Macrothink Institute™

Journal of Applied Biotechnology
ISSN 2327-0640
2017, Vol. 5, No. 1

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

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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 β-galactosidase is characterized by its ability to hydrolyze β (1-3) and β (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 β-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)

β-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 meris-tem zones of roots, cotyledons, vascular tissues, tricho- mes, and pollens (Wu & Liu, 2006). Various studies have indicated remarkable increases in the activity of β-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 β–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 β -galactosidase enzyme (Dhaked et al., 2005).

β- Galactosidaseare 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 *Aspergillus* sp. and *Kluyveromyces sp*. β-Galactosidase from *Kluyveromyces* lactis is one of the most widely used enzymes (Lee et al., 2003; Klewicki, 2007).

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 (Priyadarsini, 2014).

Cumin (*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 eupeptic, 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, diarrohea, 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_{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_{max} value, then multiplied by 100%.

2.7 Determination of Kinetic Parameters

The maximum velocity (V_{max}) and Michaelis- Menten constant(K_m) of β –galactosidase in the crude plant extracts were determined using ONGP 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 ONGP 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_{max} and K_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.

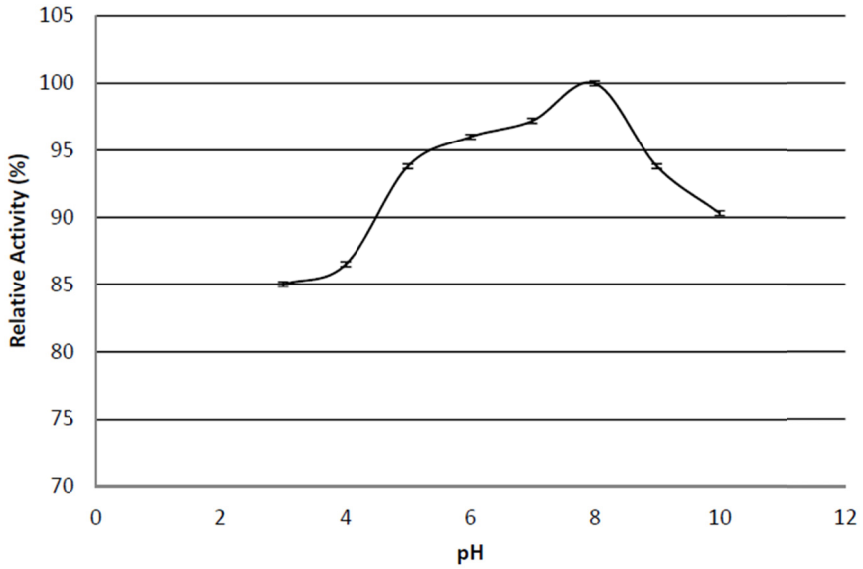

Figure 1. Relative activity (%) of β-galactosidase in the crude extract of *Cuminum cyminum* at different pH values. Mean ± SD (n=3)

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.

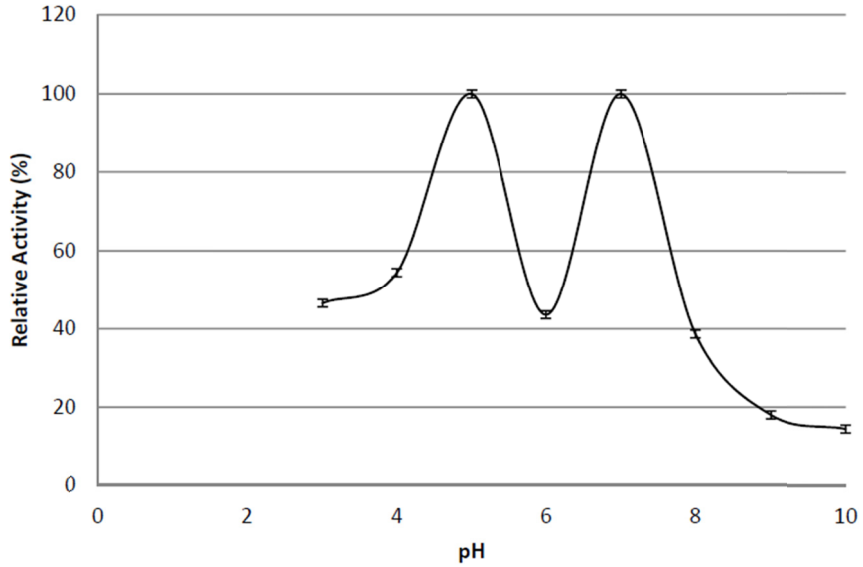

Figure 2. Relative activity (%) of β-galactosidase in the crude extract of *Curcuma longa* at different pH values. Mean ± SD (n=3)

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_{max}. The reaction velocity increases with temperature until a peak, where V_{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 - galactosidasein 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.

