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RESEARCH ARTICLE

Quality assessment and storage stability of developed lactose-hydrolyzed yoghurt using β-galactosidase

Hardwari Lal, Sharanagouda B, Sumit Mahajan, Shiv and Indu Panchal (✉)

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Abstract: The present study aimed to develop lactose-hydrolyzed yoghurt by using lactase enzyme in a yoghurt mix to produce simultaneously fermented and lactose-hydrolyzed yoghurt . Different levels of enzyme addition were tried, namely 0.20, 0.40, 0.60, 0.80, and 1.0 ml/L. Different parameters like Physico-chemical, rheological, microbiological, and sensory characteristics of the LHY were studied. It was observed that the penetration value, whey syneresis, and acidity of the lactose-hydrolyzed yoghurt (LHY) increased with the increased level of lactose hydrolysis. Whereas, setting time and sensory attributes were decreased correspondingly with the increased degree of lactose hydrolysis. Based on the sensory evaluation, 0.6ml/L lactase enzyme, which produced 66.69% lactose hydrolysis in LHY, was adjudged as very much comparable to the control. The developed products of lactose-hydrolyzed yoghurt were stored at 4±1! and evaluated at 7-day intervals for 28 days. There was a decrease in pH and an increase in acidity throughout the storage period at refrigeration temperature, and such a product was well stored up to the 14^{th} day of storage.

Keywords: Lactase enzyme, Lactose hydrolysis, Lactose hydrolyzed yoghurt

Introduction

Milk is considered a wholesome food for everyone. However, the problems associated with milk consumption are milk allergy and lactose intolerance. As per a recent survey 65% of Indians and 68% of the world population are suffering from lactose intolerance (Rishika 2024), which may be due to genetic factors. Yoghurt is one of the most popular fermented milk products in the world, due to various desirable characteristics of the product that are readily accepted by consumers and also due to the image of the product as 'healthy' (Baba et al. 2018). Yogurt is a universally well-known, unique fermented dairy product, manufactured using a special symbiotic yoghurt starter that produces characteristic flavour and body and texture. Since yogurt mix is often supplemented with milk solids non-fat (MSNF) to increase total solids in the mix, such a mix may contain more amount of lactose, which is hydrolyzed by the enzyme lactase present in the epithelial cells of the brush border of the small intestine. The lactose-intolerant consumer whose digestive system is deficient in lactase enzyme cannot utilize lactose-causing disorders such as gastro-intestinal discomfort like bloating, diarrhea, flatulence, abdominal pain, loss of appetite, nausea, etc. (Nagrala et al. 2019). In such a case, lactose in the yoghurt mix can be hydrolyzed using an external source of lactase from either bacteria or fungi. The advantages of lactose hydrolyzed products are improved therapeutic, functional, and nutritional properties with reduced lactose content and making yogurt more digestible by lactose-sensitive and lactase-deficient individuals. To get the therapeutic benefit of yoghurt consumption, it is suggested that the yoghurt containing viable probiotic cells of 10-20x10^9 cfu/ml should be consumed at least 100 g per day (Gina 2024). Some studies have suggested that yogurt consumption might help to improve lactose intolerance. The presence of β-galactosidase activity in the cells of the lactic bacteria would be responsible for better lactose digestion (Vasudha et al. 2024). The addition of β-galactosidase (lactase) in the manufacturing of low-lactose fermented milk products like yoghurt usually occurs at different stages. In the conventional process, enzymatic catalysis first occurs in a substrate at a pH between 6.5 and 6.8 and temperatures between 4 and 6ºC, to minimize microbiological contamination. This temperature takes the contact time to at least 30h. Such lactose-hydrolyzed yoghurt mix was inoculated with starter cultures (two-stage process) to produce yoghurt (Martins et al. 2012).

In this study, a single-stage process to obtain a yogurt with low lactose content was proposed, involving simultaneous enzymatic

College of Dairy Science and Technology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar 125001, Haryana (India)

Indu Panchal (✉)
Assistant Professor, Department of Dairy Engineering, College of Dairy Science and Technology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar 125001, Haryana (India).
Email: indu.codst@luvas.edu.in

hydrolysis of lactose using lactase (β-galactosidase) enzyme in order to overcome the drawbacks of the two-stage process. Hence, a proposed study has been planned to evaluate the effect of simultaneous lactose hydrolysis and fermentation by yogurt culture on the quality and storage stability of the developed yoghurt.

Material and methods

Sagar brand Skim milk powder (SMP) and Sodium-Alginate and Glycerol Monostearate (GMS) in the ratio of 1:1 used in the preparation of yoghurt were procured from the local market. Enzyme lactase (β-galactosidase), having 3000 ALU/ml activity, was used for hydrolyzing lactose. A mixed culture of *Streptococcus salivarius subsp. thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* in the ratio of 1:1 were used in the preparation of yoghurt. Media like Nutrient Agar (NA), Violet Red Bile Agar (VRBA), Potato Dextrose Agar (PDA), MRS, and M17 were used for microbial studies.

