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FULL PAPER

# Manufacture of therapeutic yogurt using β-galactosides enzyme extracted from tomato (*Lycopersicom Esculentum L*)

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The aim of this work was to extract and purify β-galactosidase from tomato (*Solanum lycopersicum*). Ten different extraction methods were explored to find the optimal extraction method for the enzyme. The best specific activity of crude enzyme was 212.27 U/mg protein in sodium phosphate buffer (0.1M and pH6). The purifying operations were carried out using 60% ammonium sulfate precipitation. Lactase enzyme 600UL/M was used for hydrolyzing lactose of milk. Maximum (85–90%) hydrolysis of milk was achieved using a 0.5 percent enzyme concentration at pH 6.5 and incubation at 45 °C for 4 hours. Milk whose lactose was hydrolyzed by β-galactosidase was used for the production of stirred flavored yogurt. The sucrose supplied to the products was calculated to compensate the sweetness gained as a result of lactose hydrolysis. The percentages of sucrose saving in stirred flavored yogurt were 4.21, 5.6, 9.4, 11.7, 13.2, 15.3, 18.5 and 21.7 % for lactose hydrolysis percent 23.6, 41.5, 52.2, 6.4, 67.6, 76.6, 83.3 and 87.2%, respectively. The sweetness sensory evaluation of the stirred flavored yogurt showed non-significant differences among hydrolyzed-lactose and the untreated samples.

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**KEYWORDS**

β-galactosidase; lactose hydrolysis; yogurt.

## Introduction

Lactase and transglycosylases are a group of enzymes that can cleave linked galactose residues from various compounds and are commonly used to cleave lactose into galactose and glucose [1,2]. It is widely distributed in nature and can be found in many microorganisms, plant and animal tissues [3,4]. β -galactosidases (EC 3.2.1.23) have various biological functions, including the breakdown of structural polysaccharides in plant cell walls, which can lead to their loosening and subsequent cell elongation [5]. The treatments of lactose malabsorption and manufacturing of lactose hydrolyzed milk represented a few medical and industrial applications [6,7,8]. The lactose-free milk products for lactose intolerant people and the generation of galactosylated products are two key applications for these enzymes [9,10]. β -galactosidases have been found in a variety of plant organs and tissues, and are defined by their ability to hydrolyze non-reducing terminal-D-galactosyl residues from -D-galactosides [1]. It was purified from a variety of plant sources, including chickpeas [11], almonds [9], apricots [10], *Vigna unguiculata* [11], and apricot seed [12]. β -galactosidase is important for fruit ripening. Rice [13], pepper [14], and arabidopsis all have β -galactosidase activity during fruit growth and ripening [15]. Many studies revealed a significant increase in

