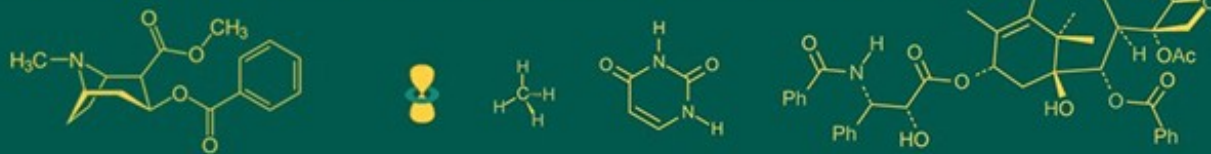


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Sensorial quality effects of lactose-hydrolyzed flavored milk during the storage period

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ABSTRACT

Hydrolysis of milk lactose to glucose and galactose by immobilized β -D-galactosidase offers an alternative to expand the commercial use of this feedstock. An attempt has been made to use the same beads for a second cycle of lactose hydrolysis. Higher enzyme activity was shown by fresh beads (74 NLU) as well as reused beads (53 NLU) containing (4 mL) immobilized β -D-galactosidase. Maximum lactose hydrolysis was achieved after 12 hours of incubation. Flavored milk was prepared by using lactose-hydrolyzed milk (LHFB and LHRB) obtained after 12 hours. The flavor of LHM was enhanced because of the monosaccharide molecules. Significant changes were observed in lactose, glucose, and galactose, and sweetness and consistency were also affected, as indicated by sensorial properties during the storage period.

Keywords: B-D-galactosidase, Lactose hydrolyzed milk, Flavored milk, Lactose intolerance

Introduction

Lactose, a disaccharide is made up of condensation of glucose and galactose linked together by β 1-4 glycosidic bonds. It is poorly soluble and lacks sweetness. Lactose-intolerant people cannot digest lactose properly because they lack the intestinal β -D-galactosidase enzyme, which makes it difficult to digest milk and milk products. Hence, the enzymatic hydrolysis of lactose has become an important biotechnological process in the food industry. Nowadays, interest in enzymatic hydrolysis of lactose has progressively increased worldwide (Harju, 2003; Jelen *et al.*, 2003) [1, 2]. β -D-galactosidase can alleviate lactose intolerance by hydrolyzing lactose into glucose and galactose, which are sweeter, more soluble, and more digestible than lactose (Speer, 1998) [3]. According to Zadow (1986) [4], hydrolyzing 70% of the lactose in milk increases its sweetness by an amount comparable to adding approximately 2% sucrose.

β -D-galactosidase can be obtained from a wide variety of sources such as microorganisms (bacteria, yeast, and fungi), plants, and animals. Depending upon their sources, properties differ markedly. Using yeast as a source of lactase offers several advantages over other sources. The most widely used yeast sources of lactase are *Kluyveromyces* species. Among them, *Kluyveromyces lactis* is commonly used to produce β -D-galactosidase in the dairy industry (Dabhole *et al.*, 1998) [5]. Yeast-derived enzymes are well suited for hydrolyzing lactose in milk (Chen *et al.*, 2009) [6]. This enzyme is active at pH 6.0–7.0, so it is most useful for treating food products near neutral pH, such as milk (Finocchiaro *et al.*, 1980; Joshi *et al.*, 1989) [7]. β -D-galactosidase is widely used in milk and milk products to improve sweetness, solubility, flavor, and digestibility (Richmond *et al.*, 1981; Grosová *et al.*, 2008a) [9, 8].

