows in the valley bottoms in the desert areas particularly at the Southern part nearest Saudi-Jordan borders (Al- Mudawarah) and in Sinai Peninsula in Egypt [36, 37, 38]. It is used traditionally as a medicinal herb in Jordan and Egypt. Artemisia has multiple beneficial bioactivities such as antiviral, antipyretic, antihemorhagic, anticoagulant, antitumor, anti-anginal, anti-ulcerogenic and anti-hepatitis [39]. In this original study, the plant extracts of tomato (Lycopersicom esculentum). were used as a source for the enzyme β-galactosidase. The enzyme activities, kinetics and the effects of heavy metals were investigated as a primary step for the use of β-galactosidase in industrial, biotechnological and medical applications in future.

Materials and Methods

Materials

Fresh plant sample of tomato (Lycopersicom esculentum). was collected from Iraqi local markets. Characterization of β-galactosidase was conducted at the Biochemistry lab in Baghadad University.

Plant Extract Preparation

plant extract was prepared from tomato (Lycopersicom esculentum) and used as source for β-galactosidase. Plant were homogenized in 0.2 M sodium-phosphate buffer (pH 6.0) in a blender for 4 min. The homogenate was filtered using cloth sheet and then was centrifuged for 20 min at 10,000 rpm. The supernatants were used for β-galactosidase assay as crude enzyme solution [28].

Protein Estimation

The protein content has been specified in a spectro-photometric way at 595nm using the Bradford method [29] with the use of the Bovine Serum Albumin (BSA) as standard.

Enzyme Assay

Crude plant extract was prepared tomato (Lycopersicom esculentum) and used as source for β-galactosidase β-galactosidase activity was assayed by measuring the rate at which it hydrolyzes ONPG by the method described by Sekimata et al. [33]. In the presence of β- D-galactosidase, ONPG is hydrolyzed to D-galactose (colorless) and o-nitrophenol (ONP) (yellow). The reaction mixture of β-galactosidase contained 0.4 ml of 0.1 M acetate buffer (pH 4.0), 0.5 ml of 2 mM of

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substrate and 0.1 ml of enzyme solution. After incubation for 15 min at 37°C, the reaction was terminated by addition of 1 ml of 0.1 mM Na2CO3 and monitored at 420 nm. One unit of enzyme activity is defined as the amount of enzyme that liberates 1.0 µmol of ONP per minute under the assay conditions.

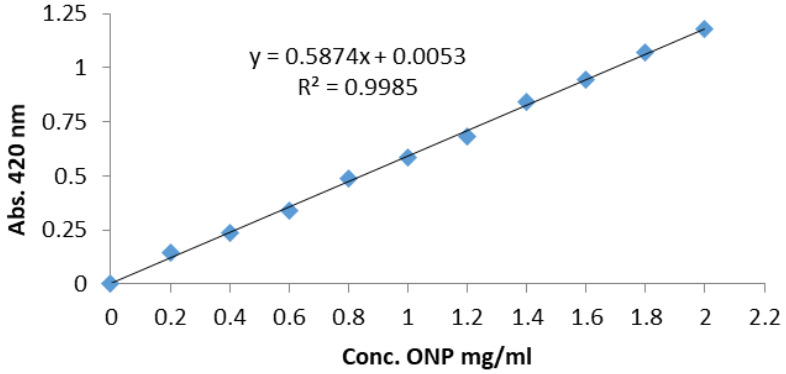

Figure1: standard curve of ONP using to quantified o-nitrophenol released in the presence of β-D-Galactosidase

Determination of Kinetic Parameters

To determine the maximum velocity (Vmax) and Michaelis-Menten constant (Km) of β-galactosidases, ONGP was used as substrate, and the effect of substrate concentration on enzyme activity were studied at pH 6.0 and at temperature 50°C. The concentration of ONGP was increased from 1 mM to 9 mM. The enzyme activity was assayed by monitoring the absorbance at 420 nm. Line weaver-Burk Plot (Reciprocal plots) used to determine Vmax, and Km values [33].

Activation energy determination:

Activation energy of enzyme for converting ONPG to product was determined using Arrhenius equation.

Determination of Isoelectric point (pI): Method that described by [16] in preparation of solutions and pI determination by isoelectric focusing, was used.