mRNA-galactosidase expression during fruit ripening in many fruits [13]. β -galactosidases have been found in a variety of plant tissues, including seeds [9,17], stems [17], root meristem zones, trichomes, cotyledons, vascular tissues, and pollens [12]. On the other hand, it is involved in the alteration of plant cell walls during elongation and differentiation [18,19]. Because of its easy availability, economic effectiveness, and versatility, plant β -galactosidase would be most suited for industrial applications [20]. Lactose is the primary carbohydrate component of milk (3-8 percent w/v) and cheese whey (70-80 percent of the solid component) [9]. β -galactosidase from almond seeds was utilized to make delactosed milk for lactose intolerant people [5]. Millions of tons of whey are generated each year around the world. Unfortunately, half of global production is wasted straight into watery habitat, posing a major waste management issue. Because of the high biological oxygen demand (BOD) of the lactose stream, this condition causes significant environmental pollution [8,21]. By converting whey lactose into very useful sweet syrup and generating important food and pharmaceutical products applications (e.g. substitute for corn syrup in soft drinks, fermented beverages, baking and animal feed), enzymatic hydrolysis of whey lactose by β - galactosidase can reduce more than 75% of water pollution [22,23]. Lactose is used in a restricted number of nutritious goods due to its low solubility, low sweetening power, and ability to cause nutritional malabsorption, which is known as lactose intolerance [24]. Furthermore, milk products with a high lactose content, such as ice cream, frozen milk, whey spreads, and condensed of the milk, can result in excessive lactose crystallization, resulting in a sandy or gritty texture. Using β -galactosidase to treat these products can lower lactose concentrations to acceptable levels, improving some technological and sensory qualities of dairy foods, such as digestibility, softness, and creaminess [25]. The simultaneous synthesis of galactooligosaccharides (GOS), which are employed as prebiotic dietary components, is another benefit of enzymatic lactose hydrolysis. These indigestible chemicals serve as dietary fibers. They encourage the formation of intestinal bifidobacteria, which has a positive impact on the intestine and liver. The need for GOS production, as well as the creation of a cost-effective and efficient GOS manufacturing process, has risen dramatically in recent years [23]. Due to the technique of strengthening the yogurt mix with nonfat milk solids, commercial yogurt usually contains significant levels of lactose. By hydrolyzing the lactose in flavored yogurt, more sweetness can be achieved without increasing the calorie content [26]. The goal of this work was to make hydrolyzed lactose milk by treating it with β -galactosidase and to make various hydrolyzed lactose dairy products, such as stirred flavored yogurt. Tomato (*Lycopersicom esculentum*) plant extracts were used as a source for the enzyme β -galactosidase in this work. The enzyme activities are being studied as a first step toward using -galactosidase in future industrial, biotechnological, and medicinal applications.

## Materials and methods

### *Extraction of the enzyme*

The tomato (*Solanum lycopersicum*) has been used as a β- galactosidase source in the form of a crude plant extract. The following solutions were used in order to establish the optimal solution for enzyme extraction: distilled water, 0.5% sodium carbonate solution pH7.2, 0.2 M sodium acetate buffer solution pH5, 10%sodium chloride pH 5, 0.20 M phosphate buffer pH5, 0.05 M ascorbic acid pH 5, 0.01M citric acid pH 5, 0.2% potassium chloride, 0.2% calcium chloride, and 20% glycerol. Fifty grams of plant fruits were homogenized in 50 mL of each solution for four minutes in a blender. The homogenate

was filtered using a cotton sheet and centrifuged for 20 minutes at 10000 rpm at 40 °C. As a crude enzyme solution, supernatants were used in the β -galactosidase assay [27].

*Estimation of the protein β- galactosidase*

The protein concentration was determined by spectrophotometry at 595 nm, using the Bradford method [28] and a standard of Bovine Serum Albumin (BSA).

*Enzyme assay*

The rate at which the enzyme can hydrolyze the o-nitrophenyl—D-glucopyranoside (ONPG) has been used to determine its activity [24]. The ONPG is hydrolyzed to D-galactose (colorless) and o-nitrophenol (ONP) in the presence of β -D-galactosidase (i.e. yellow). The reaction mixture for the β -galactosidase enzyme includes 0.40 mL of 0.10 M acetate buffer (pH4), 0.50 mL of 2mM substrate, and 0.10 mL of the enzyme solution. After a 15-minutes incubation at 37 °C, the reaction was halted by adding 1 mL of 0.10 mM $Na_2CO_3$ and the absorbance was measured at 420 nm. The ONP standard was used to determine the amount of o-nitrophenol emitted (Figure 1). A single enzyme activity unit is defined as the amount of enzyme that liberates 1mol of o-nitrophenol per minute under the test conditions.

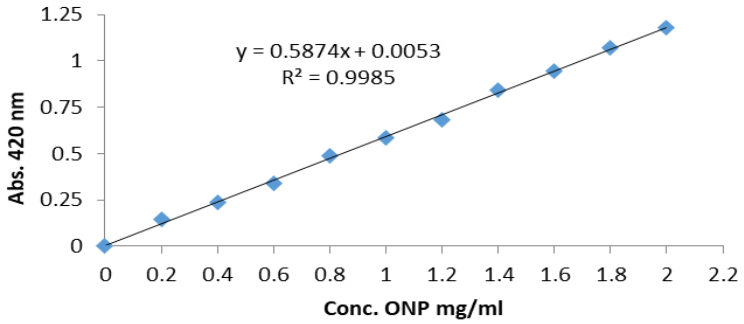


**FIGURE 1** The calibration curve for quantify the amount of o-nitrophenol emitted in the presence of D-Galactosidase, an ONP standard curve