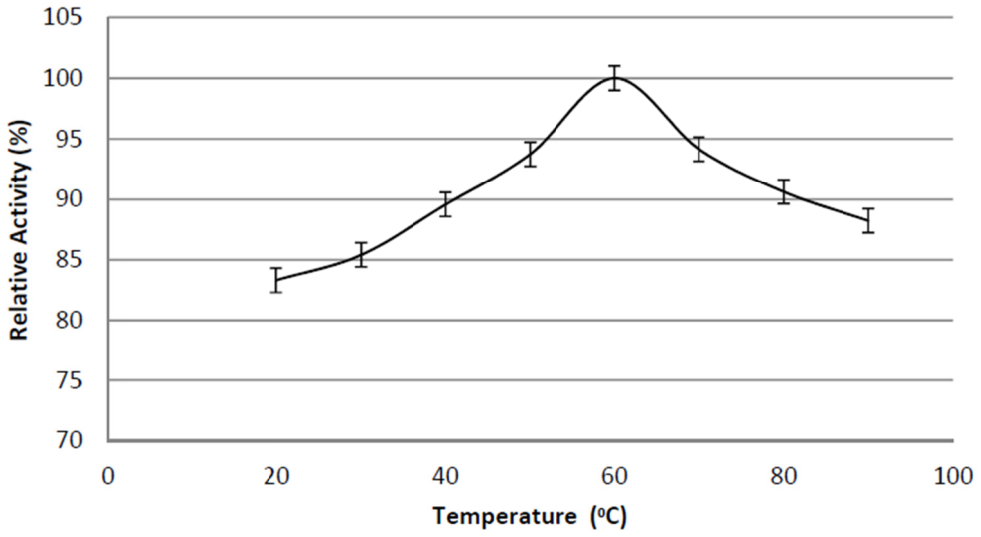

Figure 3. Relative activity (%) of β-galactosidase in the crude extract of *Cuminum cyminum* at different temperature (°C). Mean ± SD (n=3)

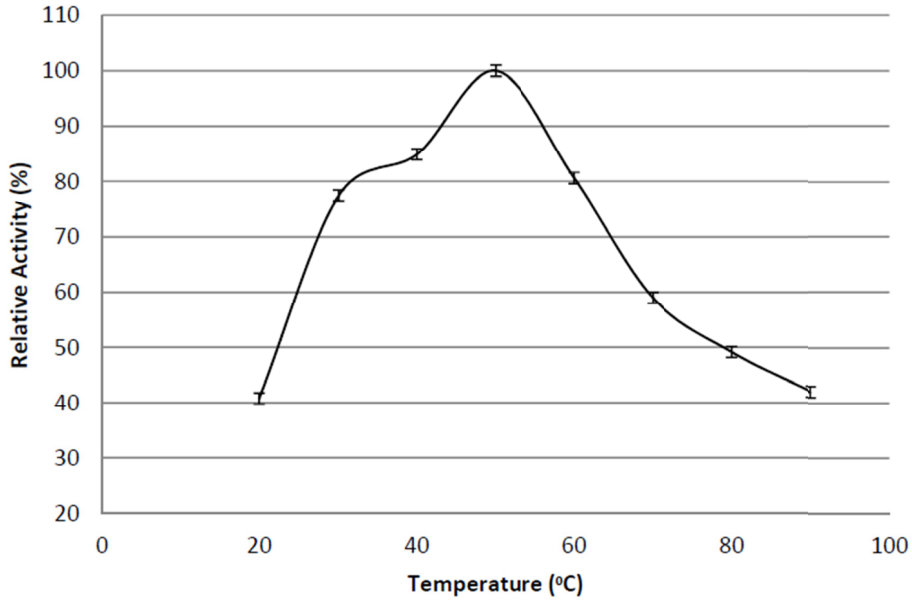

Figure 4. Relative activity (%) of β-galactosidase in the crude extract of *Curcuma longa* at different temperature (°C). Mean ± SD (n=3)

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-galactosidasein 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_{max} at 40-50 °C (Devi et al., 2007).