Estimation of Carbohydrate content Phenol-sulfuric acid method which described by Dubois et al wasused. [17]

Effect of metals ions, inhibitors and other substance on the enzyme activity

Stock solutions of CaCl2, HgCl2, CuSO4, FeCl3, KCN, NiCl2, MnCl2 and FeSO4 were prepared in acetate buffer (at optimum pH for each β-galactosidase extract) were added separately to the reaction mixture to a final concentration of 0.001 mol/Liter. Inhibitors such as beta-mercapt-oethanol (0.01 mol/Liter) and ethylene-diamine tetracetic acid (EDTA) (0.0001 mol/Liter) were also assayed. The residual enzyme activity was assayed and expressed as a percentage of the activity determined in acetate buffer alone (control).

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Statistical Analyses

Every experiment has been applied in triplicates and results have been represented as values of average ± standard deviations (SD) with the use of the Microsoft excel 2007.

Results and Discussion

. Protein Content

The protein content in the extract of tomato (Lycopersicom esculentum) was measured by Bradford method using BSA as standard protein (Figure 2). The result showed that the extract of tomato (Lycopersicom esculentum) has (0.56 mg/ml) amount of protein.

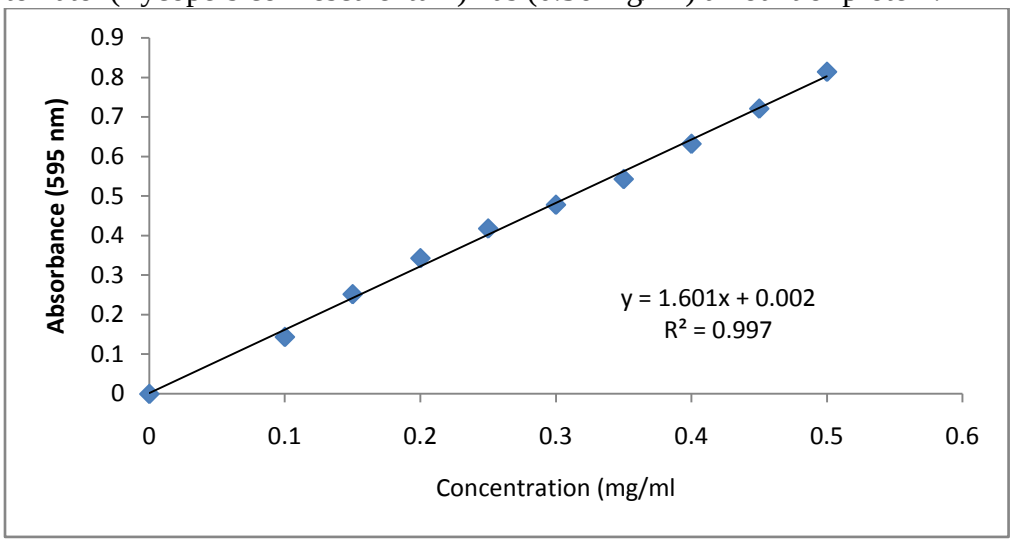

Figure 2. Determination of protein content (mg/ml) in crude extract of using BSA as standard.

Isoelectric Point of β-Galactosidase:

One protein band was appeared after coloring the gel with Commassie Brilliant Blue R-250 as shown in figure 4. Isoelectric point of β-galactosidase was found to be 4.4 when estimated by Isoelectric focusing which depends on progressive hydrogen number (pH) of the gel that is sustainable and stable due to the charged small particles (Ampholytes) and that means the existence of higher percentage of acidic amino acids in the protein as compared to basic amino acids. Our result is in agreement with [26].