*Optimization of pH of extraction method*

To find the best pH for extraction of β -galactosidase, a range of pH values between 4 and 8 were tested using 0.10 M concentration values of the following buffer systems: sodium acetate (pH 4–5.5), sodium phosphate (pH 6–7.5), and Tris–HCl (pH 8). The specific activity enzyme activities/conc. of protein) for crude extracts at different pHs were used to identify the optimal pH for extraction [3].

*Purification of β-glucosidase*

All of the purification procedures were completed at temperatures between 4 and 8 °C.

*Precipitation by ammonium sulfate*

Ammonium sulfate concentrations of 40 to 90% were used to determine the best concentration of ammonium sulfate for precipitation [26]. After weighing the ammonium sulfate amounts, the enzyme was slowly mixed with using a stirrer overnight at 4 °C until it became homogenous. Then, the precipitate was dissolved in 0.1M of Na-phosphate buffer (pH 7) and dialyzed against polyethylene glycol after centrifugation at 3500 rpm for 30 minutes at 4 °C (M wt. 20000). The enzyme activity and protein concentration were calculated.

*Enzymatic hydrolysis*

To achieve lactose hydrolysis in milk, many parameters such as time, temperature, pH, and enzyme levels were tuned. A 20 percent (w/v) sodium bicarbonate solution was used to adjust the pH of a known quantity (250 mL) of

milk to pH 6.5. The enzyme was added at 0.5 percent (v/v) to this solution and thoroughly stirred. Milk was incubated at 45 °C from 30 minutes to 4 hours. After a set of time intervals, the hydrolyzed milk was removed and the enzyme activity was stopped by heating at 60 °C for 2 minutes in a water bath. The lactose hydrolysis of milk incubated for a specific time interval was evaluated after a quick cooling to room temperature. The optimal period for maximum milk hydrolysis was calculated. Different temperatures were used (20, 30, 40, 50, and 60 °C) after standardizing the optimum time for lactose hydrolysis. A 0.5% (v/v) 600 U/M of enzyme solution was added to the milk whose pH was adjusted to 6.5, and incubated for 4 hours at various temperatures. The rate of milk hydrolysis was calculated, and the ideal temperature for maximum milk hydrolysis was determined. An experiment was accomplished using 20 percent citric acid and 20 percent sodium bicarbonate solutions to change the pH of milk to 6.0, 6.5, 6.75, 7.0, and 7.5. The enzyme 600 U/M was added to the milk samples at a concentration of 0.5% (v/v) and incubated for 4 hours. The optimal pH for maximal milk hydrolysis was calculated. In the second experiment, the pH of milk was adjusted to 6.5 and enzyme concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6% (w/v) were added individually and incubated at 45 °C for 4 hours. The degree of hydrolysis and the optimum level of enzyme concentration were determined after 4 hours of incubation.

*Glucose determination*

Glucose was determined according to colorimetric method of Trinder (1969) [29]. Ten µL of the sample or standard solution (100 mg $dl^{-1}$ glucose) was added to 1000 µL of a reagent solution consisting of glucose oxidase (5000-unit $l^{-1}$) and peroxidase (500-unit $l^{-1}$) with phenol as a chromogen. After incubation at 37 °C for 10 min, the change in absorbance at 510 nm was recorded. Glucose concentration was calculated according to the following equation:

$$\text{Glucose conc. (mg dl-1)} = \frac{\Delta 510\ (\text{Sample})}{\Delta 510\ (\text{Standard})} \times \text{Standard conc.}$$

*Lactose determination*

Lactose in milk and whey was determined using the method of phenol-sulphuric acid carbohydrate assay [37].

