Preparation of lactose hydrolysed milk using β-galactosidase enzyme extracted from potential Lactobacillus cultures

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Abstract: In the study, pH 6.5 and 40°C were selected on the basis of maximum β-galactosidase activity produced by L. helveticus MTCC 5463 (V3), L. rhamnosus (NK2) and L. casei (NK9) during optimizing the pH and temperatures using Response Surface Methodology (RSM). During evaluation of lactose hydrolysis using partially purified β-galactosidase enzyme, V3 showed highest reduction of lactose and maximum production of glucose and galactose at 2 % rate of β-galactosidase upto 12h of incubation. Partially purified β-galactosidase enzyme showed maximum lactose hydrolysis at 2% rate of addition in sterilized reconstituted skim milk for V3, NK2 & NK9 cultures up to 12h of incubation. Lactose hydrolysed milk which were prepared using partially purified enzyme were added at 2% in sterilized reconstituted skim milk for 12h at 37°C. Based on sensory scores, B (Commercially available enzyme sample), A (Reconstituted sterilized skim milk), and C (Partially purified β-galactosidase produced from V3) showed highest sensory score and were more acceptable compared to D (NK2), E (NK9) and F (Market sample: Lactose free milk) after 12h of incubation at 37°C by adding 2% partially purified β-galactosidase enzyme in sterilized reconstituted skim milk.

Keywords: β-galactosidase; Lactobacillus; Lactose hydrolysed milk; Ultrasonication,

Introduction

Lactose hydrolysis in milk and other dairy products by the enzyme, β-galactosidase [EC 3.2.1.23] is of considerable interest to the dairy industry. β-galactosidases produced by microorganisms are being used in food technology for hydrolysis of lactose in milk and milk by-products. The enzyme has attracted much attention in view of lactose intolerance in human population and due to importance of milk in human diet (Xavier et al. 2018). β-galactosidase is widely used in food industry to improve sweetness, solubility, flavor and digestibility of dairy products. The lactase enzymes most commonly used in the food industry are β-galactosidases, which are generally inhibited as glucose concentration increases (Jensen et al. 2015, Husain, 2010, Carevic et al. 2018). Enzymatic hydrolysis of lactose by β galactosidase is one of the most popular technologies to produce lactose reduced milk and related dairy products for consumption by lactose intolerant people (Haider and Husain, 2008). β-galactosidases from bacterial sources has been widely used for the hydrolysis of lactose because of the ease of fermentation, high activity of the enzyme and good stability (Picard et al. 2005). β-galactosidase from food grade probiotic microorganisms are safe for human use. Probiotic bacteria producing high level of β-galactosidase is very significant (Chanalia et al. 2018). Lactic acid bacteria (LAB) that used as starters for production of dairy products are the main factors of fermentation and protection of fermentative foods and also have a significant role in texture and flavour of food products. Maximum production of β-galactosidase was obtained with pH at 6 and Temperature at 45°C. Lactobacillus delbrueckii was showed that it is ideal for lactose intolerant people and can be used for probiotics (Sharma and Singh, 2014). Bosso et al. (2016) evaluated the enzyme from Kluyveromycetes lactis (liquid) and Aspergillus oryzae (lyophilized) was investigated the temperature and β-galactosidase concentration on the lactose hydrolysis in UHT milk was higher than in skimmed milk. With respect to the thermal stability, a decrease in hydrolysis rate was observed at pH 6.0 at 35°C for K. lactis enzyme, and at pH 6.0 at 55°C for the enzyme from A. oryzae. Chanalia et al. (2018) reported thermodynamic parameters, which were calculated and suggested that β-galactosidase is less stable at higher temperature (60°C). Approximately 75 % of Earths population is lactose intolerant and in India (particularly southern part of India)
70% of the population are lactose intolerance. To meet the challenge, lactose hydrolysed milk is an alternative solution for the proposed study. However the source of enzyme (β-galactosidase) from biological material like LAB (Lactic acid bacteria) which is an added advantage from safety point of view having GRAS (Generally recognized as safe) status.