Preparation of Yoghurt

Yoghurt mix having 3.0% milk fat and 11.0% MSNF was prepared by using mixed milk procured from students' experimental plant, LUVAS, Hisar. Skim milk powder was used to standardize the SNF in the yoghurt mix to 11.0%. Emulsifier-stabilizer powder (1:1 ratio) was added to the yoghurt mix at a rate of 0.3% of the yoghurt mix. The mix was then preheated to 60°C and homogenized at 1500 psi and 500 psi for 1st and 2nd stages respectively using a two-stage homogenizer. Then pasteurized by heating the homogenized yoghurt mix to 90°C±1/5 min in water bath maintained at 92±1°C, then cooled 42±1°C using a chilled water bath. Then the pasteurized mix was inoculated with yoghurt starter culture *Streptococcus selverious spp. thermophillus* and *Lactobacillus delburkii spp. bulgaricus* (1:1 ratio) added at the rate of 1.5% of yoghurt mix and incubated at 42°C±1°C, until the pH reached 4.4. The yogurt was cooled immediately to 5±1°C with the help of a chilled water bath and stored in a refrigerator (5±1°C) for further analysis (Poorani et al. 2022).

Preparation of Lactose Hydrolysed Yoghurt (LHY)

For the preparation of LHY, Lactase enzyme (along with starter culture) at different levels were tried viz., 0.20, 0.40, 0.60, 0.80, and 1.0 ml/L to get 50, 60, 70, 80, and 90% lactose hydrolysis in LHY. Whereas, control was prepared without adding Lactase enzyme by following the method of Sharanagouda et al. (2022). Such prepared LHY samplers were cooled immediately to 5±1°C with the help of a chilled water bath and stored in a refrigerator (5±1°C) for further analysis.

Physico-chemical, Rheological, and Microbiological characteristics of yoghurt

Physico-chemical characteristics viz. pH, fat content, milk solid not fat (MSNF), acidity, lactose content, and syneresis for the control and lactose hydrolyzed yoghurt were determined. pH of the sample was measured using a digital pH meter (Elico Pvt. Ltd.). Fat and MSNF content as per FSSAI (2015) and the acidity of samples were determined as per AOAC (2012). Lactose content was analyzed by using Lane-Eynon method (NDRI Lab manual, 2012). Syneresis was measured as per the procedure of Nikitina et al. (2019). The curd tension was measured by adopting the procedure of Khadse et al. 2020. Rheological properties like viscosity, storage modulus (G'), loss modulus (G") and loss factor were measured as per Khabibullaev et al. (2020) using the dynamic Rheometer (MCR-302e, Anton Paar) using a parallel plate configuration (PP-50) of 50 mm diameter at 20°C. The sensory analysis was carried out by serving control and experimental samples of yoghurt to the panel of five judges by providing a 9-point hedonic scale scorecard (Nagaraj et al. 2009). The keeping quality of developed LHY and control samples was tested for 28 days of storage at refrigeration conditions (4±0.5°C) with an interval of 7 days. The standard plate count (SPC), coliform, and yeast and mold counts were enumerated as per Matin et al. *(*2018).

Statistical analysis

One-way ANOVA was used for statistical analysis to find out significant differences between the treatments. Pearson's correlation was used for the relationship between different treatments. Data were analyzed statistically on the SPSS-16.0 (SPSS Inc., and Cochran, II USA) software package. The differences were tested by calculating the critical difference at the 5 % level of significance.

Results and Discussion

Effect of enzymes concentration on degree of lactose hydrolysis and rate of acid production

The results in Table 1 indicate that as the enzyme concentration in the yoghurt mix increased from 0.0 to 1.0 ml/L, the degree of lactose hydrolysis also statistically significantly increased ($p \leq 0.05$) correspondingly from 50.00% to 76.58% in the lactose-hydrolyzed yoghurt (LHY). The mean initial acidity for both control and experimental samples was 0.17% LA. As enzyme concentration increased, the mean acidity of LHY increased from 0.67% to 0.97% LA after 180 min.of incubation. Notably, at enzyme concentrations of 0.8 and 1.0 ml/L, the desired acidity of at least 0.7% was achieved within 120 minutes, while samples with 0.2, 0.4, and 0.6 ml/L required 150 minutes to reach the same level. Although the acidity increased to desired level in 120 minutes across different enzyme concentrations were not statistically significant from one another, but they were significantly different from the control (p≤0.05). The control yoghurt achieved the required acidity after 180 minutes.

Effect of enzyme concentration on physico-chemical properties of LHY

Table 2 presents the mean values for the physico-chemical properties, viz., setting time, whey syneresis, and curd penetration for both control and experimental samples. The setting time, measured in minutes, decreased as the enzyme concentration increased from 0.0 to 1.0 ml/L, changing from in control 180 to 120 min. in lactose-hydrolyzed yoghurt. It was noted that the increase in lactose hydrolysis at enzyme concentrations up to 0.6 ml/L was statistically non-significant (p≤0.05), as was the comparison between 0.8 and 1.0 ml/L. However, all enzyme treatments were significantly different from the control (p≤0.05). Whey syneresis increased with higher enzyme concentrations, from 10.0 g at 0.0 ml/L to 29.0 g at 1.0 ml/L. The differences in syneresis at concentrations of 0.0, 0.2, and 0.4 ml/L were not statistically significant, but each was significantly different from the results at 0.6, 0.8, and 1.0 ml/L (p≤0.05). The curd strength was measured by penetration depth in mm of a cone penetrometer. The mean penetration values for control and experimental samples at 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml/L of lactase were 195, 230, 260, 285, 315, and 400 mm/5 sec, respectively. This indicates that penetration increased in the experimental samples compared to the control, with higher lactase concentrations corresponding to greater penetration values. Statistical analysis confirmed that these differences were significant (p≤0.05) between the control and all experimental samples.