*Hydrolysis of milk lactose*

Batch hydrolysis process was used in which one liter of milk was pasteurized for 15 seconds at 72 °C, cooled to 30 °C and treated with 31.5 units of β-galactosidase for 2.5 hours. Aliquots of 0.25 mL were taken at 30 minutes intervals to estimate the concentration of produced glucose.

*Lactose hydrolysis calculation*

In order to calculate the percentage of lactose hydrolysis in milk and whey, lactose percentage was determined in milk and whey prior to enzymatic treatment. After lactose hydrolysis, the concentration of produced glucose was estimated as described previously.

As known, when one mole of lactose (342 gm) is hydrolyzed, it produces one mole (180 gm) of each of glucose and galactose. The amount of hydrolyzed lactose can be calculated as follows:

$$\text{Amount of hydrolyzed lactose (gm)} = \text{amount of produced glucose (gm)} \times 342/180$$

$$\text{Hydrolyzed lactose (\%)} = \frac{\text{amount of hydrolyzed lactose}}{\text{amount of initial lactose}} \times 100$$

*Yogurt manufacturing*

Cow's milk, containing 3.5% fat, was used for yogurt production. Lactose percentage was estimated to be 5.7%. Milk was pasteurized at 72 °C for 15 seconds. Yogurt was

manufactured using three milk samples: (1) unhydrolyzed-lactose milk (control), (2) milk with 25.83% hydrolyzed lactose, and (3) milk with 20% hydrolyzed lactose. All samples were then inoculated with 4% of the starter and incubated at 43±2oC for 3.5 hours. To produce stirred flavored yogurts, yogurt gel was stirred in a blender for 1-2 minutes followed by adding 8% of sucrose to the control sample [44]. The quantities of sugar which were added to the second and third samples were reduced according to the percentage of lactose hydrolysis degree to reach the same sweetness of the control sample and overall treatments, 15% (w/v) of blended strawberry fruits were added. Samples were stored at 4-5 °C and subjected to sweetness sensory evaluation.

*Sucrose saving calculations*

The sweetness of sucrose and lactose are known to be 100 and 16, respectively. The products of lactose hydrolysis are glucose and galactose. Their relative sweet nesses are 75 and 32, respectively. The following steps were used for calculating sucrose saving:

a- The sweetness produced from glucose = amount of glucose × 75.

b- The sweetness produced from galactose = amount of galactose × 32.

c- The sweetness loss due to lactose disappear = amount of hydrolyzed lactose × 16.

d- The total gain of sweetness = (a+b)-c.

As known, the relative sweetness of sucrose is 100%.

Thus, the amount of saved sucrose = (a+b)-c / 100.

The percentage of saved sucrose in each product can be calculated using the following equation:

$$\text{Saved sucrose (\%)} = \frac{\text{amount of saved sucrose}}{\text{amount of original added sucrose}} \times 100$$

*Statistical analysis*

Each experiment was carried out in triplicates, and the data were expressed as average standard deviations (SD) using Microsoft Excel 2007.

## Results and discussions

The extraction of enzyme from tomato (*Solanum lycopersicum*) was conducted using ten different solutions. In comparison to other extraction solutions, the results in Table 1 show that extraction with 0.2 M phosphate buffer (pH 7) is more efficient. The enzymatic activity and specific activity of the enzyme extracted using the aforementioned solution were 7.31 U/mL and 96.60 U/mg protein, respectively. The high level of specific activity, as well as the enzyme activity, is linked to an increase in ionic power, which has a large influence on disassembling the ionic bonds linking the enzyme and other components, a factor that accelerates enzyme dissolution, as shown in Table 1.

