PRODUCTION AND CHARACTERIZATION OF B-GALACTOSIDASE ENZYME IN THE PLANT EXTRACT FROM (ZIZIPHUS SPINA-CHRISTI) AND ITS APPLICATION IN MILK

S. A. Hussien, K. S. Doosh

Article Info:
Received: Jan. 09, 2021
Revised: Feb. 11, 2021
Accepted: April 08, 2021
Published: June 30, 2021
DOI: 10.59807/jlsar.v2i1.20

How to Cite:


Copyright: © 2021 by the authors. Submitted for possible open-access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

Abstract: In the development of a medicinal plant, β-galactosidase (EC 3.2.1.23) is essential (Ziziphus spina-christi). The enzyme activity was measured by its ability to hydrolyze the substrate 2-nitrophenyl β-D-galactopyranoside (ONPG). The maximum enzyme activity was at 50 °C and at pH 5.5. The enzyme’s Km and Vmax values were 3.65 mM and 0.18 μmol / min, respectively. HgCl2 and KCN completely inhibit the activities of β-galactosidase (Ziziphus spina-christi). Lactose in milk was reduced by 38.5 and 70 percent by β-galactosidase from (Ziziphus spina-christi), respectively, after 4h incubation. This result showed that the β-galactosidase enzyme in the extract of leaves (Ziziphus spina-christi) can be used for industrial and medical applications.

Keywords: Ziziphus spina-christi, β-galactosidase, Enzymatic Kinetics, Heavy Metals.

1. Introduction

β-galactosidase (also known as β-D-galactohydrolase), known as lactase and transgalactosylases [1], is a group of enzymes capable of splitting β-connected galactose residues from different compounds and is generally used to break lactose into galactose and glucose [2]. It has been widely distributed in nature and is present in many microorganisms, plant and animal tissues [3], [4]. β-galactosidases have multiple biological functions, including degradation of structural polysaccharides in plant cell walls, thus allowing them to loosen and elongate the cell [5]. They have many medical and industrial applications including lactose malabsorption therapy, and lactose hydrolyzed milk processing [6], [7].

These enzymes have two significant applications: the removal of lactose from milk products for people who are lactose intolerant and the manufacture of galactosylated products [8], [9]. β-galactosidases have been found in a broad range of plant organs and tissues and are identified by their ability to hydrolyze non-reduction terminal β-D-galactosyl residues [3]. Different plant sources have been filtered, such as chick pea [10], almond [11], apricot [12], Vigna unguiculata [9], apricot seed [4], β-galactosidase plays important roles in the maturing of fruits. The activity of β-galactosidase in rice [11], [13], [14], has been documented during fruit production and ripening. Many studies suggested a remarkable increase in the degree of expression of mRNA β-galactosidase in many fruits during fruit ripening [12]. β-galactosidases have been reported to be widely distributed in many plant tissues, including seeds [15], stems [16], and root meristem zones, trichomes, cotyledons, vascular tissues, and pollen [17], [18].
On the other hand, it also participates in the alteration of the cell wall during plant cell elongation and differentiation [19], [20]. Due to its easy availability, cost-effectiveness and easy adaptability, plant β-galactosidase would be better suited for industrial applications [21]. For the preparation of delactosed milk for those lactose intolerant individuals, β-galactosidase from almond seeds was used [15]. Heavy metals are necessary and significant for the growth of plants and play a major role in many of the critical compounds [21]. Some of these metals, such as Zn2+, Cu2+, Mn2+, Ni2+, and Co2+, are micronutrients essential for plant growth, while others have unknown biological functions, such as Cd2+, Pb2+ and Hg2+ [22], respectively.

All heavy metals have significant toxic effects at high concentrations, and are considered contaminants for the environment [23]. They can alter the rate of reaction and affect the kinetic properties of enzymes that cause changes in plant metabolism, or any excessive amount of heavy metals can cause oxidative stress [24]. The plant extracts of (Ziziphus spina-christi) used in this original research. The enzyme β-galactosidase has been used as a source for. As a primary step for the future use of β-galactosidase in commercial, biotechnological and medical applications, enzyme activities, kinetics and the effects of heavy metals have been studied.

2. Materials and Methods

Materials: New sample of a plant (Ziziphus spina-christi). It had been obtained from local markets in Iraq. Characterization of β-galactosidase was performed at the University of Baghdad Biochemistry Laboratory.