Effect of lactose hydrolysis on rheological properties of LHY

In the present study, various rheological properties, including viscosity, storage modulus (G'), loss modulus (G"), and loss factor were studied. As shown in Table 3, the viscosity of the control yoghurt sample decreased from 29,460 to 8,058.2 mPa·s when the temperature was increased from 25°C to 50°C as mentioned in Table 3. It was noted that viscosity decreased for both the control and all treatments as the temperature rose. These trials were conducted at a constant shear rate, and statistical analysis

Table 1 Effect of enzyme concentration on degree of lactose hydrolysis and rate of acid production during simultaneous fermentation in Lactose-hydrolyzed yogurt (LHY)

Enzyme Conc. (ml/L)	Lactose hydrolysed (%)	The period in min.						
		0	60	90	120	150	180	210
		Percentage of lactic acid						
0.00	$50.00^{a} \pm 0.69$	$0.17^{a}\pm0.001$	$0.21^{a}\pm0.006$	$0.32^{a}\pm0.006$	$0.38^{a}\pm0.006$	$0.50^{a}\pm0.012$	$0.67^{a}\pm0.006$	0.79
0.20	$56.64^{b} \pm 0.67$	$0.17^{a}\pm0.001$	$0.27^{b}\pm0.006$	$0.40^{b}\pm0.006$	$0.60^{b}\pm0.012$	$0.73^{b}\pm0.006$	$0.80^{b}\pm0.012$	--
0.40	$61.48^{c} \pm 0.43$	$0.17^{a}\pm0.001$	$0.29^{c}\pm0.006$	$0.43^{c}\pm0.012$	$0.62^{b}\pm0.012$	$0.75^{b}\pm0.006$	$0.92^{c}\pm0.006$	--
0.60	$66.69^{d} \pm 0.42$	$0.17^{a}\pm0.001$	$0.29^{c}\pm0.006$	$0.45^{c}\pm0.006$	$0.63^{b}\pm0.006$	$0.79^{c}\pm0.006$	$0.94^{c}\pm0.006$	--
0.80	$70.85^{e} \pm 0.55$	$0.17^{a}\pm0.001$	$0.32^{d}\pm0.006$	$0.47^{c}\pm0.006$	$0.65^{b}\pm0.012$	$0.81^{c}\pm0.006$	$0.95^{c}\pm0.012$	--
1.00	$76.58^{f} \pm 0.38$	$0.17^{a}\pm0.001$	$0.32^{d}\pm0.006$	$0.48^{c}\pm0.012$	$0.66^{b}\pm0.006$	$0.83^{c}\pm0.006$	$0.97^{c}\pm0.006$	--
CD (p≤0.05)	1.688	----	0.018	0.025	0.028	0.022	0.025	--
CV%	1.473	0.589	3.53	3.328	2.68	1.666	1.616	--

Mean ± S.E.

Note: n=3; Superscripts with similar alphabetes are not significant to each other when read column wise

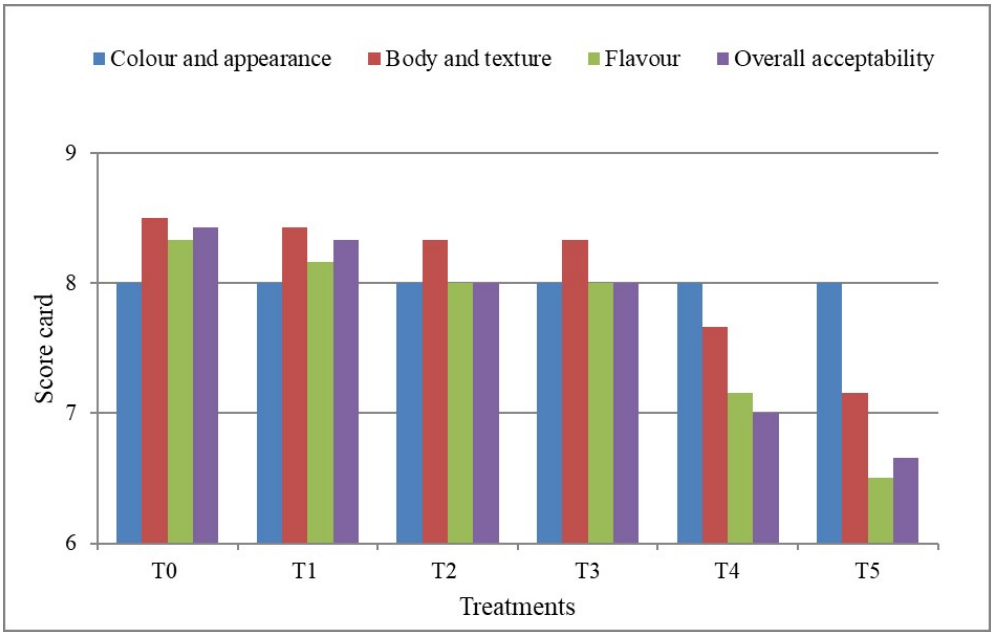
Table 2 Effect of enzyme concentration on physico-chemical properties of LHY

Enzyme conc. (ml/L)	Setting time (min.)	Syneresis (g water/100g product)	Curd Penetration (mm/5 sec)
0.00	$180^{c} \pm 2.88$	$10.0^{a} \pm 0.57$	$195^{a} \pm 2.88$
0.20	$150^{b} \pm 2.88$	$12.6^{a} \pm 0.75$	$230^{b} \pm 5.77$
0.40	$150^{b} \pm 2.88$	$12.9^{a} \pm 1.15$	$260^{c} \pm 2.88$
0.60	$150^{b} \pm 2.88$	$22.0^{b} \pm 1.15$	$285^{d} \pm 5.77$
0.80	$120^{a} \pm 2.88$	$25.0^{bc} \pm 0.57$	$315^{e} \pm 2.88$
1.00	$120^{a} \pm 2.88$	$27.0^{c} \pm 1.73$	$400^{f} \pm 5.77$
CD (p≤0.05)	8.993	3.34	14.22
CV%	3.448	10.175	2.815

Mean ± S.E.