3.3 Determination of K_m and V_{max}

The optimization of substrate concentration was performed using ONPG as substrate. V_{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_{max} values of β-galactosidase in the crude extracts of *Cuminum cyminum* were 4.16 mM and 0.087 μmol/min, respectively (Figure 5).

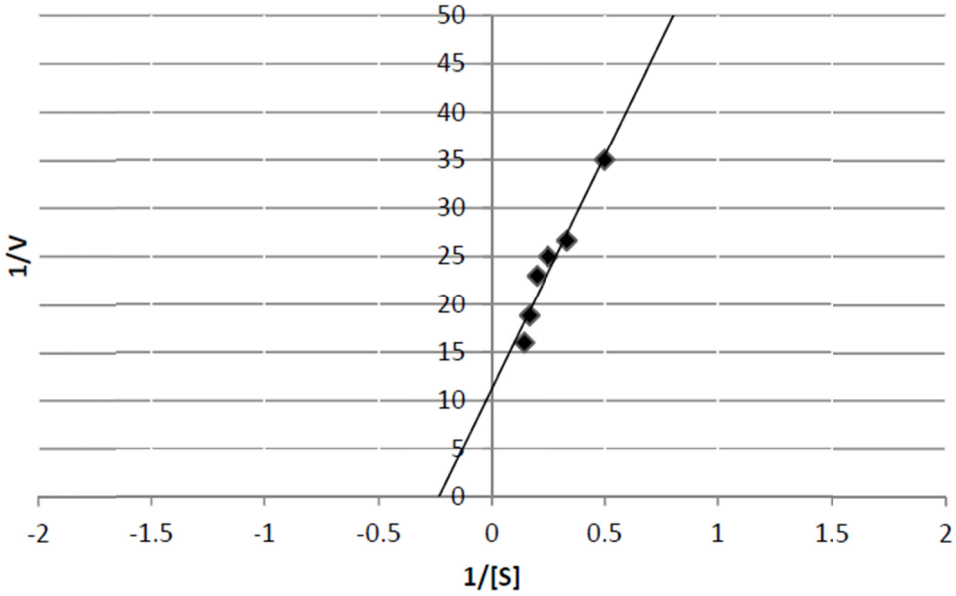

Figure 5. Determination of V_{max} and K_m values for β-galactosidase in the crude extract of *Cuminum cyminum* using ONPG as a substrate

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

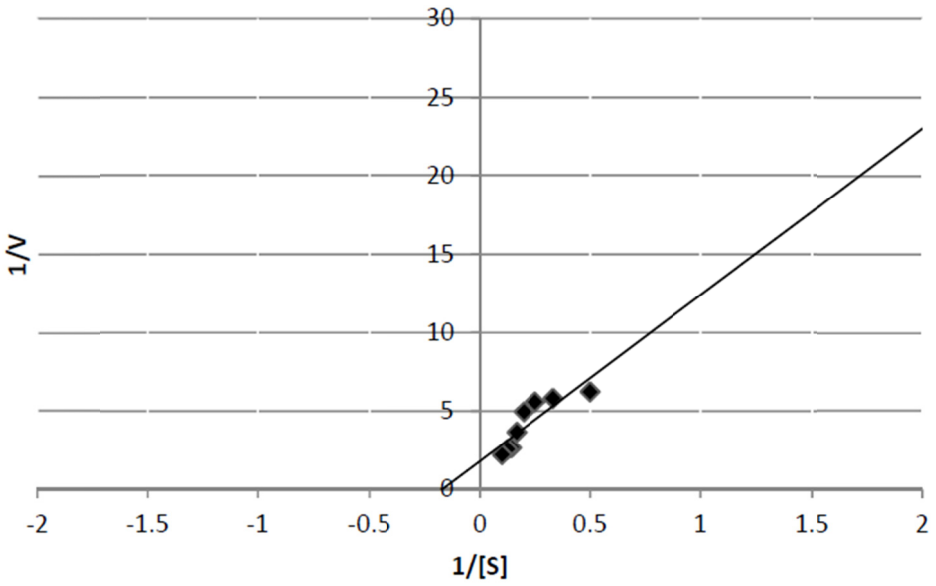

Figure 6. Determination of V_{max} and K_m values for β-galactosidase in the crude extract of *Curcuma longa* using ONPG as a substrate

However, the K_m values of the enzyme β-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 β-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_{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_{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_{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 Lactobaccilluslactis) 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).