**Table 1** Extraction of β-galactosidase from tomato by various solutions

| No. | Method of Extraction | Enzyme Activity U/mL | Protein Conc. mg/mL | Specific Activity U/mg |
|---|---|---|---|---|
| 1 | Water | 3.26 | 0.07 | 48.22 |
| 2 | NaCl (10%, pH 5) | 4.68 | 0.07 | 66.27 |
| 3 | Na2CO3 (0.5%, pH 7.2) | 4.82 | 0.06 | 77.04 |
| 4 | KCl 0.2% | 3.96 | 0.06 | 62.12 |
| 5 | CaCl2 0.2% | 5.64 | 0.07 | 80.57 |
| 6 | Buffer acetate (0.2 M, pH 5) | 4.17 | 0.06 | 70.23 |
| 7 | Buffer phosphate (0.2 M, pH 7) | 7.31 | 0.08 | 96.60 |
| 8 | Glycerol 20% | 3.29 | 0.10 | 32.44 |
| 9 | Ascorbic acid (0.05 M, pH 5) | 3.17 | 0.09 | 34.92 |
| 10 | Citric acid (0.01 M, pH 5) | 3.05 | 0.09 | 35.82 |

*Effect of pH on the activity of the β-galactosidase*

Each of the enzymes has an optimal value of the pH under which it shows its optimal performance. Any change in the value of the pH results in altering the structure of the enzyme and impacting their activities. With increase or decrease of pH, specific amino acids are protonated or deprotonated, as a result, altering the activity and the conformation of the proteins [25]. The activity of the β-galactosidase has been discovered to differ according to the values of the pH (4 to 8) and the optimal value of the pH of enzyme activity has been equal to 6 in Na- phosphate buffer solution (0.1 M) with specific activity 212.27 U/mg protein (Figure 2).

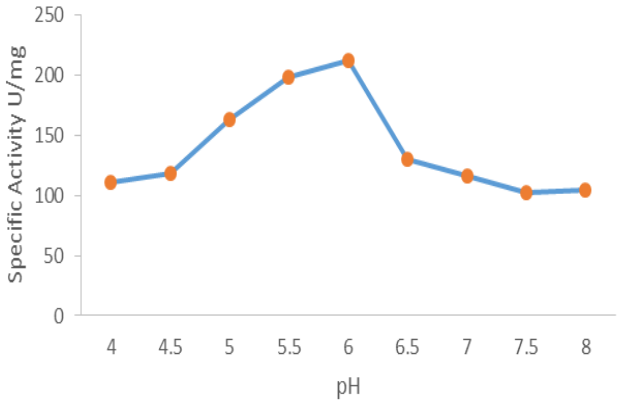


**FIGURE 2** The specific activity of β- galactosidase in tomato crude extract at various pH levels

These findings corroborate previous findings that the optimum pH of plant- β-galactosidase is in the weak acidic range. It was revealed that extracting three iso-enzymes of β-galactosidase from apricots had an ideal pH value of 4 to 6 [10], 5.50 [11] in almonds, and 4.5 [30] in black bean. While the optimal pH of the βgalactosidase from chickpea seeds was 2.8 [25], the optimal pH of the β-galactosidase from peach was 3.0[31].

*Purification of β-galactosidase*

The crude extract was precipitated with ammonium sulfate in various saturation ratios (30-90%) and dialyzed against polyethylene glycol (M.wt. 20000) overnight to remove an additional salt for purifying β-galactosidase. The results indicated that β-galactosidase was efficiently precipitated with ammonium sulfate at a 70% saturation ratio (Figure 3). When compared to crude extraction, enzyme specific activity increased to 105.82 U/mg after dissolving precipitate, with a purification fold of 1.83 and a yield of 88.81 percent (Table 2). Ammonium sulfate is the most extensively used reagent for salting out proteins because of its high solubility, which allows for the creation of a solution with a high ionic strength (36,11). The β-galactosidase was purified from almonds and the precipitation step was done with 15-60%. Purifying the crude extract of apricot seeds with ammonium sulphate (30%-70%) increased specific activity and purification fold to 29.07 U/mg protein and 2.92, respectively [31]. β-galactosidase was purified from peach using 85% ammonium sulfate saturation and increased specific activity by 45% and purification by 21-fold [12]. The precipitation method using 80% saturation with ammonium sulfate was used for partial purification of β-galactosidase from watermelon, which yielded 60% recovery and two-fold purification. The discrepancy could be due to the gradation of the salt used for purification, or the concentration of the salt and the purification's saturation time.