Immobilized enzymes offer an alternative to minimize these drawbacks (López *et al.*, 2005; Mateo *et al.*, 2007; Rodrigues *et al.*, 2008; Mendes *et al.*, 2011) [10, 11, 12, 13]. Immobilizing enzymes can increase their stability and protect them from chemical and biological degradation. Immobilized biocatalysts can be reused several times, which reduces process costs (Genari *et al.*, 2003) [14]. Compared with free enzymes, immobilized enzymes offer several benefits, including easy separation from the reaction solution, no product contamination by the enzyme, operational and long-term stability, continuous processing, and multi-enzyme reaction systems (Dervakos and Webb, 1991). Thus, enzyme immobilization is widely used in industrial applications because it enhances stability, improves reusability, reduces inhibition by reaction products, and increases selectivity toward non-natural substrates, making the process cost-effective and viable (Iyer *et al.*, 2008; Mateo *et al.*, 2007) [15, 11]. Entrapment of enzymes in calcium alginate is an important immobilization method. It is recognized as a rapid, nontoxic, inexpensive, and versatile method for enzyme immobilization and can be used in food and dairy products (Fraser *et al.*, 1997) [16].

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Material and Method

Materials

Collection of milk

Fresh cow milk was collected from the farmer

Immobilized materials

1. *Enzyme (B-D-galactosidase)* was procured from Christian Hansen Pvt. Ltd., New Delhi. The enzyme activity was 5200 neutral lactase units (NLU).
2. *Sodium Alginate and Calcium chloride* were procured from Sigma-Aldrich, USA

Methods

Preparation of calcium alginate beads using the immobilization method

Immobilization of β -D-galactosidase using calcium alginate beads was carried out according to the procedure by Das *et al.* (2014) [17]. Calcium alginate beads were prepared by using sodium alginate (3%) and β -D-galactosidase (4 ml). The enzyme-alginate mixture was added dropwise using a peristaltic pump into 200 mL of aqueous calcium chloride solution (3%). Beads containing immobilized enzyme were left in the calcium chloride solution to harden properly for 30 minutes. The calcium alginate beads were separated from the solution using a strainer and washed thoroughly with distilled water three times to remove excess CaCl_2 . Beads containing β -D-galactosidase were added directly to milk to prepare lactose-hydrolyzed milk.

Preparation of controlled and lactose hydrolyzed flavored milk

Fresh cow milk was used to prepare flavored milk, following the method and flow diagram given by De (1980) [18], as shown in Fig. 1. Fresh cow milk was procured from the cattle yard (LRC). Milk was filtered through a strainer, and standardization was adjusted to 3% fat and 8.5% SNF. Milk was preheated to 60 °C and homogenized at 2500 psi. Then, the milk was pasteurized at 70 °C for 15 seconds, and sugar was added at 6% on a milk basis. The pasteurized milk was cooled immediately to 40 °C, and color was added at 0.15% on a milk basis. Flavor was also added at 0.5 ml/1000 ml of milk. Pasteurized flavored milk was filled in a glass bottle (200 ml) and capped with a lid using a corking machine. Pasteurized flavored milk samples were stored at refrigerated temperature (5-7 °C) for 7 days. For sterilization, all filled bottles were sterilized in an autoclave at 121 °C/15 for 20 minutes, then cooled and stored at room temperature.

Qualitative analysis of lactose, glucose, and galactose content by RP-HPLC method

Lactose hydrolysis produced lactose, glucose, and galactose. Hydrolyzed milk was used to prepare flavored milk and stored for 7 days, and samples were withdrawn at 2-day intervals. The hydrolyzed and unhydrolyzed milk were analyzed for lactose, glucose, and galactose concentrations using the HPLC method as described by Chen *et al.* (2002) [19]