Materials and methods

The Lactobacillus cultures used in the present study i.e. L. helveticus MTCC 5463 (V3), L. rhamnosus (NK2) and L. casei (NK9) were obtained from the Culture Collection of Dairy Microbiology Department, SMC College of Dairy Science, Anand Agricultural University, Anand. The Lactobacillus cultures were propagated in sterilized reconstituted skim milk (10% TS) and stored at 5±2°C. The transfer of stored cultures was given every week during the course of the study. Most of the bacteriological media, chemicals and reagents were purchased either from Hi-Media (India), Sigma (USA), SDFCL (India), Chr. Hansen (Denmark).

Optimization of pH and temperatures for β-galactosidase activity

All the cultures were activated by growing in MRS broth tubes. The activated cultures were added to 100 ml MRS broth (HiMedia, India) at the rate of 2%. After mixing thoroughly, the cultures were incubated at 37°C for 24h. After that, samples were taken out for β-galactosidase activity.

Enzyme Extraction

After 24h, the cells were harvested by centrifuging at 5000 rpm for 15 min at 4°C. The supernatant was considered to be containing extracellular enzymes. The cell pellet was crushed and washed twice with a 0.05 M sodium phosphate buffer (pH 6.8) and centrifuged at 5000 rpm for 15 min at 4°C. The washed pellets were resuspended in 10 mL of 0.05 M sodium phosphate buffer (pH 6.8) for intracellular enzyme extraction using sonication method (Makwana et al. 2017; Prasad et al. 2013):

Sonication treatment

The cell suspensions were sonicated for 5 min intervals (pulse 15 seconds Off / 30 seconds On at 55% amplitudes) in ice bath using sonicator (LABMAN, India), according to the method mentioned by Prasad et al. (2013). The extract was then centrifuged at 5000 rpm under 4°C for 15 min and the supernatant containing the crude enzyme was stored at -20°C until used for enzyme assays.

Enzyme assay

The β-galactosidase was determined by the reaction mixture composed of 0.5 mL of supernatant containing extracted enzyme and 2.0 mL of 15 mM O-nitrophenyl β-D-galactopyranoside (ONPG) in 0.05 M sodium phosphate buffer (different pH). After incubation for 20 min at different incubation temperature, 0.5 mL of 0.1 M sodium carbonate was added to the mixture to stop the reaction. Absorbance was measured at 420 nm with a spectrophotometer (Systronics PC based double beam spectrophotometer 2202, India) (Makwana et al. 2017; Prasad et al. 2013). Optimization of pH and Temperature were carried out using RSM.

Preparation of lactose hydrolysed milk using β-galactosidase

Sterilized skim milk added with i.e. 1.0, 1.5 & 2% of partially purified β-galactosidase enzyme and incubated at 37°C for 0, 4, 8 & 12h. After incubations, 2 ml sample and 1.5 ml double distilled water were kept in water bath at 60°C for 10 min. Then, 0.25 ml of Carrez 1 (Potassium ferrocyanide), 0.25 ml of Carrez 2 (Zinc acetate) and 1 ml of acetonitrile were added, gently mixed it and kept in incubation at 37°C for 1 h. After 1h, the samples were centrifuged at 5000 rpm for 10 min under 20°C. The supernatant were filtered, using syringe filter (0.22 µ syringe filter). Samples were kept at -20°C until measured through RP-HPLC (Shimadzu LC-20, Japan) for lactose, glucose and galactose.

Estimation of Lactose, Glucose and Galactose through RP-HPLC

20 µl processed sample was injected in the HPLC (Shimadzu LC-20, Japan) through microinjector (HAMILTON Bonaduz AG, Switzerland. RP-HPLC (Shimadzu LC-20, Japan) was performed as described by Rodriguez-Figueroa et al. (2012); Papadimitriou et al. (2007). An isocratic HPLC system was used fitted with C18 column (Se Quant® ZIC®-cHILIC) white pore analytic column (3µ, 250×4.6 mm). Sample was applied using microinjector (HAMILTON Bonaduz AG, Switzerland) with 20µl loop. Eluent was 1% (v/v) of TFA in mixture of 70:30 of acetonitrile and deionised water. Separation was conducted at room temperature at flow rate 0.5 ml / min with eluent for 25 min. Absorbance of elute was monitored through refractive index detector (Shimadzu, RID-10A). All the samples were analysed in triplicate.