Plant Extract Preparation

The plant extract (Ziziphus spina-christi) was prepared and used as the source of β-galactosidase. The plant was homogenized for 4 minutes in a blender with a sodium phosphate buffer of 0.2 M (pH 6.0). The homogenate was filtered using a sheet of cloth and then centrifuged at 10,000 rpm for 20 min. The supernatants were used as the crude enzyme solution for the β-galactosidase assay [25].

Protein Estimation: With the use of Bovine Serum Albumin (BSA) as standard, the protein content was defined in a spectro-photometric manner at 595 nm using the Bradford method [26].

![Figure 1. Determination of protein content (mg/ml) in crude extract of leaves Artemisia judaica using BSA as standard.](image)

Enzyme Assay: Plant extract (Ziziphus spina-christi) was prepared and used as a source for β-galactosidase β-galactosidase activity by measuring the rate at which it hydrolyzes ONPG using the Sekimata et al. method [10]. ONPG is hydrolyzed to D-galactose (colorless) and o-nitro phenol (ONP) (yellow) in the presence of β-D-galactosidase. β-galactosidase reaction mixture contained, 0.5 ml of a 2 mM substrate solution and 0.1 ml of an enzyme. After incubation at 37 °C for 15 min, the reaction was terminated by adding 0.1 mM Na2CO3 to 1 ml and monitored at 420 nm. The amount of enzyme that releases 1.0 μmol of ONP per minute under the conditions of the assay is known as one unit of enzyme activity.
Determination of Kinetic Parameters: ONPG was used as a substrate to calculate the maximum velocity (Vmax) and Michaelis-Menten constant (Km) of β-galactosidases, and the effect of substrate concentration on enzyme activity was studied at pH 6.0 and 50 °C, respectively. The ONPG concentration was raised from 1 mM to 9 mM. The enzyme activity was assayed by monitoring the absorbance at 420 nm. Line weaver-Burk Plot (Reciprocal plots) was used to determine Vmax and Km values [27].

Effect of pH on Enzyme Activity: According to Gulzar and Amin, the optimum pH of β-galactosidases was calculated by incubating it at 50 °C in various buffers with different pH values varying from 2.0 to 9.0 [11]. In each buffer system, the enzyme assay has been performed separately. Relative operation was measured (%).

Effect of Temperature on Enzyme Activity: Galactosidases’ optimum temperature was determined by incubating the reaction mixtures at different temperatures ranging from 25 °C to 80 °C, and the activity was represented by relative activity (%).

Effect of metals ions, inhibitors, and other substances on the enzyme activity: In the acetate buffer (at optimum pH for each β-galactosidase extract) stock solutions of CaCl2, HgCl2, CuSO4, FeCl3, KCN, NiCl2, MnCl2 and FeSO4 were added separately to the reaction mixture at a final concentration of 0.001 mol / liter. Inhibitors such as beta-mercapt-oethanol (0.01 mol / Liter) and tetra acetic acid ethylene-diamine (EDTA) (0.0001 mol / Liter) have also been studied. The activity of the residual enzyme was assessed and expressed as a percentage of the activity calculated in the acetate buffer alone (control).

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

3. Results and Discussion

Protein Content: Protein content in the extract (Ziziphus spina-christi) was calculated using BSA as a standard protein using the Bradford method (Figure 2). The results showed that the amount of protein in the extract (Ziziphus spina-christi) was (0.56 mg / ml).

Effect of pH on β-galactosidase Activity: Each enzyme has an optimum pH at which it works best. Any change in pH will affect the structure of the enzyme and affect its activity. Some amino acids are deprotonated or protonated as the pH rises or decreases, altering the conformation and behavior of proteins [25], [27]. The operation of β-galactosidase has been found to differ according to pH values (Figure 3). The optimum activity pH of the enzyme was 5.5 and the pH of the enzyme was stable from 2.5 to 8.5. The relative behavior of β-galactosidase at pH 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.5, 7.0, 7.5, 8.0 and 8.5 was 26.29%, 35.34%, 46.83%, 80.15%, 91.75%, 100% 96.94%, 84.21%, 54.77%, 33.82%, and 19.45%, respectively.
These findings were in accordance with the observations that the optimum pH of the β-galactosidase plant was within the acid range [28]. The extraction from apricots of three β-galactosidase isoenzymes was found to have an optimum pH between 4.0 and 6.0 [9] and in almonds to be 5.5 [6] but the optimum pH value of peach β-galactosidase was 3.0 [29]. Kidney beans 4.0 [16] and Hymenaea courbaril 3.5 [29]. The results showed that the enzyme is appropriate for lactose hydrolysis present in whey or milk where the pH ranges between 4.5 and 6.8 [4].