Flavored milk using lactose-hydrolyzed milk

Lactose-hydrolyzed milk was used to prepare flavored milk, following the preparation method and flow diagram given by De (1980) [18], as shown in Fig. 1. Lactose-hydrolyzed milk was obtained after 12 hours and used to prepare flavored milk. Due to enzymatic hydrolysis, the pH shifted slightly toward the acidic side, so the pH of the milk was maintained by adding dibasic sodium phosphate at 0.03%. Then, the milk was heated at 70 °C for 15 seconds, and sugar was added at 5% on a milk basis. During lactose hydrolysis, glucose and galactose were produced in lactose-hydrolyzed milk, and glucose contributed more sweetness to the product, so less sugar was added than in flavored milk prepared from fresh milk. The pasteurized milk was cooled immediately to 40 °C, and color was added at 0.15% on a milk

basis. Flavor was also added at 0.5 ml/1000 ml of milk. Pasteurized flavored milk was filled in a glass bottle (200 ml) and capped with a lid using a corking machine. Flavored milk samples were then stored at refrigerated temperature (5-7 °C) for 7 days. All filled bottles of flavored milk were sterilized in an autoclave at 121 °C for 15 to 20 minutes. Then samples were cooled and stored at room temperature.

Statistical analysis

All determinations were carried out in triplicate, and data were subjected to analysis of variance. In the experiments, one-way and two-way Analysis of Variance (ANOVA) with subsequent post hoc tests ($P > 0.05$) were conducted as described by Snedecor *et al.* (1994) [20].

Result and Discussion

1) Effect on lactose, glucose, and galactose content in flavored milk

Figure 2 shows that, initially, lactose content was $4.562 \pm 0.35\%$ in control flavored milk and significantly ($p > 0.05$) higher than in lactose-hydrolyzed flavored milk (LHFB and LHRB). Whereas, LHFB flavoured milk was observed very lower lactose content than its reutilized counterpart. By the end of the 3rd and 5th days of storage, lactose content in unhydrolyzed flavored milk substantially decreased as the storage period increased. This may be due to microorganisms consuming lactose and converting it into by-products, i.e., lactic acid and formic acid. Whereas, there was a sharp decrease in lactose, as well as glucose and galactose, in lactose-hydrolyzed milk i.e LHFB and LHRB). After the 7th day of storage, lactose was completely converted into lactic acid in control flavored milk. No significant ($p > 0.05$) changes were observed in glucose and galactose throughout the storage period. Glucose content in both lactose-hydrolyzed flavored milk prepared using fresh beads and flavored milk prepared using reused beads was significantly ($p < 0.05$) higher than in control flavored milk. Glucose content was also consistently higher than galactose concentration, which decreased with increasing storage time. Chen *et al.* (2002) [19] reported that galactose content was significantly lower than glucose because galactose was used to form galacto-oligosaccharides. Similarly, this is consistent with the findings of Mendoza, Olano and Villamiel (2005) [21]. The rate and amount of lactic acid production were higher in lactose-hydrolyzed milk than in the control; this may be attributed to the availability of a greater quantity of easily fermentable sugar (glucose), as reported by Knanagaeva *et al.*, 1980 [22]; Baeve, 1981 [23]; Whalen *et al.*, 1988 [24]

Effect of lactose hydrolysis on sensory evaluation

A composite scoring card for sensory analysis of lactose-hydrolyzed (LHFB and LHRB) and unhydrolyzed flavored milk, as given by BIS (IS: 7768 1975) [25], was used with slight modifications. The main focus was on metallic, rancid, and oxidized flavors.

Effect of storage period on color and appearance in flavored milk

Freshly prepared hydrolyzed flavored milk (LHFB and LHRB) and unhydrolyzed were studied for the effect of storage on color and appearance during storage at refrigerated temperature (5 ± 1 °C) for 12 days. The color and appearance of all flavored milk samples (lactose hydrolyzed and unhydrolyzed) were not significantly affected ($p > 0.05$); therefore, panelists observed no changes on the 0th day. After the 3rd and 5th days of storage, panelists observed a similar trend: no significant ($p > 0.05$) changes across all flavored milk samples. Also, panelists did not report a significant ($p > 0.05$) increase in color and appearance scores with increasing storage time. At the end of the 7th day of storage, panelists again did not observe any changes in color and appearance, even though lactose-hydrolyzed milk was used in the preparation of flavored milk, compared with the control flavored milk. Therefore, panelists found no significant ($p > 0.05$) differences in scores among the flavored milk samples. These findings agree with Prajapati *et al.* (1986) [26], Usha *et al.* (2005), and Geetha and Rao

