Characterization and Effects of β–Galactosidase in the Crude Extracts of *Cuminum cyminum* and *Curcuma longa* in Preparation of Delactosed Milk and Whey

Omar M. Atrooz (Corresponding author)

Department of Biological Sciences, Faculty of Science, Mutah University, Mutah, Jordan
E-mail: omihandd@gmail.com

Received: October 23, 2016   Accepted: November 20, 2016   Published: November 22, 2016
Doi: 10.5296/jab.v5i1.10212   URL: http://dx.doi.org/10.5296/jab.v5i1.10212

Abstract

β-galactosidase (EC 3.2.1.23) was extracted from *Cuminum cyminum* and *Curcuma longa*. The crude extracts of these plants were then characterized in term of pH, temperature, and enzyme kinetic. The crude extracts were also used in hydrolysis of lactose in milk and whey. The enzyme activity was measured by its ability to hydrolyze the substrate o-nitrophenyl β-D-galactopyranoside (ONPG).

It was found that β-galactosidase in the crude extracts of *Cuminum cyminum* exhibited maximum activity at pH 8.0 and optimum temperature at 60 °C. While, β-galactosidase in the crude extracts of *Curcuma longa* have optimum pH at 5.0 and 7.0 and optimum temperature at 50 °C. The *K_m* and *V_max* values of the β-galactosidase in the crude extracts of *Cuminum cyminum* and *Curcuma longa* were 4.16 mM and 0.087 μmol/min, and 2.63 mM and 0.333μmol/min, respectively.

The results showed that 96.84-97.08% of lactose was hydrolyzed in cow’s milk and whey when treated with crude extracts of *Cuminum cyminum* and 90-98.6% when treated with crude extracts of *Curcuma longa*.

Keywords: β-galactosidase, Crude extracts, Enzymatic kinetics, Delactosed milk

1. Introduction

β-galactosidase (β-D-galactosidegalactohydrolase, EC 3.2.1.23), has tremendous potential in research and application in various fields like food, bioremediation, biosensor, diagnosis and treatment of many disorders (Princely et al., 2013). β-Galactosidases are well known biocatalyst to catalyze hydrolytic and transgalactosylation reactions. Glycoside hydrolases (GHs) are group of enzymes that hydrolyze glycoside bonds between two or more
carbohydrates or between one carbohydrate and another type of macromolecule (Naumoff, 2011). The enzyme \( \beta \)-galactosidase is characterized by its ability to hydrolyze \( \beta \) (1-3) and \( \beta \) (1-4) galactosyl bonds in polysaccharides, oligosaccharides and disaccharide molecules (Lactose), and also catalyzes enzymatic condensation and transglycosylation (Maksimainen et al., 2012; Natarajan et al., 2012).

The \( \beta \)-galactosidase hydrolytic activity contributed to many application in the food industry for reducing the lactose content in milk almost for decades, while the transgalactosylation has been used to synthesize di, tri, or higher galacto-oligosaccharides (GOS) (Sheik & Gunasekaran, 2010).

\( \beta \)-galactosidases are widely distributed in plant tissues e.g. leaves (Hirano et al., 1994) seedlings (Li et al., 2001), hypocotyls (Kotake et al., 2005) and meristem zones of roots, cotyledons, vascular tissues, trichomes, and pollens (Wu & Liu, 2006). Various studies have indicated remarkable increases in the activity of \( \beta \)-galactosidase during ripening of many fruits (Lazan et al., 2004).

Lactose malabsorption/intolerance is common among approximately 70% of the world’s adult population and it is caused by the intestinal insufficiency of the enzyme \( \beta \)-galactosidase (lactase) (Zuzana & Michal, 2006). Deficiency of this enzyme causes the accumulation of undigested lactose in small bowel, leading to increased influx of fluids inside the intestinal lumen. The unabsorbed lactose is passed into the large intestine, where in addition to increasing fluid volume of gastrointestinal content, is metabolized by the colonic bacteria, resulting in the production of short chain fatty acids and hydrogen gas, and associated symptoms of lactose intolerance (Kaur et al., 2006). Lactose-intolerant individuals often avoid dairy products and thus eliminate a major source of calcium and energy from their diet, thereby inviting other complications like osteoporosis (Domingues et al., 2005). However, the problem can be solved by removing lactose from the diet by the addition of exogenous \( \beta \)-galactosidase enzyme (Dhaked et al., 2005).

