Physico-chemical properties of *Khoa* prepared from lactose hydrolyzed buffalo milk

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Abstract Lactose is a reducing sugar which is abundantly found in mammalian milk. Lactose intolerance affects more than 70% of the world population, being apparent by the absence of β -galactosidase enzyme, thus leading to the inability to consume dairy products. In the present work, *Khoa* was prepared from lactose hydrolysed milk and its physico-chemical, textural and microbiological quality were examined during storage at 5–7 °C for 28 days. The sensory quality of low lactose *Khoa* was comparable with that of the control *Khoa* up to the 14th day of storage. Significant differences ($p < 0.05$) between the acidity, hydroxyl methyl furfural (HMF) content, lightness, redness, springiness, chewiness and hardness values of the low lactose *Khoa* and the control *Khoa* were observed. The standard plate count (SPC), coliform and yeast and mould counts of the low lactose *Khoa* were within Food safety and standards authority of India (FSSAI) standards throughout the 28 days of storage. Therefore, the low lactose *Khoa* developed in this study had different physicochemical properties from the control sample with better shelf life.

Keywords *Khoa* · Lactose hydrolyzed milk · Physico-chemical properties · Storage · β -galactosidase

Introduction

Milk is considered to be complete food providing the essential nutrients such as protein, lactose, fat, minerals and vitamins to newly born infants and mammals. India ranks first in milk production, a 165.4 million tonnes annually with a per capita availability of 355 grams per day surpassing the world average (Savita et al. 2018). Out of the total milk produced in India, half of it is used as fluid milk and the remainder is converted into traditional milk products *i.e.* paneer, ghee, *Khoa* etc. (Bhasin 2010).

Lactose is a disaccharide (β -D-galactopyranosyl-(1-4)- α -D-glucopyranose) ranging from 4.5 to 5.0 g/100 ml in milk. Sometimes the synthesis of β -galactosidase in humans decreases or even disappears with an increase in age. Many people who lack this enzyme are prone to lactose intolerance. Almost 70% of the world's population have a genetic deficiency of lactase enzyme and are unable to digest lactose (Messia et al. 2007). The undigested lactose is then digested by colonic micro flora to form short chain fatty acids in the intestine causing bloating, abdominal cramps, flatulence, nausea and loss of appetite in individuals (Di Stefano et al. 2001; Vernia et al. 2001). Lactose intolerance problems can be controlled by using β -galactosidase which hydrolyses lactose into glucose and galactose and provides a sweet flavour. Glucose is a source of energy and galactose plays a vital function in brain development (Adam et al. 2004). Sandiness and gritty texture defects in dairy products like ice cream, sweetened condensed milk is due to lactose crystallization (Dekker et al. 2019) which can also be reduced by lactose hydrolysis. Lactose hydrolysed milk can be further utilized in preparation of fermented dairy products such as cheese, yoghurt, dahi and heat desiccated products such as *Khoa*. During lactose hydrolysis, galacto-oligosaccharides are

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produced which favours the growth of intestinal microflora to provide a therapeutic effect and improves the technological and sensory characteristics of dairy products. Lactose hydrolysis in milk imparts better digestibility and sweet taste, leading to a recent rise in the demand for lactose hydrolyzed dairy products.

Khoa is a heat-desiccated Indian traditional dairy product prepared by continuous boiling of milk until the desired concentration of milk solids (60–70%) is attained (Kumar et al. 2016). According to the rules of FSSAI (2011), it must contain not less than 30% of fat on dry matter basis and be devoid of added starch, sugar and colouring matter with not more than 0.1% citric acid by weight. The Bureau of Indian Standards (BIS) has also provided standards for three types of *Khoa*, viz. Pindi, Danedar and Dhap in terms of total solids, fat, ash, acidity, coliforms and yeast and mould counts (IS 1980). These varieties are used for making value added *Khoa* based products such as burfi, peda, kalakand, gulabjamun etc. (Choudhary 2015; Choudhary et al. 2017a). Factors affecting the quality of *Khoa* depend on desiccation conditions, type of milk, fat/SNF ratio, lactose content etc. *Khoa* made from cow milk is sticky due to insufficient release of free fat whereas that made from buffalo milk has a soft and smooth body and is highly suitable for *Khoa* based sweets because of high fat content (Vogra and Rajorhia 1983). *Khoa* prepared from buffalo milk has a mildly cooked, rich nutty flavour and sweet taste with a slightly oily or granular texture.

The effect of quality of buffalo milk on compositional and physico-chemical parameters of *Khoa* during storage has been evaluated (Choudhary et al. 2019), however, the effect of lactose hydrolysis on the same has never been determined. Therefore, study was planned to generate relevant information on the changes in physico-chemical parameters of low lactose *Khoa* during storage at 5 °C.