Sensory evaluation

Lactose hydrolysed milk was prepared adding partially purified enzymes @ 2% in sterilized reconstituted skim milk and incubated for 12h at 37°C. After incubation, lactose hydrolysed milk was stored in refrigeration condition (4±2°C). The overall quality and acceptability of Lactose hydrolysed milk samples were assessed by a consumer oriented panel after 12 h of incubation. All products were coded and arranged in random order. The product was subjected to the sensory evaluation by an expert trained panel of seven judges for colour and appearance, odour, flavour and taste and body (consistency) on the basis of 100 point score card prescribed by BIS (IS:7768, 1975). Six combinations were decided (3: cultures, 1: CAE and one positive and negative control) in the study. After 12h of incubation at 37°C, the lactose hydrolysed milk stored at refrigeration temperature and then were brought to

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10°C before providing to the panelists. The score given by panelists on 100 point score card were considered for judging the acceptability of the product.

Results and discussion

A statistical software programme (Design Expert 8.0.3) was employed to optimize pH and Temperature. Two factors were in the range of pH (5.5 to 7.5) and temperature (30 to 50°C). The software suggested 13 runs and the response obtained from 13 runs by V3, NK2 and NK9 has been shown in Table 1.

Influence of different Levels of pH and temperature on β-galactosidase activity produced by V3, NK2 and NK9 cultures

In any enzymatic assay, pH and temperature play an important role in deciding the optimum activity of enzyme. The parameters chosen to assess the quality of β-galactosidase activity were pH and temperature. The data of the β-galactosidase enzyme along with their formulations as per their run order were presented in table 1. The quadratic model for pH and temperature were fitted through successive regression analysis. The Model F values for pH and temperature of V3, NK2 and NK9 cultures were 41.54, 231.24 and 63.37 respectively as shown in Table 2. The calculated F values greater than the Table F values at 5 % level of confidence indicates the significance of model terms. Furthermore, the coefficient of determination ($R^2$) which reflects the proportion of variability in data explained or accounted by the model for pH and temperature of V3, NK2 and NK9 cultures were 0.9674, 0.9940 and 0.9784 respectively. A larger $R^2$ value approaching to 1.00 suggests a better fit of the quadratic model. The adequate precision value for pH and temperature of V3, NK2 and NK9 cultures were 14.965, 32.731 and 17.655 respectively which were greater than 4, highlighting the suitability of the model to navigate the design.

Effect of pH and temperature on β-galactosidase activity

The β-galactosidase activity scores for V3 culture were ranged from 0.641 (O.D.) to 3.452 (O.D.), followed by NK2 culture ranged from 0.388 (O.D) to 2.984 (O.D) and NK9 culture ranged from 0.556(O.D) to 3.14(O.D). The β-galactosidase activity scores were found best at pH 6.5 and temperature 40°C (V3=run 3, NK2=run 5, NK9=run 7) while scored lowest for pH 7.9 and temperature 40°C for V3 and NK2 cultures and pH 5.5 and temperature 30°C for NK9 culture (Table 2). The values were presented in Table 2 which revealed that pH and temperature had significant negative effect on β-galactosidase activity on quadratic level. The square of factor (quadratic) indicates the effect of factors at highest level used in the product preparation. A significant ($P>0.01$) negative effect on V3, NK2 and NK9 cultures were found with the higher level of pH ($A^2$) and temperature ($B^2$).