\( \beta \)-Galactosidases are found in microorganisms (bacteria, fungi, yeasts), animal organs, and plants especially in almonds, peaches, apricots, and apples (Haider & Husain, 2007). The major industrial enzymes are obtained from \textit{Aspergillus} sp. and \textit{Kluyveromyces} sp. \( \beta \)-Galactosidase from \textit{Kluyveromyces} lactis is one of the most widely used enzymes (Lee et al., 2003; Klewicki, 2007).

\textit{Curcuma longa}, a perennial herb and member of the Zingiberaceae (ginger) family, grows to a height of three to five feet and is cultivated extensively in Asia, India, China, and other countries with a tropical climate (Nagpal & Sood, 2013).

Turmeric is used extensively in foods for both its flavor and color, as well as having a long tradition of use in the Chinese and Ayurvedic systems of medicine, particularly as an anti-inflammatory and for the treatment of flatulence, jaundice, menstrual difficulties, hematuria, hemorrhage, and colic (Priyadarshini, 2014).

Cumin (\textit{Cuminum cyminum} L.) is an aromatic plant belongs to Apiaceae family and is used to flavor foods, added to fragrances, and used in medical preparations (Iacobellis et al., 2005). Its fruit, known as cumin seed, is yellow to brownish-gray in color and it contains oleoresin. It is cultivated mainly in the Middle East, India, and Pakistan. Ground cumin seeds are used
commercially to flavor many ethnic cuisines (e.g. Indian, Latin American, and Mexican). It has been used in the treatment of mild digestive disorders as a carminative and euphietic, as astringent in bron copulmonary disorders, and as a cough remedy, as well as an analgesic (Rai et al., 2012; Johri et al., 2012). In the Indian traditional medicines, cumin has various effects like abortive, antiseptic, bitter tonic, carminative, stomachic, diuretic, bactericidal and fungicidal and also used as gastrointestinal, gynecological, in broncho pulmonary disorders and in respiratory disorders and also for the treatment of toothache, diarrhea, liver function, antioxidant, and anti-flatulent properties (Sharma et al., 2012; Deepak, 2013). The aims of this work were to determine the enzymatic activities and kinetics of β-galactosidase in Cuminum cyminum and Curcuma longa extracts and to use their crude extracts in preparation of delactosed milk and whey.

2. Materials and Methods

2.1 Plant Samples

Plants samples of Cuminum cyminum and Curcuma longa and fresh cow’s and goat’s milk have been purchased from local markets between February-April, 2015.

2.2 Materials

Sodium carbonate anhydrous (FLUKA, Spain), 2-Nitrophenyl β-D-galactopyranoside (ONPG) (FLUKA, Switzerland), Sodium acetate (Riedel-De Haen. Sigma-Aldrich Laborche mikalien GmbH, Seelze), Di-Sodium hydrogen phosphate anhydrous (Fluka-Chemik, Switzerland), Sodium dihydrogen phosphate (Panreac, Barcelona-Spain).

2.3 Preparation of Crude extracts

Crude extracts were prepared from plant samples of Cuminum cyminum and Curcuma longa as a source for β-galactosidase. The plant samples were homogenized in 100 mM sodium phosphate buffer (pH 6.0) in a blender for 4 min. The homogenate was filtered using cloth sheet and then was centrifuged for 15 min at 13,000 rpm.

The supernatants were used as crude enzyme solution for β-galactosidase assay (Ogasawara et al., 2007).

2.4 Enzyme Assay

The hydrolytic activity of β-galactosidase was determined using the method of Ali et al. (1995) and o-nitrophenyl-β-D-galactopyranoside (ONPG) as a substrate. ONPG is hydrolyzed to D-galactose (colourless) and o-nitrophenol (ONP) (yellow). The β-galactosidase was assayed by following the release of p-nitrophenol from ONPG substrate. The reaction mixture consisted of 0.5 ml of 6 mM substrate, 1 ml of 0.1 M sodium acetate buffer (pH 5.2), and 50 μl of crude enzyme solution. The reaction was allowed to proceed for 15 min at 37 °C and was terminated by adding 1.0 ml of 1.0 M sodium carbonate. The absorbance of liberated p-nitrophenol was measured at 420 nm. All the experiments were done in triplicate and the results are expressed as mean values ± standard deviations (SD).

2.5 Effect of pH on Enzyme Activity

In order to determine the optimal pH of β-galactosidases in the crude extracts of Cuminum cyminum and Curcuma longa, the enzyme was incubated at various buffers with different pH ranging from 3.0 to 9.0 (Gulzar & Amin, 2012). The enzyme assay was performed
separately in each buffer system. Relative activities (%) were calculated by dividing velocity value at each pH point by V\text{\text{\textsuperscript{max}}} value, then multiplied by 100%.