Effect of Temperature on β-galactosidase Activity: Each enzyme has an optimum temperature that reaches Vmax at reaction. The velocity of the reaction increases with temperature until a peak velocity is reached, where the maximum number of molecules with enough energy to pass over the energy barrier and form the reaction products [26]. As a result of temperature-induced denaturation of the enzyme due to modifying the native folded structure of proteins to uncoil into random shape, further increase in temperature would lead to a decrease in the reaction velocity. The hydrogen bonds are broken at high temperature, and therefore the enzyme 's molecular conformation is changed. The effect of oβ-galactosidase in temperature as a result of temperature-induced denaturation of the enzyme due to modifying the native folded structure of proteins to uncoil into random shape, further increase in temperature would result in decreasing the reaction velocity. The hydrogen bonds are broken at high temperature and hence

At higher temperatures, the loss of activity of the enzyme may be attributed to its unfolding and subsequent loss of the active site due to denatured proteins [24]. The same optimum temperature for nasturtium, peach and Hymenaea courbaril obtained (50 ° C) [24]. Extraction of three isoforms of the was found Mung bean seedlings
have an optimal temperature of β-galactosidase between 50 °C and 53 °C [30]. The optimum temperature was slightly different in many plants, such as chick pea, cowpea and almond, 60 °C [10], [4], [11], 40 °C [12], apricots and 70 °C apricot seed. Most of the above studies provided optimum temperature for the majority of β-galactosidase in the 40-60 °C range. Optimum temperature determination is an important factor in the selection of enzymes for commercial, biotechnological, and medical applications. Most industrial enzymes reportedly have Vmax at 40-50°C [31]. The optimum temperature of Artemisia judaica is 50 °C, which means it can in industrial and medical applications, it is used.

Kinetics Analysis: Initial reaction rates at various ONPG concentrations ranging from 1 mM to 9 mM were calculated to determine the enzyme kinetic parameters (Km and Vmax of β-galactosidase). The data were analyzed by plotting 1 / V value against 1/[S] value according to the Line weaver Burk map, and the kinetic parameters were determined from the graph. The effects of the enzyme’s Km and Vmax values 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 (Ziziphus spina-christi) using ONPG as a substrate.

Km is the substrate concentration at which the rate of reaction is half- full. In enzyme kinetic, Km is important because its importance includes not only the substrate’s affinity for the enzyme but also the rate at which the enzyme-bound substrate in the catalytic reaction is converted to the product. The value of Km can thus be interpreted as a crude measure of the affinity of the Enzyme Substratum [32]. The enzyme Km value was higher than previously recorded, 1.67 mM for carrot [33], 1.77 mM for tomato [34], 1.85 mM for apricot β-galactosidase [11], 1.73 mM for chick pea [10] and 1.19 mM for radish [35], but was lower than for other plants, such as 5.16 mM for peach [36] and 10.53 mM for almond [4], respectively. A reaction rate or velocity (V) is the amount of substrate molecules converted into product per unit time. With substrate concentration, the rate of an enzyme-catalyzed reaction increases until a maximum velocity (Vmax) is reached. Substrate saturation of all available binding sites on the enzyme molecules present reflects the leveling off of the reaction rate at high substrate concentrations [37]. The Vmax value of the β-galactosidase enzyme in the crude extract was, however, higher than stated earlier. The value of Vmax for β-galactosidase I, β-galactosidase II and β-galactosidase III isolated from apricots were found to be 0.52, 0.70 and 0.38 μmol / min, respectively [15], but below that of other plants such as 5.2 μmol / min for rice [37].