(2008). The color and appearance of flavored milk samples stored at 5 °C were not significantly affected ($P < 0.05$); therefore, the panelist score showed no change. Osman *et al.* (2009) [27] reported similar findings. Appearance scores did not differ significantly with storage, while a significant ($p < 0.01$) change was observed between the drink types, i.e., control and low calorie. The score remained in the liked very much to liked slightly range throughout the 7-day storage period for the control sample, whereas it shifted to extremely liked for lactose-hydrolyzed flavored milk. Therefore, the products were sensorially acceptable when stored at 5 °C for 7 days.

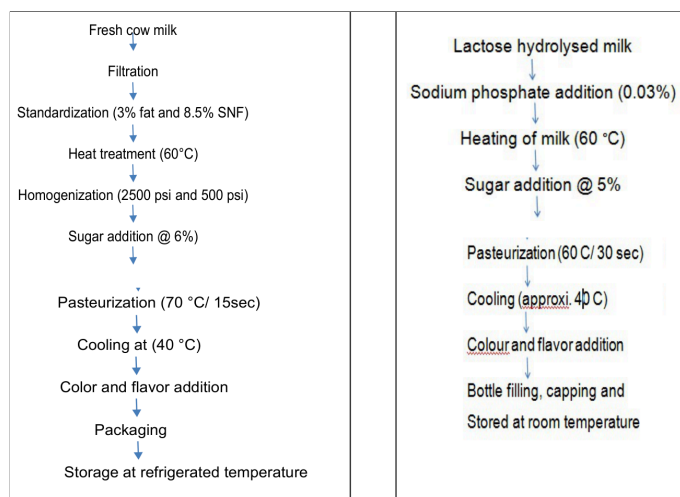


Figure 1. Preparation of Flavored milk using Lactose

Effect of storage period on consistency in flavored

Freshly prepared hydrolyzed flavored milk (LHFB and LHRB) and unhydrolyzed milk were studied for the effect of storage on consistency at refrigerated temperature (5 ± 1 °C) for 12 days. The consistency of flavored milk prepared using fresh beads and flavored milk prepared using reused beads had significantly higher scores than control flavored milk on the 0th day. Consistency was also affected during storage, with a greater effect observed in hydrolyzed flavored milk (LHFB and LHRB) than in unhydrolyzed flavored milk. The lactose-hydrolyzed milk (LHFB) sample had the highest consistency score, higher than LHRB flavored milk, on the 3rd and 5th day of

storage. This may be due to greater moisture loss during preparation and storage, making the product slightly thicker. Another reason sodium phosphate was added to lactose-hydrolyzed milk during preparation was to increase viscosity. Similarly, hydrolyzed flavored milk (LHFB and LHRB) received a higher score from the panelists than the unhydrolyzed flavored milk (LHFB and LHRB) at the end of the 7th day, respectively. However, the consistency of control flavored milk was not significantly affected by the increase in storage period. Present findings are in agreement with Prajapati *et al.*, 1986 [26], Usha *et al.* (2005, and Geetha and Rao (2008). They reported that Body and mouthfeel scores also declined significantly ($p < 0.01$) with storage. Scores declined faster in the control than in the low-calorie drink.