β-galactosidase activity for V3 culture = $+3.37+0.058A+0.046B+0.13AB-1.41A^2-0.83B^2$

β-galactosidase activity for NK2 culture= $+2.97+0.036A+7.12E-003B-0.029AB-1.31A^2-0.99B^2$

β-galactosidase activity for NK9 culture= $+3.07+0.029A-2.359E-003B+0.0.046AB-1.33A^2-0.90 B^2$

Where, A and B refer to pH and temperature (°C) respectively. The response surface plots for the values obtained were shown in Fig. 1 which are based on the above model with varying levels of the two variables studied within the experimental range.

| Run | A: pH | B: Temperature (°C) | β-galactosidase activity (O. D) V3 Culture | β-galactosidase activity (O. D) NK2 Culture | β-galactosidase activity (O. D) NK9 Culture |
|-----|-------|---------------------|-------------------------------------------|---------------------------------------------|-------------------------------------------|
| 1   | 5.5   | 50                  | 0.750                                      | 0.501                                       | 0.588                                     |
| 2   | 5.5   | 30                  | 0.708                                      | 0.464                                       | 0.556                                     |
| 3   | 6.5   | 40                  | 3.452                                      | 2.976                                       | 3.03                                      |
| 4   | 7.5   | 30                  | 0.846                                      | 0.736                                       | 0.595                                     |
| 5   | 6.5   | 40                  | 3.368                                      | 2.984                                       | 2.991                                     |
| 6   | 5.1   | 40                  | 0.865                                      | 0.486                                       | 0.603                                     |
| 7   | 6.5   | 40                  | 3.371                                      | 2.98                                        | 3.14                                      |
| 8   | 6.5   | 26                  | 1.985                                      | 1.043                                       | 1.54                                      |
| 9   | 7.9   | 40                  | 0.641                                      | 0.388                                       | 0.584                                     |
| 10  | 6.5   | 54                  | 1.829                                      | 1.113                                       | 1.358                                     |
| 11  | 7.5   | 50                  | 1.396                                      | 0.657                                       | 0.81                                      |
| 12  | 6.5   | 40                  | 3.366                                      | 2.95                                        | 3.121                                     |
| 13  | 6.5   | 40                  | 3.313                                      | 2.942                                       | 3.045                                     |
Optimization of pH and temperature for β-galactosidase enzyme production

Optimization of pH and temperature for β-galactosidase production were carried out with the objective of determining the best possible combination(s) of pH and temperature (°C) that would lead to maximum β-galactosidase production. The goals

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**Table 2** Partial Coefficients of regression equations of suggested models for β-galactosidase activity scores at different pH and temperature

| Factor       | β-galactosidase activity V3 Culture | β-galactosidase activity NK2 Culture | β-galactosidase activity NK9 Culture |
|--------------|-------------------------------------|-------------------------------------|-------------------------------------|
| Linear       | A                                   | 0.0584                              | 0.0362                              | 0.0293                              |
|              | B                                   | 0.0464                              | 0.0071                              | -0.0024                             |
| Interactive  | AB                                  | 0.127                               | -0.029                              | 0.0458                              |
| Quadratic    | $A^2$                               | -1.412*                             | -1.3067*                            | -1.332*                             |
|              | $B^2$                               | -0.835*                             | -0.9862*                            | -0.903*                             |
| $R^2$        |                                     | 0.9674                              | 0.9940                              | 0.9784                              |
| Model f value|                                     | 41.54                               | 231.24                              | 63.37                               |
| Intercept    |                                     | 3.374                               | 2.9664                              | 3.0654                              |
| APV          |                                     | 14.965                              | 32.731                              | 17.655                              |
| Model        |                                     | Quadratic                           | Quadratic                           | Quadratic                           |

*P < 0.01; APV = Adequate Precision Value; $R^2$ = Coefficient of determination

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**Fig. 1** Response surface of β-galactosidase activity score as influenced by level of pH and temperature of A= V3, B= NK2, C= NK9 cultures
that were set for obtaining the best combination were illustrated in Table 3. The ranges of the minimum and maximum values for each of the attribute observed in the previous phase for each of the selected constraints/responses were fed to the software package. The data were analyzed in Design Expert Package. Considering the constraints and their upper and lower limits, the RSM suggested the one most suited solution as shown in Table 4.