Effect of metal ions and inhibitors on β-galactosidase Activity in (Ziziphus spina-christi) Extract: figures (6) show effects of some metal ions and inhibitors on the β-galactosidase activity extracted from (Ziziphus spina-christi). The data indicated that the activities of β-galactosidase isolated from (Ziziphus spina-christi) are completely inhibited by HgCl2 and KCN. CaCl2, NiCl2 and CuSO4 reduced the activities of β-galactosidase by 2.7, 55.9 and 31.5% respectively. FeCl3 and FeSO4 increased the activities of β-galactosidase by 49 and 52.4%, respectively.
MnCl$_2$ increased the activities of β-galactosidase from (Ziziphus spina-christi) by 12.8%, [37]. noted that the presence of divalent actions such as Fe$^{2+}$ and Mn$^{2+}$ increased the activity of β-galactosidase from Lactobacillus crispatus in the reaction mixture [24]. Extracted β-galactosidase from Kluyveromyces marxianus IFO541 and K. marxianus var. lactis 1-2. They found that the crude enzyme from K. marxianus IFO541 was activated largely by Mn$^{2+}$ and inhibited by Ca$^{2+}$. However, the crude enzyme from K. marxianus var. lactis 1-2 was strongly activated by Ca$^{2+}$ and minimally activated by Mn$^{2+}$. β-galactosidase from mung bean reduced by Fe$^{2+}$ (15.1%), Ca$^{2+}$ (15.1%), Cu$^{2+}$ (23.4%) and Mn$^{2+}$ (21.1%), but strongly inhibited by Hg$^{2+}$ [38]. The activity of β-galactosidase from the Bacillus coagulans RC53 was inhibited by Fe$^{2+}$ (17.1%), Cu$^{2+}$ (91.1%), Ni$^{2+}$ (76.3%) and Hg$^{2+}$ (65.7%), while the activity of the enzyme was activated by Ca$^{2+}$ (7.5%) and Mn$^{2+}$ (19.6%) [27]. [39] noted that the activity of β-galactosidase from Bifidobacterium infants HL96 was activated by Fe$^{2+}$ and Mn$^{2+}$ while, the enzyme was partially inhibited by Ca$^{2+}$ and it was completely inhibited by Hg$^{2+}$ and Cu$^{2+}$. [38] reported that the activity of β-galactosidase from Bacterium pseudoalteromonas increased by Mn$^{2+}$ (40%), but strongly inhibited by Cu$^{2+}$. Figure (6) shows the effect of inhibitors on the β-galactosidase activity extracted from (Ziziphus spina-christi). The data indicated that the beta-mercaptoethanol had very little effect on the activity of β-galactosidase (Ziziphus spina-christi). However, EDTA inhibited the activity of the enzyme from (Ziziphus spina-christi) by 18.5 and 4.3% respectively [11]. Finding that EDTA inhibited β-galactosidase activity from mung bean. EDTA [10] did not affect the β-galactosidase activity from chickpea. The β-galactosidase activity of persimmon fruit was significantly inhibited by EDTA [29].

4. Conclusion

In the final The enzyme activity was its ability to hydrolyze the substrate 2-nitrophenyl β-D-galactopyranoside (ONPG). The maximum enzyme activity was at 50 °C and at pH 5.5. The enzyme’s Km and Vmax values were 3.65 mM and 0.18 μmol / min, respectively. HgCl$_2$ and KCN completely inhibit the activities of β-galactosidase (Ziziphus spina-christi). Lactose in milk was reduced by 38.5 and 70 percent by β-galactosidase from (Ziziphus spina-christi), respectively, after 4h incubation, the β-galactosidase enzyme in the extract of leaves (Ziziphus spina-christi) can be used for industrial and medical applications.

Supplementary Materials:
No Supplementary Materials.

Author Contributions:
S. A. Hussien and K. S. Doosh; methodology, writing — original draft preparation, S. A. Hussien; writing — review and editing. All authors have read and agreed to the published version of the manuscript.
Funding:
This research received no external funding.

Institutional Review Board Statement:
The study was conducted in accordance with the protocol authorized by the University of Anbar, Ethics Committee, Iraq.

Informed Consent Statement:
No Informed Consent Statement.

Data Availability Statement:
No Data Availability Statement.

Conflicts of Interest:
The authors declare no conflict of interest.

Acknowledgments:
The authors are thankful for the help, the College Dean of the College of Agriculture, University of Anbar, Iraq. We would also like to thank the undergraduate students for their valuable help and technical assistance in conducting this research.

Disclaimer/Journal’s Note:
The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of JLSAR and/or the editor(s). JLSAR and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

5. References