Note: n=3; Superscripts with similar alphabetes are not significant to each other when read column wise

Fig. 1 Effect of level of enzyme concentration in simultaneous lactose hydrolysis and fermentation process on sensory quality of Lactose Hydrolysed Yoghurt.



revealed significant differences ($p \leq 0.05$) in viscosity between the control and experimental samples with respect to temperature. In contrast, the angular frequency for the control (T_0) and all treatments (T_1, T_2, T_3, T_4, T_5) remained statistically non-significant ($p \leq 0.05$).

The storage modulus (G') of the control (T_0) was significantly higher than that of treatments T_3 and T_4. However, treatments T_1, T_2, and T_5 exhibited significantly higher G' values ($p \leq 0.05$) compared to the control and treatments T_3 and T_4. Regarding the loss modulus (G"), the control (T_0) was significantly higher than treatments T_3 and T_4, yet lower than T_1, T_2, and T_5. Specifically, the loss modulus for T_1 and T_2 was significantly greater ($p \leq 0.05$) than that of T_3, T_4, and T_5. Additionally, the loss factor of the control was significantly higher ($p \leq 0.05$) than that of treatment T_1, while it was non-significantly different from treatment T_2. Among all treatments, T_4 exhibited a significantly higher loss factor ($p \leq 0.05$) compared to the other treatments (T_0, T_1, T_2, T_3, and T_5). There was no significant difference ($p \leq 0.05$) in the loss factor between treatments T_3 and T_5, but both were significantly higher ($p \leq 0.05$) than T_0, T_1, and T_2.

Effect of lactose hydrolysis on the sensory characteristics of LHY

Five enzyme concentrations, viz., 0.20, 0.40, 0.60, 0.80, and 1.00 ml/L, were tested to produce acceptable quality lactose-hydrolyzed yoghurt. The mean sensory scores for the control and experimental samples are illustrated in Figure 1. The mean scores for color and appearance across all samples were not significantly different from one another ($p \leq 0.05$). However, samples with 0.80 and 1.00 ml/L of lactase enzyme showed significantly lower scores ($p \leq 0.05$) for body and texture, flavor, and overall acceptability compared to the other samples. The control sample achieved the highest scores for all sensory attributes. Based on the sensory evaluation, 0.6 ml/L lactase enzyme addition was found to be very comparable to the control and was selected for further storage studies.

Effect of lactose hydrolysis on microbiological quality of LHY

The results of microbial enumeration, including standard plate count (SPC), coliforms, yeast and mold count (YMC), and starter count for control and lactose-hydrolyzed yoghurt, are presented in Table 6. The SPC for the control was significantly different ($p \leq 0.05$) from all other treatments (T_1, T_2, T_3, T_4 and T_5), with T_5 showing the highest SPC count among all treatments ($p \leq 0.05$). No significant difference was observed between the SPC counts of T_2 and T_3, although both were significantly higher ($p \leq 0.05$) than those of control and T_1. The YMC of the control was significantly lower ($p \leq 0.05$) than all other treatments, with T_5 again exhibiting the highest count ($p \leq 0.05$). Treatments T_2 and T_3 had non-significant differences in YMC but were significantly higher ($p \leq 0.05$) than control and T_1. Notably, coliforms were completely absent in all treatments, including the control. For the starter counts, control showed significantly lower values ($p \leq 0.05$) compared to all other treatments. Among the treatments, T_5 had the highest starter count ($p \leq 0.05$), while T_2 and T_3 had non-significant differences in their counts but were significantly higher ($p \leq 0.05$) than control and T_1.

Changes in microbiological and chemical quality of LHY during refrigerated storage temperature

The changes in microbiological quality including SPC and Yeast and Mold count of lactose hydrolyzed yoghurt during storage at refrigeration temperature (4±1°C) are presented in Fig 2 and 3