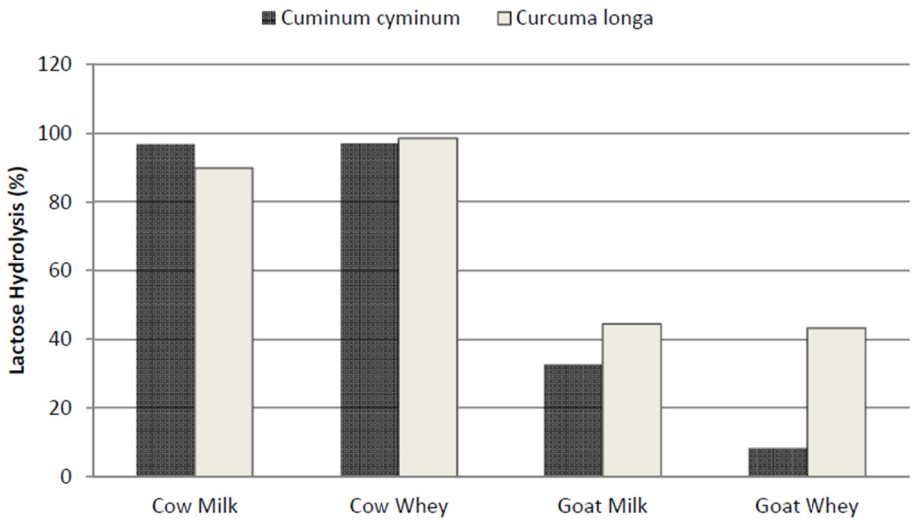

Figure 7. Lactose hydrolysis (%) in cow's and goat's milk and whey in presence of crude extract of *Cuminum cyminum* and *Curcuma longa*

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

Alcântara, P. H., Martim L., Silva, C. O., Dietrich, S. M., & Buckeridge, M. S. (2006). Purification of a β-galactosidase from cotyledons of *Hymenaea courbaril* L. (Leguminosae).

Enzyme properties andbiological function. *Plant Physiol Biochem., 44*, 619-627. http://dx.doi.org/10.1016/j.plaphy.2006.10.007

Ali, Z. M., Armugam, S., & Lazan, H. (1995). β -galactosidase and its significance in ripening mango fruit. *Phytochem, 38*, 1109-1114. http://dx.doi.org/10.1016/0031-9422(94)00804-3

Biswas, S., Kayastha, A. M., & Seckler, R. (2003). Purification and characterization of athermostable beta-galactosidase from kidney beans (*Phaseolus vulgaris* L.) cv. PDR14. *J Plant Physiol, 160*, 327-337. http://dx.doi.org/10.1078/0176-1617-00748

Deepak. (2013). Importance of *Cuminum cyminum* and *Carumcarvi L.* in Traditional Medicaments - A Review. *Indian Journal of Traditional Knowledge, 12*, 300-307.

Devi, R. S., Narayan, S., Vani, G., & Devi, C. S. (2007). Gastroprotective effect of *Terminaliaarjuna*bark on diclofenac sodium induced gastric ulcer. *Chem Biol Interact., 167*, 71-83. http://dx.doi.org/10.1016/j.cbi.2007.01.011

Dhaked, R. K., Alam, S. I., & Singh, L. (2005). Characterization of β -galactosidase from an Antarctic Bacillus sp. *Indian J. Biotechnol, 4*, 227-231.