Effect of storage period on sweetness in flavored milk

The table shows that, as expected, the rate of sweetness scores awarded by the panelist to the control sample was not significantly ($p > 0.05$) lower than that of hydrolyzed flavored milk (LHFB and LHRB) on the 0th day. Lactose-hydrolyzed flavored (LHFB) milk was rated sweeter than flavored milk prepared using reused beads. When samples were analyzed on the 3rd and 5th days of storage, panelists again gave a higher score to lactose-hydrolyzed flavored milk (LHFB), followed by LHRB flavored milk and then the control flavored milk. This may be because lactose was hydrolyzed more in LHFB, producing more glucose and galactose and a sweeter taste than LHRB flavored milk. In contrast, unhydrolyzed flavored milk showed no changes in glucose and galactose, but it had a lower sweetness score than hydrolyzed flavored milk (LHFB and LHRB) on the 7th day of storage. The rates of change in sweetness for LHFB and LHRB flavored milk were higher than those of control flavored milk at the end of the 7th day of storage. Control flavored milk also had a lower score than hydrolyzed (LHFB and LHRB) flavored milk. Lactose-hydrolyzed flavored milk had significantly higher sweetness attributes than regular skim milk because hydrolysis of lactose to glucose and galactose increases sweetness. Jelen *et al.* (2003) [2] reported that lactose-hydrolyzed milk was hydrolyzed enzymatically, giving the milk its natural sweetness due to glucose and galactose. Zadow (1986) [4] reported that hydrolyzing 70% of lactose in milk increases sweetness by an amount corresponding to adding about 2% sucrose. Chapman *et al.* (2001 found that the main difference between lactose-free ultra-pasteurized milk and regular ultra-pasteurized milk was the intensity of sweetness.

Table 1. Sensory score of flavored milk (lactose hydrolysed and unhydrolysed)

Control	Maximum Score	Storage Period In Days			
		0	3	5	7
Flavor	40	36.71 $\pm$ 0.22 ^{aA}	36.57 $\pm$ 0.11 ^{aA}	36.57 $\pm$ 0.12 ^{aA}	36.42 $\pm$ 0.12 ^{aA}
Color and appearance	30	28.28 $\pm$ 0.14 ^{aA}	28.29 $\pm$ 0.12 ^{aA}	28.31 $\pm$ 0.23 ^{aA}	28.34 $\pm$ 0.11 ^{aA}
Consistency	20	18.18 $\pm$ 0.12 ^{aA}	18.24 $\pm$ 0.11 ^{aA}	18.36 $\pm$ 0.24 ^{aA}	18.42 $\pm$ 0.22 ^{aA}
Sweetness	10	7.61 $\pm$ 0.24 ^{aA}	7.60 $\pm$ 0.23 ^{aA}	7.60 $\pm$ 0.11 ^{aA}	7.66 $\pm$ 0.12 ^{aA}
LHFB	Maximum Score	Storage Period In Days			
		0	3	5	7
Flavor	40	38.14 $\pm$ 0.12 ^{aA}	38.32 $\pm$ 0.21 ^{aA}	38.47 $\pm$ 0.11 ^{aA}	38.55 $\pm$ 0.21 ^{aA}
Color and appearance	30	28.28 $\pm$ 0.22 ^{aA}	28.29 $\pm$ 0.12 ^{aA}	28.32 $\pm$ 0.23 ^{aA}	28.35 $\pm$ 0.22 ^{aA}
Consistency	20	18.47 $\pm$ 0.23 ^{aA}	18.59 $\pm$ 0.11 ^{aA}	18.61 $\pm$ 0.22 ^{aA}	18.79 $\pm$ 0.21 ^{aA}
Sweetness	10	8.75 $\pm$ 0.12 ^{aA}	9.45 $\pm$ 0.12 ^{aA}	9.85 $\pm$ 0.22 ^{aA}	9.97 $\pm$ 0.23 ^{aA}
LHRB	Maximum Score	Storage Period In Days			
		0	3	5	7
Flavor	40	37.85 $\pm$ 0.12 ^{aA}	38.18 $\pm$ 0.01 ^{aA}	38.38 $\pm$ 0.12 ^{aA}	38.50 $\pm$ 0.23 ^{aA}
Color and appearance	30	28.28 $\pm$ 0.23 ^{aA}	28.29 $\pm$ 0.02 ^{aA}	28.32 $\pm$ 0.13 ^{aA}	28.35 $\pm$ 0.24 ^{aA}
Consistency	20	18.37 $\pm$ 0.21 ^{aA}	18.46 $\pm$ 0.12 ^{aA}	18.55 $\pm$ 0.02 ^{aA}	18.62 $\pm$ 0.21 ^{aA}
Sweetness	10	8.31 $\pm$ 0.22 ^{aA}	9.43 $\pm$ 0.21 ^{aA}	9.47 $\pm$ 0.01 ^{aA}	9.77 $\pm$ 0.23 ^{aA}

Data are presented as means $\pm$ SEM ($n = 3$).