The final enzyme assay was conducted by employing the formulation as shown in Table 4. The result was suggested by the software (Table 4). Both values were nearer to suggested values and it is not possible to control temperature and pH up to 2 decimal places. Thus, we carried out experiment at pH 6.5 and temperature i.e. 40°C in actual conditions. We found the highest β-galactosidase activity produced by V3, NK2 and NK9 cultures at above conditions. The process was replicated seven times. The selected factors and the average values of the results were derived. The values of the selected constraints/responses as shown in Table 4 were compared statistically using paired t-test with that of the predicted values as shown in Table 4. The calculated values of all these selected constraints suggest that the calculated values of ‘t’ for all the constraints were less than the table values, thus it was inferred that there was no significant (P>0.05) difference between the predicted and actual values of responses as shown in Table 5.

### Table 3 Criteria/responses chosen for process optimization of pH and temperature for β-galactosidase enzyme production

| Sr. No | Constrain | Units | Goal   | Lower limit | Upper limit | Level of importance |
|--------|-----------|-------|--------|-------------|-------------|---------------------|
| 1      | pH        | -     | In range | 5.5         | 7.5         | 3                   |
| 2      | Temperature | °C | In range | 30          | 50          | 3                   |
| 3      | β-galactosidase activity (V3) | OD | Maximum | 0.641       | 3.452       | 3                   |
| 4      | β-galactosidase activity (NK2) | OD | Maximum | 0.388       | 2.984       | 3                   |
| 5      | β-galactosidase activity (NK9) | OD | Maximum | 0.556       | 3.14        | 3                   |
It was therefore confirmed that the selected combination of the factors was the best in terms of the responses delineated at the study.

Rajakala and Karthigai, (2006) evaluated that optimizing the conditions for whey lactose hydrolysis to prepare whey based soups or syrups. β-galactosidase enzyme was extracted from Kluyveromyces maxianus and partially purified. The whey lactose hydrolysis was carried out at two different temperatures, i.e. 25 and 37°C, using whey with different pH (6.6 and 7.0). Out of eight combinations of alkali metal ions (K⁺, Na⁺), pH (6.6 and 7.0) and...
In the Fig. 2, Lactose reduction (%) by V3 culture was significantly (P<0.05) increased at 2% rate of β-galactosidase addition after 12h of incubation (Fig. 4). Lactose reduction (%) was significantly maximum at 2% rate of β-galactosidase addition after 12h of incubation for V3 (6.467 %), NK2 (1.567 %) and NK9 (1.747 %), compared to 1.5 % and 1 % respectively. Significantly maximum glucose production (%) was also observed after 12h of incubation (1.345 %), followed by 8h (1.034 %) and 4h (0.010 %). From the Fig. 3, it was also observed that glucose production (%) of V3, NK2 and NK9 cultures were significantly (P<0.05) increased with the different inoculation rates (1, 1.5 and 2 %) along with incubation periods (0, 4, 8 and 12h).

Galactose production (%) by V3 was significantly (P<0.05) increased at 2% rate of β-galactosidase after 12h of incubation (Fig. 4). Galactose production (%) was significantly maximum at 2% rate of β-galactosidase addition after 12h of incubation for V3 (6.467 %), NK2 (1.567 %) and NK9 (1.747 %), compared to 1.5 % and 1 % respectively. Significantly maximum galactose production (%) was also observed after 12h of incubation (3.246 %), followed by 8h (2.273 %) and 4h (1.048 %). From the Fig. 4, it was also found that galactose production (%) of V3, NK2 and NK9 cultures were significantly (P<0.05) increased with the different inoculation rate (1, 1.5 and 2 %) along with incubation periods (0, 4, 8 and 12h).