Domingues, L., Loma, N., & Teixeira, J. A. (2005). *Aspergillusniger* β –galactosidase production by yeast in a continuous high cell density reactor. *Process Biochem., 40*, 1151-1154. http://dx.doi.org/10.1016/j.procbio.2004.04.016

Enéas-Filho, J., da Costa Barbosa, G. K., Suderio, F. B., Prisco, J. T., & Gomes-Filho, E. (2001). Isolation and partial purification of β -galactosidases from cotyledons of two cowpea cultivars. *Rev. Bras. Fisiol. Veg., 13*, 251-261. http://dx.doi.org/10.1590/S0103-31312001000300001

Gulzar, S., & Amin, S. (2012). Kinetic Studies on β-Galactosidase Isolated from Apricots (*Prunus armeniacakaisa*). *American Journal of Plant Sciences, 3*, 636-645. http://dx.doi.org/10.4236/ajps.2012.35077

Haider, T., & Husain, Q. (2007). Preparation of lactose-free milk by using salt-fractionated almond (*Amygdalus communis*) b-galactosidase, *J. Sci. Food Agric, 87*, 1278-1283. http://dx.doi.org/10.1002/jsfa.2840

Harvey, R. A., & Ferrier, D. R. (2011). Lippincott's Illustrated Reviews Biochemistry text chapter on Enzymes. (pp. 53-68, 5th ed.). USA: Ed. Lippincott Williams & Wilkins, Inc.

Hirano Y., Tsumuraya Y., & Hashimoto, Y. (1994). Charac-terization of Spinach leaf α-L-Arabinofuranosidases and β-Galactosidases and Their Synergistic Action on an En-dogenous Arabinogalactan-Protein. *Physiologia Planta-rum, 92*(2), 286-296. http://dx.doi.org/10.1111/j.1399-3054.1994.tb05339.x

Iacobellis, N. S., Lo Cantore, P., Capasso, F., & Senatore, F. (2005). Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi L.* essential oils. *Journal of Agricultural and Food Chemistry, 53*, 57-61. http://dx.doi.org/10.1021/jf0487351

Johri, R. K. (2011). Cuminum cyminum and Carum carvi: An update. *Pharmacogn Rev., 5*(9), 63-72. http://dx.doi.org/10.4103/0973-7847.79101

Kaur, K., Mahmood, S., & Mahmood, A. (2006). Hypolactasia as a molecular basis of lactose intolerance. *IJBB., 43*, 267-274.

Kishore, D., & Kayastha, A. M. (2012). A β -galactosidase from chick pea (*Cicer arietinum*) seeds: Its purification, biochemical properties and industrial applications. *Food Chemistry, 13*, 1113-1122. http://dx.doi.org/10.1016/j.foodchem.2012.03.032

Klewicki, R. (2007). Formation of gal-sorbitol during lactose hydrolysis with β-galactosidase. *Food Chem., 100*, 119-1201. http://dx.doi.org/10.1016/j.foodchem.2005.10.064

Konno, H., Yamasaki, Y., & Katoh, K. (1986). Characteristics of β –galactosidase purified from cell suspension cultures of carrot. *Physiol. Plant, 68*, 46-52. http://dx.doi.org/10.1111/j.1399-3054.1986.tb06594.x

Kotake T., Dina, S., Konishi, T., Kaneko, S., & Igarashi, K. (2005). Molecular Cloning of a β-Galactosidase from Radish That Specifically Hydrolyzes β-1 → 3- and β-1 → 6-Galactosyl Residues of Arabinogalactan Protein. *Plant Physiology, 138*(3), 1563-1576. http://dx.doi.org/10.1104/pp.105.062562

Ladero, M., Perez, M. T., & Garcia-Ochoa, F. (2003). Hydrolysis of lactose by free and immobilized β-galactosidase from Thermussp. strain T2. *Biotechnology and Bioengineering, 81*, 241-252. http://dx.doi.org/10.1002/bit.10466

Lazan H., Ng, S. Y., Goh, L. Y. & Ali, Z. M. (2004). Papaya β-Galactosidase/Galactanase Isoforms in Differential Cell Wall Hydrolysis and Fruit Softening during Ripen-ing. *Plant Physiology and Biochemistry, 42*(11), 847-853. http://dx.doi.org/10.1016/j.plaphy.2004.10.007

Lee, D. H., Kang, S. G., Suh, S. G., & Byun, J. K. (2003). Purification and characterization of a beta-galactosidase from peach (*Prunus persica*). *Mol Cells, 15*, 68-74.