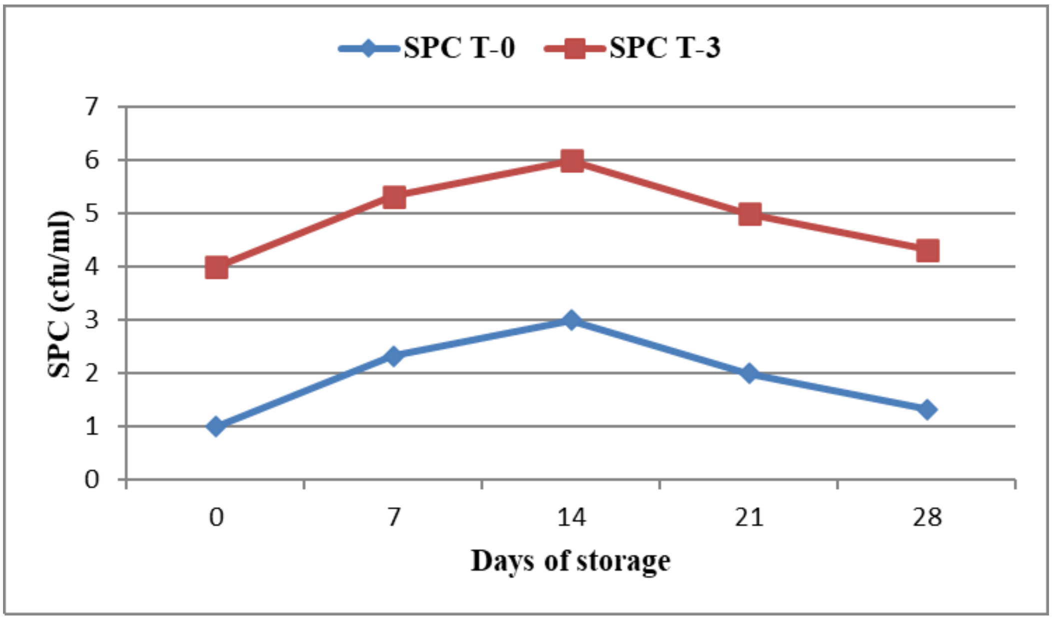


Fig. 2 Graph showing the standard plate count in simultaneous Lactose Hydrolysed Yoghurt stored at refrigertaion temperature (4±1!).

respectively. On the zero day of storage, the standard plate count (SPC) was 1.00×10^6 cfu/ml for the control and 4.00×10^6 cfu/ml for treatment T_3. The SPC increased significantly (p≤0.05) until the 14th day of storage, after which it decreased significantly for control. In contrast, the SPC for T_3 continued to decrease non-significantly (p≤0.05) up to the 28th day of storage. The yeast and mold count (YMC) on the zero day of storage was 1.00 cfu/ml for control and 4.00 cfu/ml for T_3. The YMC increased non-significantly (p≤0.05) until the 14th day, but thereafter, significant increases (p≤0.05) were observed for control up to the 28th day. The YMC for T_3 also increased non-significantly (p≤0.05) throughout the 28 days of storage period. The Coliforms were absent in all samples throughout the storage period, with no detection at any stage of storage studies. The starter counts of *S. thermophillus* (ST) on the zero day was 4.00×10^7 cfu/ml for control and 6.33×10^7 cfu/ml for T_3. The counts increased significantly (pd"0.05) until the 14th day, followed by a significant decrease for control. The counts for T_3 decreased non-significantly (p≤0.05) up to the 28th day of storage. The *L. bulgaricus* (LB) count on the first day was 5.00×10^7 cfu/ml for control and 8.00×10^7 cfu/ml for T_3. Similar to the ST counts, the LB counts increased significantly (p≤0.05) until the 14th day, then decreased significantly (p≤0.05) for control while T_3 continued to show a non-significant decrease (p≤0.05) up to the 28th day of storage.

The effect of refrigerated storage temeperature (4±1!) on developed acidity in Lactose Hydrolyzed Yoghurt throughout storage period are presented in Figure 4. It was observed that the pH of control (T_0) stored at referigeration temperature on 0, 7th, 14th, 21st and 28th days of storage decreased from 4.46 to 4.43, 4.40, 4.33, 4.23 and acidity of of the same samples for the respective storage period was increased from 0.79 to 0.80, 0.82, 0.84, 0.92% LA respectively. Similarly, the pH in lactose hydrolysed yoghurt (T_3) decreased from 4.40, 4.36, 4.30, 4.26 to 4.10 and acidity raised from 0.79, 0.82, 0.85, 0.90 to 0.99% LA on 0 day to 28th day of storage. The statistical analysis confirmed that there was significant (p≤0.05) decrease in pH was observed at 21 days and above storage periods. In the same lines the statistical analysis confirmed that there was significant (p≤0.05) increase in acidity was observed at 14 days and above storage periods.

In this study, different concentrations of lactase, ranging from 0.20 ml/L to 1.00 ml/L, were tested to assess their impact on lactose hydrolysis. Results indicated that lactose hydrolysis increased with higher lactase levels, reaching up to 76.56% (Table 1). This is likely due to the increased availability of the enzyme for lactose breakdown. The higher acidity observed in lactose-hydrolyzed yogurt (LHY) compared to the control can be attributed to the rapid conversion of glucose, which promotes starter culture growth and lactic acid production, leading to quicker yogurt setting. Similar findings were reported by Yamamoto et al. (2021) who observed faster pH reduction and decreased setting time in LHY. This is due to the ongoing activity of β-galactosidase, facilitating glucose conversion and its subsequent metabolism to acid by the starter cultures.

As shown in Table 2, increasing lactase concentrations resulted in higher acidity, likely due to the increased availability of glucose and galactose, which support greater lactic acid production. The greater acidity in LHY compared to the control could also be linked to the glucose conversion after inoculation, providing optimal conditions for starter cultures to produce acid more rapidly. These findings are consistent with those of Yamamoto et al. (2021) who reported similar pH decreases and increased lactic acid production in LHY. Additionally, Sharanagouda et al. (2022) observed higher lactic acid production with increased lactose hydrolysis.

Fig. 3 Graph showing the Yeast and Mold count in simultaneous Lactose Hydrolysed Yoghurt stored at refrigertaion temperature (4± 1°C).