Sarah et al. (2012) evaluated the correct labelling of dairy foods as “lactose-free” requires a suitably sensitive and valid analytical method for the quantification of lactose in complex food matrices. Thus, an RP-HPLC method for the simultaneous determination of lactose, glucose and galactose in original skim milk was investigated. The samples derived from an enzymatic lactose hydrolysis approach (0.5 L) using the commercial β-galactosidase Godo-YNL2. After derivatisation with p-aminobenzoic acid and sodium cyanoborohydride, the samples were injected on a RP-C18 column. Tetrabutylammonium hydrogen sulphate was used as the ion-pair reagent in the eluent system. The sugars were quantified using photometric (UV; 303 nm) and fluorescence-detection (λex 313 nm, λem 358 nm). The overall run time was 27 min. The limits of detection (LOD) were estimated at 2 mg L⁻¹ (UV detection) and at 0.13 mg L⁻¹ (fluorescence detection). The limits of quantification were 6 mg L⁻¹ (UV detection) and 0.45 mg L⁻¹ (fluorescence detection). Thus, this analytical method is suitable for sensitive lactose quantification in milk systems of less than 10 mg L⁻¹ (Sarah et al. 2012).

Sensory analysis of lactose hydrolysed milk using partially purified β-galactosidase enzyme extracted from different cultures

During hydrolysing lactose in milk, lactose is broken down into glucose and galactose. Hence, the content of glucose in the milk becomes high. Since sweetening index of glucose is five times higher than that of lactose, and sweetening index of galactose is four times higher than that of lactose, and the milk tastes sweeter without adding any sweetening substance due to glucose and galactose content. It should be free from undesirable off flavors like cowy, barny, weedy, foreign, acidic, malty, lacks freshness and other extraneous matter. Lactose hydrolysed milk samples were subjected to judging and grading for various sensory

Preparation of lactose hydrolysed milk using β-galactosidase

The β-galactosidase enzyme extracted from selected Lactobacillus cultures, i.e. V3, NK2 and NK9 were statistically analysed for the different time intervals of incubations along with different % addition of enzyme for evaluating the production of lactose hydrolysed milk. Lactose reduction and glucose & galactose production by V3, NK2 and NK9 in sterilized reconstitute skim milk at different times (0, 4, 8 and 12h) with varying concentrations of enzyme (1, 1.5 and 2%) were represented in Fig. 2, Fig. 3 and Fig. 4 respectively.

In the Fig. 2, Lactose reduction (%) by V3 culture was significantly (P<0.05) increased at 2% rate of β-galactosidase enzyme after 12h of incubation. Lactose reduction (%) was significantly higher at the rate of 2% β-galactosidase enzyme after 12h of incubation for V3 (25.445 %) than NK2 (12.474 %) and NK9 (20.750 %) as compared to 1.5% and 1% respectively. Significantly maximum lactose reduction (%) was also observed at 12h of incubation (22.511 %), followed by 8h (18.402 %) and 4h (14.064 %). From the Fig. 2, it was also observed that lactose reduction (%) of V3, NK2 and NK9 cultures were significantly (P<0.05) increased with the different inoculation rates (1, 1.5 and 2 %) along with incubation periods (0, 4, 8 and 12h).

In the Fig. 3, glucose production (%) by V3 was significantly (P<0.05) increased at 2% rate of β-galactosidase after 12h of incubation. Glucose production (%) was significantly highest at 2% rate of β-galactosidase after 12h of incubation for V3 (3.720 %), NK2 (0.134 %) and NK9 (0.214 %) as compared to 1.5 % and 1% respectively. Significantly maximum glucose production (%) was also observed after 12h of incubation (1.345 %), followed by 8h (1.034 %) and 4h (0.010 %). From the Fig. 3, it was also observed that glucose production (%) of V3, NK2 and NK9 cultures were significantly (P<0.05) increased with the different inoculation rates (1, 1.5 and 2 %) along with incubation periods (0, 4, 8 and 12h).
attributes viz. i) Color and Appearance, ii) Odour (roma), iii) acidity, iv) Flavour & taste and v) Body (consistency) vi) Overall acceptability on the basis of 100 point score card as per BIS (IS:7768, 1975). Six combinations were decided (3: cultures, 1: Commercially available enzyme (CAE) and one positive and negative control) in this study.