Materials and methods

Fresh pooled buffalo milk was collected from livestock research centre, National Dairy Research Institute, Karnal, India. Milk was heated to 85 °C for 15 s and cooled to 30 °C and then β -D-galactosidase enzyme (Christian Hansen Pvt Ltd. New Delhi, India) was added at three different levels i.e. 0.5 ml, 1.0 ml, and 1.5 ml per 2.5 l of milk and separately incubated at refrigeration temperature (5–7 °C) for 12 h to prepare low lactose milk. After the incubation period, lactose hydrolyzed milk was analyzed for gross chemical composition (fat, protein, ash, SNF (IS:SP (Part XI) 1981) and lactose (Chen et al. 2002).

Preparation of *Khoa* using lactose hydrolyzed milk

Khoa was prepared as described by Rajorhia et al. (1990). Firstly 2–3 kg of milk was boiled in a small, shallow, open, round, thick bottomed iron pan (karahi) having two loop handles, placed over a heater. Milk was then stirred vigorously and constantly with a circular motion by an iron stirrer (khunti) and during this operation all parts of the pan with which milk comes in contact are lightly scraped to prevent milk from burning and overheating. This was followed by evaporation of water until the concentration reached to 2.5 times at which heat coagulation of milk proteins occurs resulting in a viscous mass, marked by an abrupt change in colour. Heat desiccation was continued with closer attention and the speed of scraping was increased until a semi-solid consistency was obtained. The final product was ready when it showed the signs of leaving the bottom and sides of the pan and sticking together.

Physico-chemical analysis of *Khoa* during storage at refrigerated temperature (5–7 °C)

Khoa samples were stored at refrigerated temperature (5–7 °C) up to 28 days, then drawn at regular intervals and analysed for their physico-chemical and sensory characteristics during storage. The physico-chemical parameters, viz. moisture (IS:SP (Part XI) 1981), fat (Ladkani and Mulay 1974), protein (AOAC International 1995), ash (AOAC 1975) and lactose, glucose and galactose (Chen et al. 2002) content were analysed.

Sensory evaluation

Sensory evaluation of low lactose *Khoa* samples was carried out using the method of Choudhary et al. (2017b). A sensory panel including 15 selected panelists from the faculty of Dairy processing departments of ICAR-National Dairy Research Institute, Karnal with adequate knowledge on the sensory evaluation methods and products attributes. All the samples were evaluated for sensory attributes such as flavour (50), body and texture (35), colour and appearance (15) and total score on the basis of 100-point composite score card.

Titrateable acidity

The titrateable acidity was calculated according to the method of IS: SP(Part XI) (1981) and expressed as percent lactic acid.

Colour measurement

A Hunterlab Colorflex colorimeter (Hunter Associates Laboratory, Inc., Reston, VA, 100 USA) was utilized to measure the colour of *Khoa*. Firstly, the instrument was calibrated with standard black and white tiles as per the instructions of the manufacturer. Dual beam xenon flash lamp was used as the light source. The measured colours were described in terms of L^* [dark (0–50) and light (50–100)], a^* [green (negative numbers) and red (positive number)] and b^* [blue (negative numbers) and yellow (positive numbers)]. The color intensity (B) was also calculated using the Eq. 1 (Gavahian et al. 2019).

$$B = \sqrt{(a^*)^2 + (b^*)^2} \quad (1)$$

Hydroxy methyl furfural

The content of hydroxyl methyl furfural (HMF) in *Khoa* prepared from lactose hydrolysed milk was determined according to the standard procedure of Keeney and Bassette (1959) with slight changes. Three grams of lactose hydrolysed *Khoa* was mixed thoroughly with 7 ml distilled water followed by the addition of 5 mL of 0.3 mol eq/l oxalic acid in the tubes which were kept in a boiling water bath for 60 min. The tubes were then cooled and 5 ml of 40 g/100 ml trichloroacetic acid solution was added. The precipitated mixture was then filtered using Whatman filter paper number 42. The filtrate (0.5 ml) was then taken into a 5 ml test tube followed by the addition of 3.5 ml of distilled water and 1 ml of 0.05 mol/l thiobarbituric acid solution (aq) and maintained in a water bath at 40 °C for 50 min. Absorbance was measured at 443 nm on cooling and reported as $\mu\text{mol/g}$.

Texture profile analysis (TPA)

A texture analyzer TA-XT2i (M/s Stable Micro Systems, Godalming, UK) fitted with a 25 kg load cell and calibrated with a 5 kg standard dead weight was utilized to perform texture profile analysis. For determining the TPA parameters, the samples which were previously tempered to 25 °C, cut down into cylindrical shape of height 1 cm and were subjected to monoaxial compression of 0.8 cm/cm of the initial sample height using a probe (P – 70) during the first stage of the two-bite test with a cross head test speed of 2.5 mm/s. From the resulting force–time curves, various textural characteristics such as hardness, springiness, gumminess and chewiness were calculated using the Texture Expert Exceed software (v 2.55) supplied by the manufacturer along with the instrument. A minimum of three replicates per sample was run.