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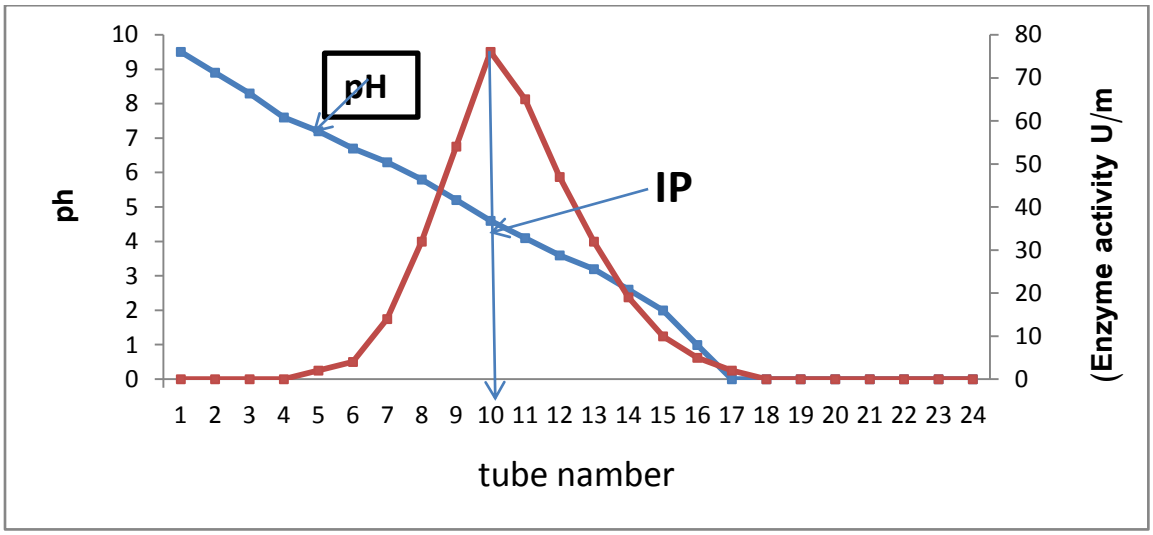

Figure (3): Isoelectric point pI of β-galactosidase purified from tomato

Carbohydrate content:

Figure (5) shows that the carbohydrate peak at 490nm is approximately identical to the protein peak at
280nm and activity peak at 420nm, this is an evidence of presence carbohydrates in the protein structure of lactase and this means that apricot lactase is a glycoprotein. Carbohydrate concentration of apricot β- galactosidase was found to be 19.6 % by employing phenol-sulfuric acid method and this result approximately agree with [27]. Carbohydrate percentage in lactase enzyme differs from one source to another.

Table (1): Carbohydrates are% of protein

Carbohydrates are% of protein	Carbohydrate content	Absorbance at 490 nm	Enzyme
19.6	20.011	0.25	B galactosidase

Activation energy of β-galactosidase:

Activation energy (Ea) of lactase purified from apricot for conversion of ONPG to product was measured to be 16.56 kcal/ mol; and this value is within the range that stated by [33] which is located between 6-18Kcal/mol. Low value of Ea is considered as an indicator of the catalytic efficiency of the enzyme to convert substrate to products. Whereas, it was found that inactivation energy of purified enzyme from apricot equals 48.46kcal/mol, this value gives an idea about un-

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stability of enzyme at high temperatures. Inactivation energy of enzymes ranged 40-175 Kcal/mol. [34]

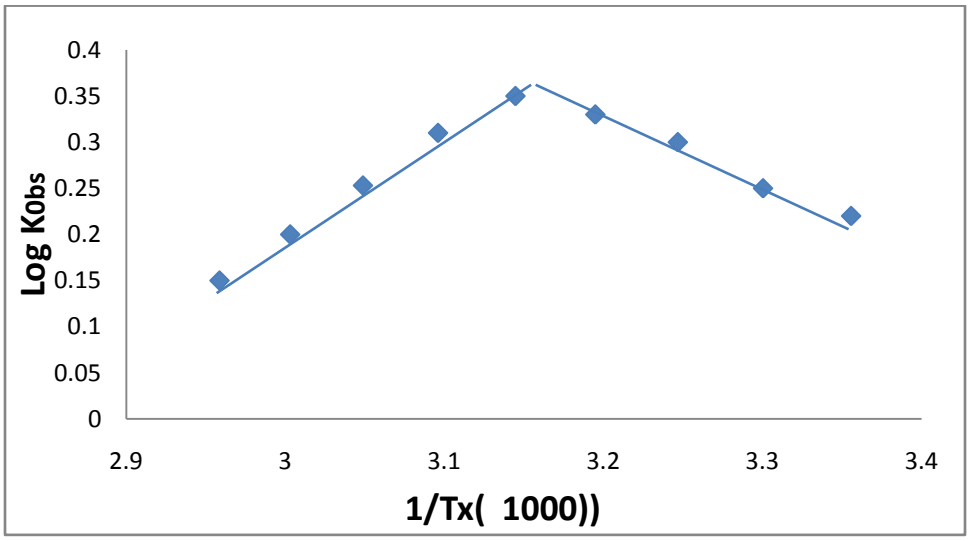

Figure (4): Arrhenius plot for determining activation energy of apricot β-galactosidase.