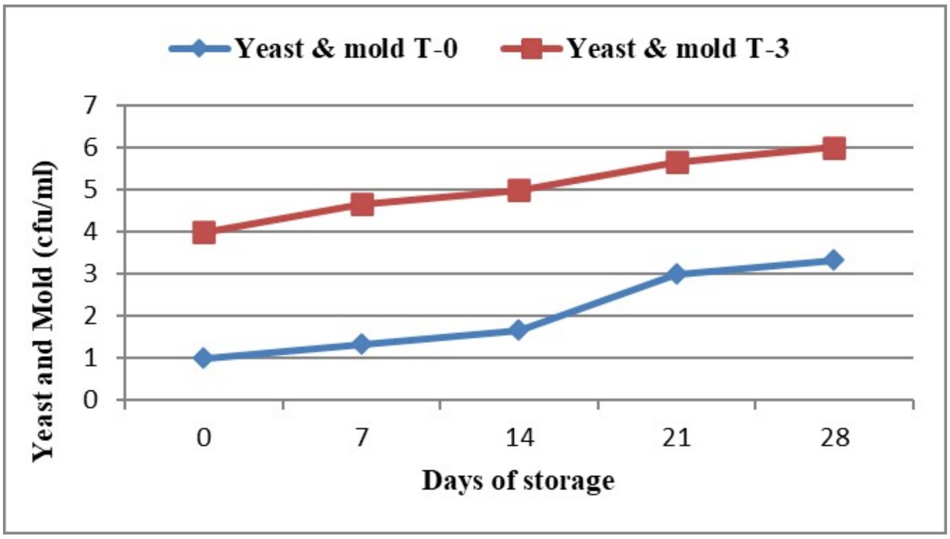
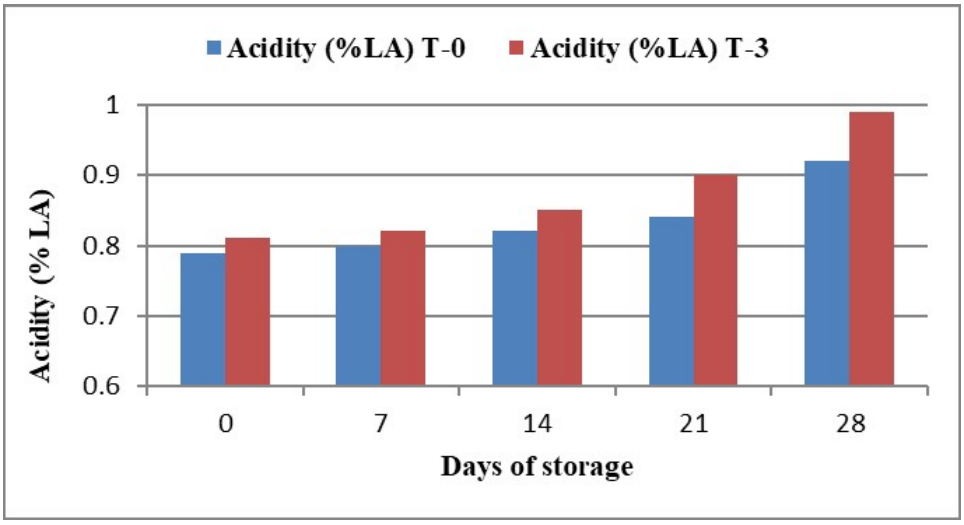


Fig. 4 Changes in percent acidity (LA) of Lactose Hydrolyzed Yoghurt during storage at refrigeration temperature (4 ± 1°C)



The physico-chemical properties evaluated in this study included whey syneresis, setting time, and curd strength. The setting time, needed to achieve an acidity of approximately 0.7% lactic acid (LA), ranged from 120 to 180 minutes. A significant ($P<0.05$) reduction in fermentation time was observed with increasing degrees of lactose hydrolysis (Table 2). As lactose hydrolysis increased, the setting time decreased significantly ($P<0.05$) compared to the control. This reduction may be due to the continuous hydrolysis of lactose during fermentation, leading to the production of easily fermentable sugars, which accelerated starter culture growth and shortened the setting time. Schmidt et al. (2016) reported similar results, noting faster setting times for LHY than the control, with the rapid fermentation of glucose from hydrolyzed lactose being a key factor (Nagaraj et al. 2009; Schmidt et al. 2016; Yamamoto et al. 2021). Additionally, whey syneresis increased significantly ($P\leq0.05$) with higher degrees of lactose hydrolysis. This could be due to protein destabilization caused by higher lactic acid content and increased monosaccharide solubility, as noted by Martin et al. (2012) and Pachekrepapol et al. (2021). Furthermore, curd strength, as indicated by cone penetration, decreased significantly ($P\leq0.05$) with increasing lactose hydrolysis. This reduction suggests a weaker protein network in LHY, likely due to the faster and higher production of lactic acid, leading to protein gel destabilization (Khabibullaev et al. 2020). However, these findings contrast with Ibrahim (2018), who reported improved viscosity and reduced syneresis in co-hydrolyzed fermented camel milk, potentially due to enhanced exopolysaccharide (EPS) production. This was possibly due to EPS interacting with water molecules, trapping them within the gel matrix and increasing the product's water-holding capacity. EPS acts as a natural thickener, increasing the viscosity and improving the body and texture of the fermented milk product.