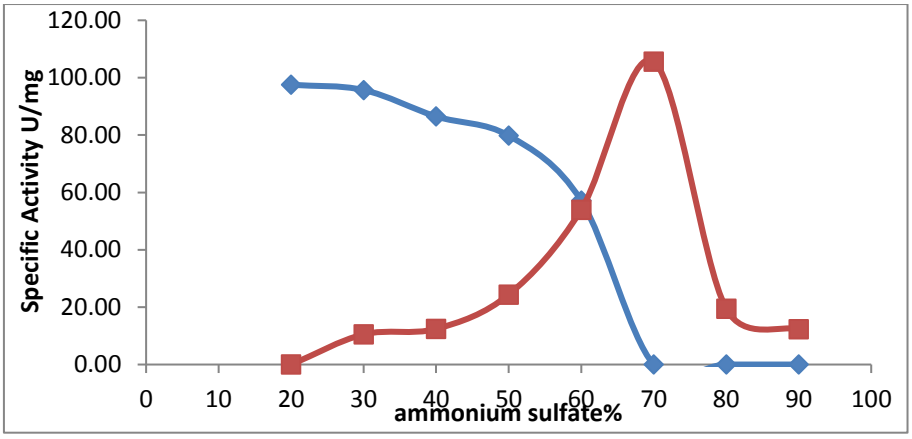


**FIGURE 3** Enzyme activity of β-galactosidase using precipitation by ammonium sulfate in different saturation ratios

*Lactose hydrolysis*

Lactose hydrolysis in milk increased as the incubation time was extended from 30 to 240 minutes. After 30–240 minutes of incubation, the degree of hydrolysis in milk ranged from 79.8% to 89.2% (Figure 2). The milk hydrolysis was affected by both the milk systems and the incubation times (p≤ 0.01). It was discovered that the degree of lactose hydrolysis was highest (89.2–91.3%) at 45 °C and lowest (41.9–42.0%) at 20 °C. At a temperature of 37 °C, maximal lactose hydrolysis was achieved in milk permeates (32). At 20 °C, the minimum hydrolysis of lactose (42%) was achieved, which was in close agreement with the previous findings (32). At pH 6.5, maximum hydrolysis (84.3–88.9%) was reported in milk, while minimum hydrolysis (55.8–66.5%) was observed at pH 6.0. In order to obtain lactose hydrolysis in milk, pH adjustment is critical (33). However, at pH 6.5, maximal lactose hydrolysis in milk permeate was acquired (33). In both milk and water, maximum lactose hydrolysis (88.2–89.2%) was achieved with enzyme concentrations of 0.5 percent, while minimum hydrolysis (59.2–63%) was achieved with enzyme concentrations of 0.1 percent (Figure 2). The hydrolysis of lactose in milk was significantly affected by different enzyme concentrations (p≤ 0.01, CD -2.139). The results showed that at a pH of 6.5 and a temperature of 45 °C, maximal hydrolysis in milk was achieved with a 0.5% enzyme concentration after 180 minutes of incubation. However, authors [34] found that increasing the enzyme concentration from 1.0 to 2.0 percent boosted whey hydrolysis. Lactose hydrolysis in cheese and whey increased as incubation time was extended from 30 to 240 minutes. After 30–180 min of incubation from 30 min to 240 min, the degree of hydrolysis in cheese and whey ranged from 70.7–89.7% and 79.8–89.2%, respectively. The degree of hydrolysis in cheese and whey was 70.7–89.7% and 79.8–89.2 percent, respectively. After 30–180 minutes incubation at pH 6.75, maximum hydrolysis (84.3–88.9%) was reported in whey, whereas lowest hydrolysis (55.8–66.5%) was observed at pH 6.0. In both cheese and whey, maximum lactose hydrolysis (88.2–89.2%) was obtained with Maxilact L-2000 enzyme concentration of 0.4%, while minimum hydrolysis (59.2–63 percent) was obtained with an enzyme concentration of 0.1%. Among the various temperatures (20-60 °C) and at a pH of 6.75 and enzyme concentration of 0.4 percent (v/v), it was noted that degree of lactose hydrolysis was high [35].