Kinetics Analysis

To determine the enzyme kinetic parameters (Km and Vmax of β-galactosidase), initial reaction rates at different ONPG concentration ranging from 1 mM to 9 mM were measured. The data were analyzed according to Line weaver Burk plot by plotting 1/V value against 1/[S] value and kinetic parameters were calculated from the graph. The results of Km and Vmax values of the enzyme were 3.65 mM and 0.18 µmol/min, respectively (Figure 4).

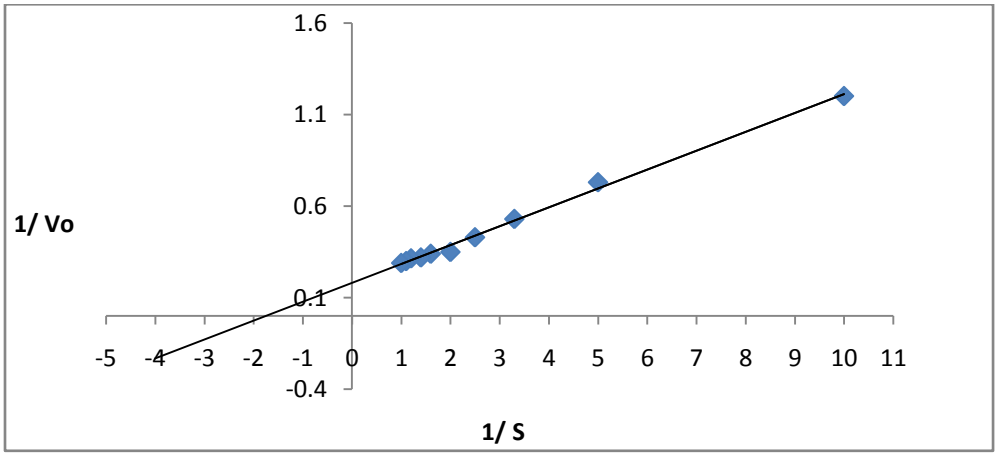

Figure 5. Determination of Vmax and Km values of the enzyme β-galactosidase in the extracts of tomato (Lycopersicom esculentum) using ONPG as a substrate.

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Km is the concentration of substrate at which the reaction rate is half- maximum. Km is important in enzyme kinetic because its value includes not only the affinity of substrate for the enzyme but also the rate at which the enzyme-bound substrate is converted to the product in the catalytic reaction. Thus, Km value can be interpreted as a crude measurement of the affinity of the substrate for the enzyme [55]. The Km value of the enzyme was higher than reported earlier, 1.67 mM for carrot [56], 1.77 mM for tomato fruit [48], 1.85 mM for apricot β-galactosidase I [11], 1.73 mM for chick pea [13] and 1.19 mM for radish seed [33], but it was lower than that of other plants such as 5.16 mM for peach [46] and 10.53 mM for almond [6]. The rate or velocity of a reaction (V) is the number of substrate molecules converted to product per unit time. The rate of an enzyme-catalyzed reaction increases with substrate concentration until a maximal velocity (Vmax) is reached. The leveling off of the reaction rate at high substrate concentrations reflects the saturation with substrate of all available binding sites on the enzyme molecules present [49]. However, the Vmax value of the enzyme β-galactosidase in the crude extract was higher than reported earlier. The Vmax value for β-galactosidase I, β-galactosidase II and β-galactosidase III isolated from apricots was found to be 0.52, 0.70 and 0.38 µmol/min, respectively [18], but it was lower than that of other plants such as 5.2 µmol/min for rice [46].

Effect of metal ions and inhibitors on β-galactosidase Activity in tomato (Lycopersicom esculentum) Extract

figures (5) show effects of some metal ions and inhibitors on the β-galactosidase activity extracted from tomato (Lycopersicom esculentum). The data indicated that the activities of β-galactosidase isolated from tomato (Lycopersicom esculentum) are completely inhibited by HgCl2 and KCN. CaCl2, NiCl2 KCN and CuSO4 reduced the activities of β-galactosidase by 63.12, 34.18 , 0 and 33.5% respectively. FeCl3 and FeSO4 increased the activities of β-galactosidase by 149 and 152.4%, respectively. Ag , EDTA and Beta-M1 increased the activities of β-galactosidase by 0 , 118.5 and 104.3 %, respectively.