Lactose-hydrolyzed yogurt (LHY) exhibits specific sensory characteristics, such as a sweet taste, rich flavor, smooth texture, and reduced sourness, attributed to lactose hydrolysis and fermentation (Ahmed et al. 2020). The ideal level of lactose hydrolysis for producing acceptable quality LHY was determined through sensory evaluation that can be observed in Figure 1. This study demonstrated that low-lactose yogurt with good quality can be efficiently produced by adding lactase enzyme simultaneously with the starter culture, a more convenient and time-saving method than pre-hydrolyzing the yogurt mix. Sensory evaluation revealed minimal differences in sweetness perception as lactose hydrolysis increased. Although hydrolyzed samples were perceived as sweeter and less acidic, the acidity and pH values were slightly higher than in the control samples. This perceived sweetness could be due to the breakdown of lactose into glucose and galactose, but the differences were not statistically significant (p≤0.05). In terms of colour and appearance, no significant differences (p≤0.05) were observed across the five enzyme addition levels (0.20 to 1.00 ml/L), though LHY had a creamier appearance than the control similar to the findings of Ahmed et al. (2020). Regarding body and texture, scores decreased non-significantly (p≤0.05) up to 0.60 ml/L enzyme addition but then significantly (p≤0.05) dropped compared to the control at higher enzyme levels. This may be due to the formation of larger whey pockets as lactose hydrolysis increases, leading to a weaker body and texture. These results align with those of Khabibullaev et al. (2020), who found that lower enzyme concentrations resulted in smaller whey pockets, while higher concentrations weakened the yogurt's structure. Flavour is a crucial factor for product acceptance. As lactose hydrolysis progressed, sweetness increased due to the production of glucose and galactose, alongside lactic acid and other flavouring compounds like acetaldehyde during fermentation (Popescu et al. 2022). It is also observed that flavour scores improved non-significantly (p≤0.05) up to 0.60 ml/L of lactase enzyme but decreased significantly (p≤0.05) at higher levels.

The overall acceptability is the combined characteristic of colour and appearance, body and texture, and flavour. All score for lactose hydrolyzed yoghurt were non-significantly different up to 0.60 ml/L of enzyme concentration and there onwards decreased significantly (p≤0.05) for body and texture and flavour. Schmidt et al. (2016) observed a decrease in sensory scores for more than 80% lactose hydrolyzed yoghurt and also reported that better sensory scores secured for up to 66.69% lactose

Table 3 Effect of temperature on Apparent viscosity of Lactose-hydrolyzed yogurt (LHY)

Temp. (°C)	Viscosity (mPa. s)					
	T_0	T_1	T_2	T_3	T_4	T_5
25	29460 ^{f} ±1.15	12854 ^{f} ±0.57	225.65 ^{f} ±2.35	491.04 ^{f} ±1.74	2227.6 ^{f} ±0.61	2233.1 ^{f} ±1.15
30	21983 ^{e} ±0.57	8815.5 ^{e} ±1.15	152.65 ^{e} ±1.17	285.37 ^{e} ±0.59	1622.6 ^{e} ±1.35	1489.8 ^{e} ±1.10
35	17184 ^{d} ±1.15	6825.5 ^{d} ±0.14	86.204 ^{d} ±2.30	152.74 ^{d} ±1.18	1116.7 ^{d} ±1.64	1107.7 ^{d} ±0.62
40	13121 ^{c} ±1.15	5232.6 ^{c} ±1.15	43.045 ^{c} ±1.15	61.949 ^{c} ±2.32	683.16 ^{c} ±0.58	740.21 ^{c} ±1.18
45	10142 ^{b} ±0.57	4028.3 ^{b} ±1.84	21.004 ^{b} ±2.31	33.166 ^{b} ±0.58	333.46 ^{b} ±1.74	362.7 ^{b} ±2.36
50	8058.2 ^{a} ±1.62	3361.4 ^{a} ±2.12	12.914 ^{a} ±0.57	17.677 ^{a} ±1.73	168.98 ^{a} ±0.57	155.97 ^{a} ±1.16
CD (p≤0.05)	3.113	3.918	5.582	4.675	3.761	4.30
CV%	0.01	0.032	3.439	1.497	0.204	0.236

Mean ± S.E.
Note: n=3; Superscripts with similar alphabetes are not significant to each other when read column wise.
T_1, T_2, T_3, T_4, and T_5 represent enzyme concentration at different level of 0.00, 0.20, 0.40, 0.60, 0.80, and 1.00 ml/L

Table 4 Effect of degree of lactose hydrolysis on different rheological properties of LHY at constant angular frequency

Treatment	Storage Modulus (G') (Pa)	Loss Modulus (G") (Pa)	Loss Factor
T_0	487.75 ^{c} ± 0.59	158.63 ^{c} ± 1.16	0.325 ^{b} ±0.001
T_1	1278.9 ^{e} ± 1.73	392.27 ^{e} ± 0.59	0.307 ^{a} ±0.001
T_2	1522.3 ^{f} ± 1.31	496.71 ^{f} ± 1.11	0.326 ^{b}±0.002
T_3	312.32 ^{b} ± 2.89	112.87 ^{b} ± 1.70	0.361 ^{c} ±0.001
T_4	37.108 ^{a} ± 1.15	15.585 ^{a} ± 1.72	0.420 ^{d} ±0.006
T_5	706.37 ^{d} ± 0.61	254.83 ^{d} ± 2.28	0.361 ^{c} ±0.001
CD (p≤0.05)	4.935	4.764	0.008
CV%	0.379	1.111	1.273

Mean ± S.E. Note: n=3; Superscripts with similar alphabetes are not significant to each other when read column wise.
Angular frequency (rad/s) = 0.1± 0.00
T_1, T_2, T_3, T_4, and T_5 represent enzyme concentration at different level of 0.00, 0.20, 0.40, 0.60, 0.80, and 1.00 ml/L.