**TABLE 2** Effect of time, temperature, pH and enzyme concentration on lactose hydrolysis of milk. Each observation is the mean of five replicates

| Degree of hydrolysis (%) | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Temperature (°C)** | **Milk** | **Time (min)** | **Milk** | **PH** | **Milk** | **Enzyme concentration (%)** | **Milk** |
| **20** | 18.32 | **30** | 23.6 | **5** | 38.84 | **0.1** | 26.43 |
| **25** | 39.47 | **60** | 41.5 | **5.5** | 44.20 | **0.2** | 47.31 |
| **30** | 47.54 | **90** | 52.2 | **6** | 50.13 | **0.3** | 67.62 |
| **35** | 76.21 | **120** | 6.4 | **6.5** | 70.03 | **0.4** | 86.43 |
| **40** | 84.54 | **150** | 67.6 | **7** | 88.39 | **0.5** | 88.21 |
| **45** | 88.36 | **180** | 81.4 | **7.5** | 80.55 | **0.6** | 89.02 |
| **50** | 82.52 | **210** | 86.3 | **8** | 60.65 | **-** | - |
| **55** | 71.6 | **240** | 87.2 | **value LSD** | **8.31 *** | - | **9.47*** |
| **value LSD** | **7.63 *** | **---** | **8.9*** | *P≤0.05 | | | |

*Effect of lactose hydrolysis on milk pH values during yogurt manufacturing*

To estimate the effect of lactose hydrolysis on the rate of milk pH decreasing during yogurt manufacturing, the pH values of yogurt samples were measured every 30 minutes of incubation, and for a period of 3 hours of incubation using lactose-hydrolyzed milk in proportions 0, 23.6, 41.5, 52.2 60.4, 67.4 83.3 and 87.2%, the average pH drop at the end of the 3 hour incubation period is 5.12, 5.02 , 4.98, 4.83, 4.78, 4.78, 4.74, 4.68, 4.61 and 4.54 (Table 4). In a study [25] achieved to estimate the effect of lactose hydrolysis on the rate of milk pH decreasing during yogurt manufacturing, the pH values of yogurt samples were measured every 30 minutes of incubation, and for a period of 3 hours of incubation using lactose-hydrolyzed milk in proportions 24.5 and 51.4%, it was found that the average pH drop at the end of the 3 hour incubation period was 5.42 and 4.79. The data showed that there was a significant decrease in pH values (p≤ 0.05) due to the combined effects of lactose hydrolysis degree and incubation time (Table 3). At the end of the incubation period (3 hours), a significant decrease was detected in hydrolyzed-lactose samples. Higher hydrolysis degree caused more decrease in pH value of the product. This may be due to the availability of more simple sugars (glucose and galactose) to be converted into lactic acid by lactic acid bacteria and lowering the pH faster than untreated milk.

**TABLE 3** Effect of lactose hydrolysis on milk pH values during yogurt manufacturing

| Incubation time (hour) | pH values* Lactose hydrolysis (%) | | | | | | | | | **LSD** |
|---|---|---|---|---|---|---|---|---|---|---|
| | **0%** | **23.6%** | **41.5%** | **52.2%** | **60.4%** | **67.6%** | **76.4%** | **83.3%** | **87.2%** | |
| **0** | 6.75 | 6.73 | 6.77 | 6.76 | 6.78 | 6.76 | 6.77 | 6.76 | 6.74 | 0.407 NS |
| **0.5** | 6.79 | 6.78 | 6.8 | 6.79 | 6.81 | 6.8 | 6.79 | 6.8 | 6.79 | 0.428 NS |
| **1** | 6.67 | 6.64 | 6.62 | 6.61 | 6.49 | 6.34 | 6.28 | 6.22 | 6.13 | 0.502 * |
| **1.5** | 6.41 | 6.34 | 6.32 | 6.17 | 6.12 | 5.67 | 5.54 | 5.45 | 5.34 | 0.733 * |
| **2** | 6.11 | 6.04 | 5.96 | 5.54 | 5.47 | 5.12 | 5.07 | 5.01 | 4.87 | 0.749 * |
| **2.5** | 5.63 | 5.51 | 5.47 | 4.87 | 4.84 | 4.8 | 4.76 | 4.72 | 4.64 | 0.692 * |
| **3** | 5.12 | 5.02 | 4.98 | 4.83 | 4.78 | 4.74 | 4.68 | 4.61 | 4.54 | 0.588 * |
| **LSD value** | 0.821 * | 0.785 * | 1.02 * | 1.17 * | 1.25 * | 1.07 * | 1.116 * | 1.372 * | 1.266 * | --- |
| P≤0.05 * | | | | | | | | | | |