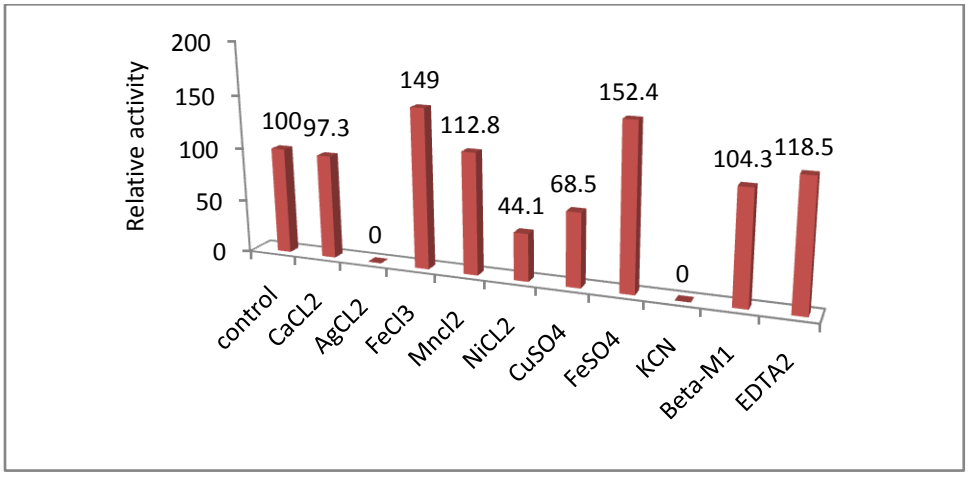

Fig. 6: Effect of metal ions on the β-galactosidase activity extracted from tomato (Lycopersicom esculentum)

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MnCl2 increased the activities of β-galactosidase from tomato (Lycopersicom esculentum) by 12.8%, [40] . noted that the presence of divalent actions such as Fe2+ and Mn2+ in the reaction mixture increased the activity of β -galactosidase from Lactobacillus crispatus [41]. extracted β-galactosidase from Kluyveromyces marxianus IFoo541 and K. marxianus var. lactase 1-2. They found that the crude enzyme from K. marxianus IFoo541 was activated largely by Mn2+ and inhibited by Ca2+. However, the crude enzyme from K. marxianus var. lactis 1-2 was strongly activated by Ca2+ and minimally activated by Mn2+. β-galactosidase from mung bean reduced by Fe2+ (15.1%), Ca2+ (15.1%), Cu2+ (23.4%) and Mn2+ (21.1%), but strongly inhibited by Hg2+ [42]. The activity of β-galactosidase from Bacillus coagulans RC53 was inhibited by Fe2+ (17.1%), Cu2+(91.1%), Ni2+ (76.3%) and Hg2+ (65.7%), while the activity of the enzyme was activated by Ca2+ (7.5%) and Mn2+(19.6%) [43]. [44] noted that the activity of β-galactosidase from Bifidobacterium infants HL96 was activated by Fe2+ and Mn2+ while, the enzyme was partially inhibited by Ca2+ and it was completely inhibited by Hg2+ and Cu2+. [42] reported that the activity of β-galactosidase from Bacterium pseudoalteromonas increased by Mn2+ (40%), but strongly inhibited by Cu2+.

Figure (6) shows the effect of inhibitors on the β-galactosidase activity extracted from tomato (Lycopersicom esculentum). The data indicated that the beta-mercaptoethanol had very little effect on the activity of β-galactosidase tomato (Lycopersicom esculentum). However, EDTA inhibited the activity of the enzyme from tomato (Lycopersicom esculentum) by 18.5 and 4.3% respectively [44] . found that the activity of β-galactosidase from mung bean was inhibited by EDTA. The activity of β-galactosidase from chickpea was not affected by EDTA [45]. The activity of β-galactosidase from persimmon fruit was inhibited greatly by EDTA [46].

References

[1] Matthews BW. The structure of E. coli β-galactosidase. Competes Rendus Biologies, 2005, 328 (6): 549-56.

[2] Alliet P Scholtens P Raes M Hensen K Jongen H Rummens JL Boehm G and Vandenplas Y. Effect of prebiotic galacto oligosaccharide, long-chain fructo oligosaccharide infant formula on serum cholesterol and triacylglycerol levels. Nutr, 2007, 23: 719-723.