The variations in color and appearance scores of lactose hydrolysed milk were given in Fig. 5. Lowest score was observed in Sample F (7.61). The average color and appearance score was 8.54. During sensory evaluation, Samples B, C and E exhibited highest score followed by D, A and F. The average color and appearance score was ranged from 7.61 to 8.89. The variations in odour scores of lactose hydrolysed milk were given in Fig. 5. Lowest score was observed in Sample F (16.0). The average odour score was 16.44. During sensory evaluation, Samples B shown highest score followed by C, A, D, E and F. The average odour score was ranged from 16.0 to 17.48. The variations in flavour & taste scores of lactose hydrolysed milk were given in Fig. 5. Lowest score was observed in Sample D (32.41). The average flavour and taste score was 33.83. During sensory evaluation, Samples B exhibited the highest score followed by A, F, C, E and F. The average flavour & taste score was ranged from 32.41 to 37.04. The variations in body (consistency) scores of lactose hydrolysed milk were given in Fig. 5. Lowest score was observed in Sample A (25.52). The average body (consistency) score was 26.15. During sensory evaluation, Samples B had shown highest score followed by C, D, E, F and A. The average body (consistency) score was ranged from 25.52 to 26.93. The variations in overall acceptability scores of lactose hydrolysed milk were given in Fig. 5. Lowest score was observed in Sample F (82.50). The average overall acceptability score was 84.95. During sensory evaluation, Samples B had shown highest score followed by A, C, E, D and F. The overall acceptability score was ranged from 82.50 to 90.33.

Adhikari et al. (2010) reported the sensory characteristics of ultra-pasteurized (UP) lactose-free milk of different fat contents, and compared them with normal milk. Nine milk samples (six UP lactose-free and three regular) containing 0, 2 or 3g milk fat/100 ml were tested by a descriptive panel. A consumer test with three UP lactose-free milk and three regular samples was also conducted. The skim milks were found to be lacking in freshness and the dairy notes were lower compared to the higher-fat-content milks. The UP lactose-free milks were different from the regular milk because of higher intensities of cooked, processed, and sweet attributes. This results is similar as our study. In another study, Nielsen et al. (2017) studied on the effects of storage conditions on the shelf-life of hydrolysed-lactose ultra-high-temperature (UHT) milk was evaluated using proteomics. Lactose content was initially reduced to approximately 40% by ultra and nano filtration before hydrolysis. Sensory descriptive analysis was used to describe the sensory characteristics of the milk during storage. Bitterness intensity significantly increased over time and was correlated with the level of peptides released via either enzymatic (proteolytic side activity of the enzyme used in lactose hydrolysis) or non-enzymatic pathways (heat and storage induction). This study demonstrated a relationship between proteolysis and decreased shelf-life of hydrolysed-lactose UHT milk compared with conventional UHT milk.

Based on above sensory scores, B, A, and C cultures showed highest sensory score and they were more acceptable compared to D, E and F after 12h of incubation at 37°C by adding 2% partially purified β-galactosidase enzyme in sterilized reconstituted skim milk. However, B, A and C were found acceptable.

Conclusions

L. helveticus MTCC 5463 (V3) produced maximum β-galactosidase activity at pH 6.5 and temperature i.e. 40°C. During evaluation of lactose hydrolysis using partially purified β-galactosidase enzyme, V3 showed maximum reduction of lactose and maximum production of glucose and galactose at 2 % rate of β-galactosidase upto 12h of incubation. Based on sensory score, B (Commercially available enzyme sample), A (Reconstituted sterilized skim milk), and C (Partially purified β-galactosidase produced from V3) were found acceptable. Therefore, β-galactosidase extracted from Lactobacillus cultures could be used for the production of lactose hydrolysed milk.

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