*Sucrose saving in yogurt manufacturing*

The initial percentage of lactose in milk which was used for stirred flavored yogurt production was 5.0%. After incubation with β-galactosidase, 23.6, 41.5, 52.2, 60.4, 67.6, 76.4, 83.3 and 87.2% of lactose hydrolysis were obtained. Since the amount of sucrose added to untreated yogurt sample was equal to 80 g $l^{-1}$, the amounts of saved sucrose were calculated to be 2.3, 3.4, 5.1, 7.3, 9.2, 11.6, 14.7 and 17.4 g $l^{-1}$ which corresponded to 4.21, 5.6, 9.4, 11.7, 13.2, 15.3, 18.5 and 21.7% of added sucrose for the above mentioned hydrolysis degrees, respectively (Table 4). Increased health benefits and less caloric values of flavored yogurt could be obtained through lactose hydrolysis by minimizing the amount of added sucrose without affecting the overall sweetness [34]. The initial percentage of lactose in milk which was used for stirred flavored yogurt production was 5.7%. After incubation with β-galactosidase, 25.83 and 51.66% of lactose hydrolysis were obtained. Since the amount of added sucrose to untreated yogurt sample was equal to 80 g $l^{-1}$, the amounts of saved sucrose were calculated to be 5.94 and 11.87 g $l^{-1}$ which correspond to 7.42 and 14.84% of added sucrose for the above-mentioned hydrolysis degrees, respectively (Table 2).

**TABLE 4** Effect of lactose hydrolysis on sweetness and saved sucrose of stirred flavored yogurt

| Treatments | Lactose hydrolysis (%) | Sweetness | Texture | Saved sucrose | |
|---|---|---|---|---|---|
| | | | | g $l^{-1}$ | % of added sucrose |
| T1 | 0 | 8.5 | 8 | 0 | 0 |
| T2 | 23.6 | 5 | 6 | 2.3 | 4.21 |
| T3 | 41.5 | 5.5 | 6 | 3.4 | 5.6 |
| T4 | 52.2 | 6 | 7 | 5.1 | 9.4 |
| T5 | 60.4 | 6 | 7 | 7.3 | 11.7 |
| T6 | 67.6 | 6.5 | 7.5 | 9.2 | 13.2 |
| T7 | 76.4 | 7 | 7 | 11.6 | 15.3 |
| T8 | 83.3 | 7.5 | 7.5 | 14.7 | 18.5 |
| T9 | 87.2 | 7.5 | 8.5 | 17.4 | 21.7 |
| LSD value | 7.92 * | 1.77 * | 1.08 * | 4.51 * | 4.86 * |

*P≤0.05

## Conclusion

The study has investigated the use of lactose hydrolyzed milk by β-calactosidase enzyme for those suffering from the problem of poor digestion of milk sugar. Manufacturing of product that has a functional therapeutic from lactose hydrolyzed milk in order to use it for medical and therapeutic purposes is what the paper is conducted for. Therefore, the study has conducted more studies to search for the enzyme from other local sources, which finished by finding the most sources of this type of available sources. Commercially producing the enzyme from local sources such as tomato peels to save foreign currency is performed during the current paper and it was found that there was an excellent amount of β-calactosidase enzyme was extracted and purified.

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