[3] Seddigh S and Bandani A R. Comparison of α and β-galactosidase activity in the three cereal pests, Haplothrips tritici Kurdjumov (Thysanoptera: Phlaeothripidae), Rhopalosiphum padi L. (Hemiptera: Aphididae) and Eurygaster integricep Puton (Hemiptera: Scutelleridae). Mun Ent Zool., 2012, 7: 904–908.

[4] Pal A Lobo M and Khanum F. Extraction, purification and thermodynamic characterization of almond (Amygadalus communis) β-galactosidase for the preparation of delactosed milk. Food Technol and Biotechnol., 2013, 51: 53-61.

[5] Li, Deng & Zhu, & Xing,. (2019). A New β-Galactosidase from the Antarctic Bacterium Alteromonas sp. ANT48 and Its Potential in Formation of Prebiotic Galacto-Oligosaccharides. Marine Drugs. 17. 599. 10.3390/md17110599.

[6] Shaikh F A Randriantsoa M Withers S G. Mechanistic analysis of the blood group antigen-cleaving endo-betagalactosidase from Clostridium perfringens. Biochemistry, 2009, 48 (35): 8396-404.

[7] Jokar A and Karbassi A. In-house Production of Lactosehydrolysed Milk by Beta-galactosidase from
Lactobacillus bulgaricus. J. Agr. Sci. Tech., 2011, 13: 577- 584.

[8] Seddigh S and Darabi M. (2014) Comprehensive analysis of betagalactosidase protein in plants based on Arabidopsis thaliana. Turk J Biol., 38: 140-150.
[15] Husain Q. β-Galactosidases and their potential applications: a review. Crit. Rev. Biotechnol., 2010, 30: 41-62.
[9] Seddigh S and Darabi M. Comprehensive analysis of betagalactosidase protein in plants based on Arabidopsis thaliana. Turk J Biol., 2014, 38: 140-150.
[10] Kishore D and Kayastha AM. A β-galactosidase from chick pea (Cicer arietinum) seeds: Its purification, biochemical properties and industrial applications. Food Chemistry, 2012,13: 1113–1122.
[11] Gulzar S and Amin S. Kinetic Studies on β-Galactosidase Isolated from Apricots (Prunus armeniaca kaisa). American Journal of Plant Sciences, 2012, 3: 636-645.
[12] Suderio F B Barbosa G K Gomes-Filho E and Eneas-Filho J. Purification and characterization of cytosolic and cell wall β-galactosidases from Vigna unguiculata stems. Braz. J. Plant Physiol., 2011, 23 (1): 5-14.
[13] Yossef HD and El Beltagey AE. Extraction, purification and characterization of apricot Seed β-Galactosidase for Producing Free Lactose Cheese. J Nutr Food Sci., 2014, 4: 270.
[14] Pal A., Lobo M. and Khanum F. (2013) Extraction, purification and thermodynamic characterization of almond (Amygadalus communis) β-galactosidase for the preparation of delactosed milk. Food Technol and Biotechnol, 51: 53-61.
[15] Ogasawara, S., Abe, K., and Nakajima, T. Pepper betagalactosidase 1 (PBG1) plays a significant role in fruit ripening in bell pepper (Capsicum annuum). Biosci. Biotechnol. Biochem., 2007, 71 (2): 309-22.
[16] Dean G H Zheng H Tewari J Huang J Young D S Hwang Y T Western T L Carpita N C McCann M C Mansfield S D Haughna G W. The Arabidopsis MUM2 Gene Encodes a β-Galactosidase Required for the Production of Seed Coat Mucilage with Correct Hydration Properties. The Plant Cell, 2007, 19: 4007–4021.
[17] Yossef HD and El Beltagey AE. (2014) Extraction, purification and characterization of apricot Seed β-Galactosidase for Producing Free Lactose Cheese. J Nutr Food Sci., 4: 270
[18] Asma S., Muhammad S., Umber Z., Rubina N., Saleem J. and Ahmad S. (2017). β-Galactosidase from Watermelon (Citrullus lanatus) Seedlings: Partial Purification and Properties. Pak. J. Biotechnol., 14 (3): 271-278.
[18] Al-Arrji, S. B. and Al-Hamadi, N. A.(2018).Extraction, Purification and Characterization of β-Galactosidase from Apricot (Prunus armeniaca kaisa) Fruit for lactose intolerance treatment. International Journal of ChemTech Research, ,10(6): 919-929.Joaquim E.F.,
[20] Ali, N.; Andleeb, S.; Mazher, B. and Ali Khan, A.(2016). Optimization of B-galactosidase Production from Yogurt and Dairy Soil Associated Bacteria Using Different Fermentation. BMRJ. 11(2):1-15.Torres DMP, Do Pilar F, Goncalves M, Texeira JA & Rodrigues LR (2010) Galacto-oligosaccharides: production, properties, applications, and significance as prebiotics. Comp Rev Food Sci Food Safety, 9: 438–454.
[21] Martin I Jimenez T Esteban R Dopico B and Labrador E.Immunolocalization of a cell wall β-galactosidase reveals its developmentally regulated expression in Cicer arietinum and its relationship to vascular tissue. J. Plant Growth Regul.,2008, 27: 181-191.
[22] Dwevedi, A., & Kayastha, A. M. Plant β-Galactosidases:Physiological Significance and Recent Advances in Technological Applications. J. Plant Biochemistry and Biotechnology, 2010, 19 (1): 09-20.