2.6 Effect of Temperature on Enzyme Activity
To determine the optimal temperature of β- galactosidases in the crude extracts of Cuminum cyminum and Curcuma longa, the enzyme assay was performed by incubating reaction mixtures at various temperatures ranging from 20 °C to 90 °C. Relative activities (%) were calculated by dividing velocity value at each temperature point by V\text{\text{\textsuperscript{max}}} value, then multiplied by 100%.

2.7 Determination of Kinetic Parameters
The maximum velocity (V\text{\text{\textsuperscript{max}}}) and Michaelis- Menten constant( K\text{\text{\textsuperscript{m}}}) of β –galactosidase in the crude plant extracts were determined using ONPG as substrate, the effect of substrate concentration on enzyme activity was studied at optimum pH and at optimum temperature for each crude extract. Keeping the amount of enzyme constant in assay mixture, the concentration of ONPG was increased from 1 mM to 9 mM. The enzyme activity was assayed by monitoring the absorbance at 420 nm. Lineweaver-Burk Plot were used to determine V\text{\text{\textsuperscript{max}}} and K\text{\text{\textsuperscript{m}}} values.

2.8 Determine Lactose Hydrolysis
Skimmed milk was prepared by centrifuging cold milk at 4000 rpm for 15 min. The fat layer was removed and skimmed milk was treated with crude plant extracts. The hydrolysis of lactose was calculated by estimating the amount of hydrolysis using lactose standard curve.

2.9 Whey preparations
Whey was deproteinized by heating milk at 55 °C for 10 min with 1M HCl until the pH dropped to 4.5, then filtered to remove the coagulated proteins. Furthermore, whey was treated with crude plant extracts. The hydrolysis of lactose was calculated by using lactose standard curve.

2.10 Statistical Analysis
All the experiments were done in triplicate and the results are expressed as mean values± standard deviations (SD) using Microsoft excel 2007.

3. Results and Discussion
3.1 Effect of pH on β – galactosidase Activity
The pH dependence of the enzymatic release of ONP from ONPG was measured between pH 3.0 and 9.0 using different buffers. The activity was determined at different pH values under standard assay conditions (Princely, 2013).

Each enzyme has an optimum pH at which it performs best. Any changing in pH will cause alteration in the enzyme structure and affecting their activity. As pH increases or decreases, certain amino acids are deprotonated or protonated, causing them to lose their net charge which also cause alteration in the enzyme structure, and the rate of the reaction, therefore, changes. Any alteration in pH will alter electrical charges on the enzyme, and thereby changing the proteins conformation and activity (Harvey & Ferrier, 2011).

The optimum pH of the β-galactosidase in the crude extracts of Cuminum cyminum was 8.0 (Figure 1). The relative activities (%) of β-galactosidase at pH 3, 4, 5, 6, 7, 8 and 9 were 85%,
86.5%, 93.8%, 96%, 97.2%, 100% and 93.8%, respectively.

Figure 1. Relative activity (%) of β-galactosidase in the crude extract of *Cuminum cyminum* at different pH values. Mean ± SD (n=3)

The optimum pH of the β-galactosidase in the crude extracts of *Curcuma longa* was 5.0 and 7.0 (Figure 2). The relative activities (%) of β-galactosidase at pH 3, 4, 5, 6, 7, 8 and 9 were 46.53%, 54.53%, 100%, 43.56%, 100%, 38.61% and 18%, respectively.

Figure 2. Relative activity (%) of β-galactosidase in the crude extract of *Curcuma longa* at different pH values. Mean ± SD (n=3)

Similar results have been reported for several β-galactosidase that were extracted from different sources such as almond with optimum pH 5.5 (Pal et al., 2013), whereas best
activity of three isoenzymes of β-galactosidase extracted from apricots was found between pH 4.0 and 6.0 (Gulzar & Amin, 2012). Optimum pH value of β-galactosidase from other plants was found lower, such as in peach it was 3.0 (Lee et al., 2003), Hymenaea courbaril 3.5 (Alcântara et al., 2006) and kidney beans 4.0 (Biswas et al., 2003). However, β-galactosidases from other sources like chick pea, mung beans and cowpea were also reported to be optimally active at acidic pH (Li et al., 2001; Enéas-Filho et al., 2001; Kishore & Kayastha., 2012). The phenomenon that β-galactosidase in the crude extract of Curcuma longa has two optimum pH may be due to the presence of isoenzymes.