Li, S. C., Han, J. W., Chen, K. C., & Chen, C. S. (2001). Purification and characterization of isoforms of β-galactosidases in mung bean seedlings. *Phytochemistry, 57*, 349-359. http://dx.doi.org/10.1016/S0031-9422(01)00022-X

Maksimainen, M., Paavilainen, S., Hakulinen, N., & Rouvinen, J. (2012). Structural analysis, enzymatic characterization, and catalytic mechanisms of β-galactosidase from *Bacillus circulans*Sp. *Alkalophilus*. *FEBS Journal, 279*, 1788-1798. http://dx.doi.org/10.1111/j.1742-4658.2012.08555.x

Nagpal, M., & Sood, S. (2013). Role of *curcumin* in systemic and oral health: An overview. *J Nat SciBiol Med, 4*(1), 3-7. http://dx.doi.org/10.4103/0976-9668.107253

Natarajan, J., Christobell, C., Kumar, D. J., Balakumaran, M. D., Kumar, M. R., & Kalaichelvan, P. T. (2012). Isolation and characterization of β-galactosidase producing *Bacillus* sp.From dairy effluent. *World Applied Sciences Journal, 17*(11), 1466- 1474.

Naumoff, D. G. (2011). Hierarchical classification of glycoside hydrolases. *Biochemistry (Moscow), 76*(6), 622-635. http://dx.doi.org/10.1134/S0006297911060022

Ogasawara, S., Abe, K., & Nakajima, T. (2007). Pepper beta-galactosidase 1 (PBG1) plays a significant role in fruit ripening in bell pepper (*Capsicum annuum*). *Biosci. Biotechnol. Biochem.,71*(2), 309-22. http://dx.doi.org/10.1271/bbb.60179

Pal, A., Lobo, M., & Khanum, F. (2013). Extraction, purification and thermodynamic characterization of almond (*Amygadalus communis*) β-galactosidase for the preparation of delactosed milk. *Food Technol.Biotechnol., 51*(1), 53-61.

Panesar, P. S., Kumari, S., & Panesar, R. (2010). Potential applications of immobilized β-galactosidase in food processing industries. *Enzyme Research,* 1-16. http://dx.doi.org/10.4061/2010/473137

Princely, N., SaleemBasha, J., John, K., & Dhanaraju, M. D. (2013). Biochemical characterization, partial purification, and production of an intracellular beta-galactosidase from Streptococcu thermophilus grown in whey. *European Journal of Experimental Biology, 3*(2), 242-251.

Priyadarsini, K. I. (2014). The chemistry of *curcumin*: from extraction to therapeutic agent. *Molecules, 19*(12), 20091-112. http://dx.doi.org/10.3390/molecules191220091

Rai, N., Yadav, S., Verma, A. K., Tiwari, L., & Sharma, R. (2012). Monographic Profile on Quality Specifications for a Herbal Drug and Spice of Commerce- *Cuminum cyminum* L. *International Journal of Advanced Herbal Science and Technology, 1*, 1-12.

Saad, R. R. (2004). Purification and some properties of β-galactosidase from *Aspergillus japonicus*. *Annals of Microbiology, 54*(3), 299-306.

Sheik, A., &Gunasekaran, P. (2010). Current trends of ß-galactosidase research and application. *School of Biological Science*s., 66, 584-590.

Solomon, E., Berg, L., & Martin, D. (2007). *Biology text chapter on Energy and Metabolism*. (pp.160-167. 8th ed.). USA: Ed. Thomson Learning, Inc.

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

Wu, A., & Liu, J. (2006). Isolation of the Promoter of a Cotton Beta-Galactosidase Gene GhGal1 and Its Expression in TransgenicTobacco Plants. *Science in China Series C: Life Sciences, 49*(2), 105-114. http://dx.doi.org/10.1007/s11427-006-0105-7

Yossef, H., & El Beltagey, A. (2014). Extraction Purification and Characterization of Apricot Seed β-Galactosidase for Producing Free Lactose Cheese. *Nutrition and Food Science, 4*(2), 1-5. http://dx.doi.org/10.4172/2155-9600.1000270

Zheng, P., Yu, H., Sun, Z., Ni, Y., Zhang, W., & Fan, Y. (2006). Production of galacto-oligosaccharides by immobilized recombinant beta-galactosidase from *Aspergillus candidus*. *Journal of Biotechnology, 1*(12), 1464-1470. http://dx.doi.org/10.1002/biot.200600100

Zuzana, M., & Michal, R. (2006). Current trends of β -galactosidase application in food technology. *Journal of Food and Nutrition Research, 45*(2), 47-54.

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