[23] Aldoobie NF and Beltagi MS. Physiological, biochemical and molecular responses of common bean (Phaseolus vulgaris L.) plants to heavy metals stress. African Journal of Biotechnology, 2013, 12 (29): 4614- 4622.
[24] Gaur A and Adholeya A. Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavymetal contaminated soils. Current Science., 2004, 86 (4): 528–534.
[25] Nedelkoska TV and Doran PM. Characteristics of heavy metal uptake by plant species with potential for phytoremediation and phytomining. Miner. Eng., 2000, 13: 549–561.
[26] Schutzendubel A and Polle A. Plant responses to abiotic stress: heavy metal induced oxidative stress and protection by mycorrhization. J Exp Bot., 2002, 53: 1351-1365.
[27] Nofal SM Mahmoud SS Ramadan A Soliman GA and Fawzy R. Anti- Diabetic effect of Artemisia judaica extracts. Res. J. Med. Med. Sci. 2009, 4 (1): 42–48.
[28] Al-Rawashdeh IM. Genetic variability in a medicinal plant Artemisia judaica using random amplified polymorphic DNA (RAPD) Markers. Int. J. Agric. Biol., 2011, 13: 279–282.
[29] Abd-Elhady HK. Insecticidal activity and chemical composition of essential oil from Artemisia judaica L. against Callosobruchus maculatus (F.) (coleoptera: bruchidae). J. Plant Prot. Res., 2012, 52 (3): 347- 352.
[30] Tilaoui M Mouse HA Jaafari A Aboufatima R Chait A and Zyad A. Chemical composition and antiproliferative activity of essential oil from aerial parts of a medicinal herb Artemisia herba-alba. Rev. Bras. Farmacogn., 2011, 21 (4): 781-785.
[31] Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilization the principle of protein dye binding. Analytical Biochemistry, 72: 248.
[32] Omar, M., A.; Mohammad H. A.; Ibrahim M. A. (2016) Characterization of β-galactosidase in the Crude Plant Extract of Artemisia judaica L. in Presence and Absence of Some Heavy Metals. American Journal of Life Sciences, 4(5): 99-10
[33] Khalid F. A., Gazi M. A., Abdulhakem D. H. (2016) Extraction of Peroxidase from Seeds of Some Plants belonging to Gramineae, Fabaceae and Solanaceae Families and Study of Biochemical Characteristics from the Selected Source. Anbar J. of Agriculture Sciences, 14(1): 314-326.
[34] Talwar G. P. and Srivastava L. M. Textbook of biochemistry and human biology, 2006, 3rd Ed. Prentice-Hall: New Delhi.
[35] Abdulhakeem D. H.; Khalid F. A.; Gazi M. A; Zazali B. (2020) A. Purification and Kinetic Properties of Purified Peroxidase from Proso Millet Seeds. International Journal of Pharmaceutical Research, 12(2): 923-934.
[36] Pagthinathan, M.; Ghazali, H. M.; Yazid, A. M.; Foo, H. L. (2019) Extraction, purification and characterisation of a milk-clotting protease from 'kesinai' (Streblus asper Lour.) leaves. International Food Research Journal, 26(3): 913-922.
[37] Alcantara PH Martim L Silva CO Dietrich SM Buckeridge MS. Purification of a β-galactosidase from cotyledons of Hymenaea courbaril L. (Leguminosae). Enzyme properties and biological function. Plant Physiol Biochem., 2006, 44: 619-627.
[38] Biswas S Kayastha AM and Seckler R. Purification and characterization of a thermo stable β-galactosidase from kidney beans (Phaseolus vulgaris L.) cv. PDR14. Journal of Plant Physiology, 2003, 160: 327–337.
[39] Richard A. Harvey and Denise R. Ferrier. Lippincott's Illustrated Reviews: Biochemistry, Fifth Edition. Lippincott Williams & Wilkins, a Wolters Kluwer business, 2011.