3.2 Effect of temperature on β- galactosidase Activity

The temperature dependence of enzyme activity was measured by assaying the enzyme samples over the temperature range of 20-90 °C for 15 min (Somyos & Phimchanok, 2009). Each enzyme has an optimum temperature at which reaction reaches $V_{\text{max}}$. The reaction velocity increases with temperature until a peak, where $V_{\text{max}}$ is reached, this increase is the result of the increased number of molecules having sufficient energy to pass over the energy barrier and form the products of the reaction (Harvey & Ferrier, 2011). Further increase in temperature will lead to decrease the reaction velocity as a result of temperature-induced denaturation of the enzyme due to changing uncoiling of the native folded structure of proteins into random configuration (Solomon et al., 2007).

The effect of temperature on β-galactosidase activity in the crude extracts were investigated by measuring enzyme activity at different temperature values ranging from 20 °C to 90 °C. The optimum temperature of β-galactosidase in the crude extract of Cuminum cyminum was obtained at 60 °C. The relative activities(%) of β-galactosidase are shown in Figure 3. The optimum temperature of β-galactosidase in the crude extract of Curcuma longa was obtained at 50 °C and the relative activities (%) of β-galactosidase are shown in Figure 4.

![Graph](image-url)  
**Figure 3.** Relative activity (%) of β-galactosidase in the crude extract of Cuminum cyminum at different temperature (°C). Mean ± SD (n=3)
The loss of activity of the enzyme at higher temperatures could be attributed to its unfolding and subsequent loss of active site (Haider & Husain, 2007). It was reported that the optimum temperatures of β-galactosidase in nasturtium, peach and Hymenaea courbaril are 50 °C (Lee et al., 2003; Alcântara et al., 2006). It has been also found that three isoforms of β-galactosidase extracted from mung bean seedlings have the optimum temperature between 50 °C to 53 °C (Li et al., 2001). The optimum temperature of β-galactosidase in the crude extract of Curcuma longa (60 °C) was similar to optimum temperature of chick pea, cowpea and almond (Enéas-Filho et al., 2001; Kishore & Kayastha, 2012; Pal et al., 2013). In other plants, the optimum temperatures were slightly different, such as in apricots at 40 °C (Gulzar & Amin, 2012) and apricot seed at 70 °C (Yossef & El Beltagey, 2014). Most of above studies provide that the optimum temperatures of most β-galactosidase were in the range 40-60°C. Determination of optimum temperature is an important factor for the selection of enzymes for industrial, biotechnological and medical applications. It has been reported that most of industrial enzymes have $V_{\text{max}}$ at 40-50 °C (Devi et al., 2007).

3.3 Determination of $K_m$ and $V_{\text{max}}$

The optimization of substrate concentration was performed using ONPG as substrate. $V_{\text{max}}$ and $K_m$ were calculated from the Lineweaver-Burk reciprocal double plot (by plotting $1/V$ value against $1/[S]$) at different ONPG concentration ranging from 1 mM to 9 mM. The results of $K_m$ and $V_{\text{max}}$ values of β-galactosidase in the crude extracts of Cuminum cyminum were 4.16 mM and 0.087 μmol/min, respectively (Figure 5).
Figure 5. Determination of $V_{\text{max}}$ and $K_m$ values for $\beta$-galactosidase in the crude extract of *Cuminum cyminum* using ONPG as a substrate

While, the results of $K_m$ and $V_{\text{max}}$ values for $\beta$-galactosidase in the crude extract of *Curcuma longa* were 2.63 mM and 0.333 µmol/min, respectively (Figure 6).

Figure 6. Determination of $V_{\text{max}}$ and $K_m$ values for $\beta$-galactosidase in the crude extract of *Curcuma longa* using ONPG as a substrate

However, the $K_m$ values of the enzyme $\beta$-galactosidase in the crude extract of *Cuminum cyminum* (4.16) were higher than reported earlier, 1.67 mM for carrot (Konno et al., 1986), 1.77 mM for tomato fruit, 1.85 mM for apricot $\beta$-galactosidase I (Gulzar & Amin, 2012), and
1.73 mM for chick pea (Kishore & Kayastha, 2012), but it was close to the $K_m$ value of peach (5.16 mM) (Lee et al., 2003) and has $K_m$ value lower than that of almond (10.53 mM) (Pal et al., 2013). While β-galactosidase in the crude extract of Curcuma longa has lower $K_m$ value (2.63) compared to other plants.