Table 5 Effect of degree of lactose hydrolysis in simultaneous lactose hydrolysis and fermentation process on microbiological quality of yoghurt

Treatments	SPC	Coliform	Yeast and mold	ST	LB
		Counts cfu/ml			
T_0	$1.00^{a} \times 10^{6} \pm 0.00$	ND	$1.00^{a} \pm 0.00$	$4.00^{a} \times 10^{7} \pm 0.00$	$5.00^{a} \times 10^{7} \pm 0.57$
T_1	$2.00^{b} \times 10^{6} \pm 0.00$	ND	$2.00^{b} \pm 0.00$	$5.67^{b} \times 10^{7} \pm 0.33$	$6.33^{b} \times 10^{7} \pm 0.33$
T_2	$2.67^{c} \times 10^{6} \pm 0.33$	ND	$3.00^{c} \pm 0.00$	$6.00^{bc} \times 10^{7} \pm 0.00$	$7.33^{bc} \times 10^{7} \pm 0.33$
T_3	$3.00^{c} \times 10^{6} \pm 0.00$	ND	$3.33^{c} \pm 0.33$	$6.33^{bc} \times 10^{7} \pm 0.33$	$8.00^{cd} \times 10^{7} \pm 0.00$
T_4	$4.00^{d} \times 10^{6} \pm 0.00$	ND	$4.00^{d} \pm 0.00$	$7.00^{c} \times 10^{7} \pm 0.57$	$8.33^{cd} \times 10^{7} \pm 0.33$
T_5	$5.00^{e} \times 10^{6} \pm 0.00$	ND	$5.00^{e} \pm 0.00$	$8.33^{d} \times 10^{7} \pm 0.33$	$8.67^{d} \times 10^{7} \pm 0.33$
CD ($p \leq 0.05$)	0.424	---	0.424	1.038	1.122
CV%	8.005	---	7.714	9.279	8.569

Mean ± S.E.
Note: n=3; Superscripts with similar alphabetes are not significant to each other when read column wise.

hydrolyzed yoghurt and are in agreement with our findings. Similarly, Ibarra et al. (2012) reported that as the level of enzyme concentration increased the sensory quality of yoghurt decreased. Based on sensory analysis 0.60 ml/L enzyme concentration was found to be optimum in the preparation of yoghurt from simultaneous lactose hydrolysis and fermentation method.

Viscosity, a key indicator of yogurt's textural quality, decreases with rising temperature. In this study, lactose-hydrolyzed yogurt viscosity dropped from 29,460 to 2,233.1 mPa.s at 25°C as hydrolysis increased, with the lowest value at 0.40 ml/L enzyme concentration (Table 3). This reduction likely stems from increased monosaccharides aiding EPS synthesis by starter cultures. Shear-thinning behavior, where the viscosity decreases with increased shear rate, was most pronounced at 0.40 ml/L enzyme concentration (Schmidt et al. 2016). Higher temperatures (25°C to 50°C) further reduced viscosity (Khabibullaev et al. 2020). Rheological analysis showed the control had higher Storage Modulus (G') and Loss Modulus (G") than treated samples (T3, T4). The weaker protein network in hydrolyzed yogurt explains these differences (Khabibullaev et al. 2020). For acceptable rheological properties in lactose-free yogurt, lower enzyme concentrations are recommended, in line with prior research work of Jaster et al. 2018).

Microbiological testing is crucial for ensuring product safety and quality. It is observed from Table 5 that the standard plate count (SPC) of lactose-hydrolyzed yogurt was significantly ($p<0.05$) higher in T_5 due to increased bacterial growth than control due to the glucose availability during fermentation (Ibrahim 2018). Yeast and mold counts followed a similar trend, with T_5 showing the highest counts, possibly due to glucose availability (Mohammad et al. 2011). Coliforms were absent in all samples, indicating proper hygiene during production (Cakmakci et al. 2012).

During refrigerated storage, acidity increased as mentioned in Figure 4, reflecting slow but continuous microbial growth (Vénica et al. 2014). *L. bulgaricus* counts decreased after 28 days, while *S. thermophiles* remained stable (Gilliland and Speck 1977). The microbial counts increased until day 14, then declined. Yeast and mold counts rose throughout storage, aligning with findings by Con et al. (1996) and Mohammad et al. (2011), while coliforms remained absent due to proper hygienic conditions.

Conclusion

In conclusion, increasing the lactase enzyme addition from 0.20 to 1.00 ml/L in the yogurt mix resulted in a rise in lactose hydrolysis from 56.64% to 76.58%, an increase in acidity from 0.80% to 0.97% lactic acid, and a reduction in setting time by approximately 33.3%. Lactose hydrolysis led to greater syneresis and penetration in the experimental samples compared to the control. The sensory evaluation indicated that a lactase addition of 0.60 ml/L was comparable to the control sample and was selected for further storage studies. Both control and experimental treatments showed decreased viscosity with rising temperatures. During the storage period under refrigeration, pH decreased and acidity increased. Lactose-hydrolyzed yoghurt was found acceptable till the 14th day of storage under refrigeration conditions.

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