[40] Haider T and Husain Q. Preparation of lactose free milk by using salt fractionated almond (Amygadalus Communis) β -galactosidase. J Sci Food Agric., 2007, 87: 1278-1283.
[40] Abood, S. C. and Hakeem, L. M. (2016). Purification and characterization of salivary amylase inhibitor extracted from barley. Iraq Journal of Agriculture sciences.47(4)1039-1048.
[41] Ajaykumar, M. ; Take, I. and Shweta, N. (2012). Isolation, Characterization and Purification of alpha galactosidase from Peas. J.Adv. Lab. Res. Biol. 2(3): 164-170
[42] Ali, N.; Andleeb, S.; Mazher, B. and Ali Khan, A.(2016). Optimization of B-galactosidase Production from Yogurt and Dairy Soil Associated Bacteria Using Different Fermentation. BMRJ. 11(2):1-15.
[43] Anja, k. W. ; emko, M. and Anja, E.(2014).A new approach to evaluate immobilization of B-galactosidase on Eupergit C:structural kinetic, and thermal characterization, Quim 37(5): 60-71.
[44] Atrooz, O. M.; Abukhalil, M. H. and AlRawashdeh, I. M.(2016). Characterization of β-galactosidase in the crude plant extract of Artemisia judaica L. in presence and absence of some heavy metals. American Journal of Life Sciences 4(5): 99-105.
[45] Edwards M Bowman YJ Dea IC and Reid JS. A β-galactosidase from nasturtium (Tropaeolum majus L.)
cotyledons. Purification, properties and demonstration that xyloglucan is the natural substrate. J. Biol. Chem., 1988, 263:4333–4337.
[46] Sanad B. Al-Arrji, Nawal A. Al-Hamadi (2017) Extraction, Purification and Characterization of β-Galactosidase from Apricot(Prunusarmeniacakaisa) Fruit for lactose intolerance treatment. International Journal of ChemTech Research, 10(6): 919-929.
[47] Sanad B. M., Jinan H. M., Iman H. A. (2018). Extraction and purification of ßeta-galactosidase from a Local Almond seeds (Prunusamygdalus). IOSR Journal of Pharmacy and Biological Sciences, 13(1): 34-39.
[48] Davies, G., Henrissat, B. Structures and mechanisms of glycosyl hydrolases. Structure, 1995, 3, 853-85.
[49] Shengwen Shen, Xing-Fang Li, William R. Cullen, Michael Weinfeld, and X. Chris Le. Arsenic Binding to Proteins. Chem. Rev., 2013, 113 (10): 7769–7792.
[50] Konno H., and Tsumuki H. Purification of a β-galactosidase from rice shoots and its involvement in hydrolysis of the natural substrate in cell walls. Physiol plant, 1993, 89: 40-47.
[51] Carrington CM and Pressey R. β-Galactosidase II activity in relation to changes in cell wall Galactosyl composition during tomato ripening. Journal of the American Society for Horticultural Science, 1996, 121: 132-136.
[52] Voet D. and Voet J. G. Biochemistry text chapter on Rate of Enzymatic Reactions. 2011, pp. 482-500. Fourth edition. USA: Ed. John Wiley & Sons, Inc.
[53] Baritaux, O., Amiot, M. J., Richard, H. and Nicolas, J. Enzymatic browning of basil (Ocimum basilicum L.) studies on phenolic compounds and polyphenoloxidase. Sciences des Aliments, 1991, 11: 49-62.
[54] Suginta W and Svasti MR. Purif ication and properties of β-galactosidase from Hibiscus sabdariffa L. var. altissima. J.Sci. Soc. Thailand., 1995, 21: 183-186