However, the $V_{\text{max}}$ values of the β-galactosidase in the crude extracts of Cuminum cyminum and Curcuma longa (0.087 and 0.333 μmol/min, respectively) were close to the $V_{\text{max}}$ values for β-galactosidase I, β-galactosidase II and β-galactosidase III isolated from apricots (0.52, 0.70 and 0.38 μmol/min, respectively) (Gulzar & Amin, 2012), but lower than $V_{\text{max}}$ values of other plants such as rice (5.2 μmol/min) (Konno & Tsumuki, 1993).

3.4 Hydrolysis of Milk and Whey Lactose Using Crude Plant Extracts

Cow’s and goat’s milk and whey were treated with crude extracts of Cuminum cyminum and Curcuma longa at 60 °C and 50 °C, respectively, for estimation of lactose hydrolysis (%).

The crude extract of Cuminum cyminum showed a 96.84% and 97.08% of lactose hydrolysis in cow’s milk and whey (respectively) (Figure 7). While treatment of goat’s milk and whey with crude extract of Cuminum cyminum showed a 32.5% and 8.3% of lactose hydrolysis (respectively) (Figure 7). On the other hand, treatment of cow’s milk and whey with crude extracts of Curcuma longa showed a 90% and 98.6% of lactose hydrolysis (respectively), and illustrated a reduction in lactose hydrolysis with 44.5% in goat’s milk and 43.4% in goat’s whey (Figure 7).

The results obtained for the treatment of cow’s milk and whey by β-galactosidase of Cuminum cyminum and Curcuma longa indicated that they possess a high efficient capacity for the hydrolysis of lactose, while for goat’s milk and whey were less efficient (< 44.5%). The results are comparable with that of other authors’ investigations. According to Pal et al. (2013) 50% of lactose hydrolysis can be achieved by incubating 100 mL of milk with 10 g of almond seed powder for 5 h at 42 °C (using purified β –galactosidase), while Ladero et al. (2003) used β-galactosidase from Thermus sp. for lactose hydrolysis (yield higher than 95%), also for Panesar et al., (2010) and Zheng et al. (2006) who used β-galactosidase extract from Bacteria (Streptococcus thermophilus and Lactobacillus lactis) to hydrolyse milk lactose. Many reports revealed the hydrolysis of lactose in whey through many source of β-galactosidase, such as Saad, (2004) used β-galactosidase from Aspergillus japonicas for lactose hydrolysis in whey (55%), after 4 h incubation at 45 °C.

The higher efficiency of the crude extracts in the lactose hydrolysis in cow’s milk and whey compared to goat’s milk and whey may be due to the differences in pH in milk and whey that may enhance the formation of GOS products.

The results indicated that the enzyme β-galactosidase extracted from Cuminum cyminum and Curcuma longa is suitable for hydrolysis of lactose present in whey or milk (Figure 7).
Figure 7. Lactose hydrolysis (%) in cow’s and goat’s milk and whey in presence of crude extract of Cuminum cyminum and Curcuma longa

4. Conclusion

The strong industrial interest in β-galactosidases arises from their ability to hydrolyze lactose into D-galactose and D-glucose, and for their transglycosylation activity. Characterization of β-galactosidase in the crude extracts of Cuminum cyminum and Curcuma longa had maximum activity at pH 8.0 and 5.0 and 8.0 and at optimum temperature 60 °C and 50 °C, respectively. Also, the results of K_m and V_max values of the enzyme β-galactosidase in the crude extracts of Cuminum cyminum (4.16 mM and 0.087 μmol/min) and Curcuma longa (2.63 mM and 0.333 μmol/min), respectively, demonstrated that these extracts have a potential activities (β-galactosidases) that hydrolyze lactose into glucose and galactose.

β-galactosidase is one of the most important enzymes used in food processing, which offers nutritional, technological, and environmental applications. The crude extracts of of Cuminum cyminum and Curcuma longa showed 90.0 to 98.6% of lactose hydrolysis in cow’s milk and in whey. While the crude extracts of Curcuma longa showed a higher activity (about 44%) of lactose hydrolysis in goat’s milk and whey compared to the treatment with crude extracts of of Cuminum cyminum (32.5%, 8.3%) respectively. In general, The findings indicate that crude extracts of Cuminum cyminum and Curcuma longa can be successfully employed for the production of low lactose milk and whey.

Acknowledgement

This project was supported by Deanship of the Academic Research (grant number 120/14/394) at Mutah University. The technical assistance of Miss Manar Al-Btoosh is greatly acknowledged.

References


Somyos, O., & Phimchanok, J. (2009). Isolation and characterization of \( \beta \)-galactosidase from the thermophile B1.2. As. J.Food Ag-Ind., 2(04), 135-143.


Copyright Disclaimer

Copyright reserved by the author(s).

This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).