Means within rows with different lowercase superscript letters respectively, are significantly different ($P < 0.05$) from each other. Means within column with different uppercase superscript letters respectively, are significantly different ($P < 0.05$) from each other.

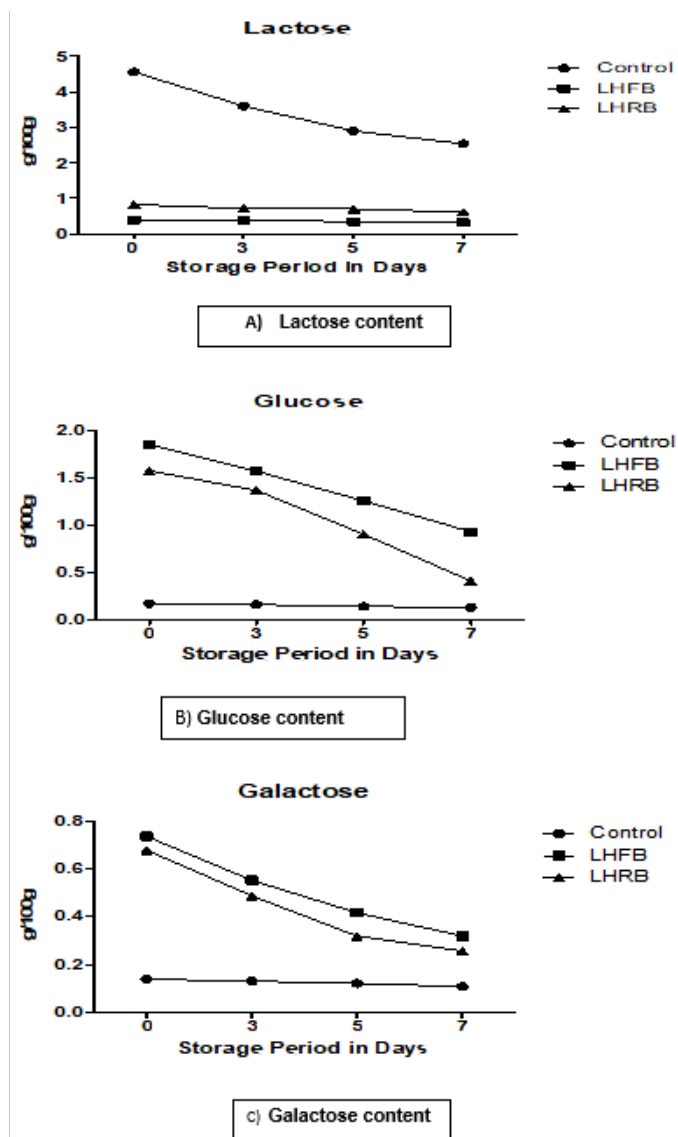


Figure 2. Figure A, B, C represents lactose, Glucose, Galactose content in flavored milk during storage expresses in (g/100g) period

Conclusion

The immobilization method reduced lactose content in milk by up to 90%, and reusing the same beads achieved an 82% reduction in lactose in milk used for the next batch. This study paves the way for efficient utilization of lactose-hydrolyzed milk in preparing flavored milk. The sensorial quality of lactose-hydrolyzed milk was acceptable compared with the control. The hydrolysis of lactose in dairy products by β -D-galactosidase enzyme can alleviate problems related to lactose intolerance and enhance the sensory attributes of lactose-hydrolyzed dairy products.

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