Microbiological analysis

Considering the fact that the *Khoa* and *Khoa* based products are subjected to a high heat treatment, they are supposed to be free from micro-organisms but the microbial contamination has been reported in the samples available in the market. They may gain entry at any stage of processing right from the farm until consumption, so it becomes imperative not only to take all kinds of preventive measures, but also to evaluate at every stage which will subsequently influence the microbiological quality. Tropical climate and high humidity favours the growth of the moulds. Considering the above facts, the detection and enumeration of microorganisms from the control and low lactose *Khoa* samples were carried out according to the standard methods of Manual of Dairy Bacteriology (ICAR 1982).

Standard plate count

Diluted *Khoa* samples were plated by using nutrient agar and incubated at 30 °C for 24 h. At the end of incubation period the plates were taken out from incubation, colonies were counted and expressed as cfu/g of *Khoa* sample.

Yeast and mould

Diluted *Khoa* sample suspensions were pour plated by using PDA agar and incubated at 30 °C for 24 h. Before pouring the growth medium to plates, 2–4 drops of tartaric acid were added and adjusted the pH of medium to 3.5. At the end of incubation period the plates were taken out from incubation, colonies were counted and expressed as cfu/g of *Khoa* sample.

Coliform

Diluted *Khoa* samples were pour plated by using VRBA and incubated at 37 °C for 24–48 h. At the end of incubation period the plates were taken out from incubation, colonies were counted using an illuminated magnifying colony counter. The colony forming units per gram (cfu/g) of the samples were obtained by multiplying the counts obtained with dilution factor.

Statistical analysis

The results obtained in the current study were subjected to one way analysis of variance (ANOVA) with replications and comparison between means was marked by using Fisher's least significant difference test employing SPSS Software version 16 (Coakes et al. 2009).

Results and discussion

Optimisation of β -D-galactosidase enzyme for the preparation of lactose hydrolyzed milk

Preliminary trials were conducted to standardize the concentration of β -D-galactosidase most appropriate for the preparation of low lactose milk and *Khoa*. It was observed that the enzyme concentration of 1.5 ml/2.5 l milk gave the maximum lactose hydrolysis (95%) from which low lactose *Khoa* was prepared for further analyses. Gulati and Deodhar (1985) reported 77% of the hydrolysis of lactose using a lactase preparation, “Lact Aid” (Lact Aid-Inc. Pleasantville, N.J. U.S.A.) at the rate of 80 o-nitrophenyl- β -D-galactoside (ONPG) units/litre in milk.

Chemical composition of milk samples

Fresh buffalo and lactose hydrolyzed milk were studied for their chemical composition and the results are illustrated in Table 1. It was observed that average fat, protein, lactose, ash and SNF of buffalo milk were 6.1, 4.34, 4.95, 0.68 and 9.2%, respectively whereas those for lactose hydrolyzed milk were 6.1, 4.47, 0.23, 0.72 and 9.5%, respectively (Table 1). The observed pH of buffalo milk and lactose hydrolyzed milk were observed to be 6.6 and 6.7, respectively (Table 1). Lactose hydrolyzed milk showed slightly higher SNF and ash content as compared to untreated buffalo milk (Table 1).

Chemical composition of *Khoa* samples

Khoa samples prepared from buffalo milk and lactose hydrolyzed milk were subjected to chemical evaluation and results are depicted in Table 2. The average fat, protein, moisture, lactose, glucose, galactose and ash content of fresh buffalo milk *Khoa* were observed as 35.24, 17.60, 21.76, 21.13, 0.34, 0.01 and 2.74%, respectively whereas those for the *Khoa* prepared from lactose hydrolyzed milk were 35.02, 17.96, 22.13, 3.63, 9.6, 8.5 and 2.87%, respectively (Table 2). No significant ($p > 0.05$) difference was observed between the fat and protein content of fresh

buffalo milk *Khoa* and low lactose *Khoa* (Table 2) although a significant difference ($p < 0.05$) in the lactose content was observed. This may be due to the hydrolysis of lactose to glucose and galactose by lactase. Similar values of chemical constituents were also observed by Choudhary et al. (2019) for the *Khoa* samples prepared from buffalo milk.

Changes in sensory quality of *Khoa* prepared from lactose hydrolyzed milk during storage

Sensory evaluation was carried out to detect any variations produced in the sensory attributes during storage by a team of 15 selected panelists from the Institute (NDRI, Karnal). Statistical analysis revealed no significant change ($p > 0.05$) in flavour and body and texture in *Khoa* prepared from lactose hydrolyzed milk compared with fresh milk *Khoa* samples until the 21st day of storage but a significant change ($p < 0.05$) was observed on the 28th day (Fig. 1a, b). However, the flavour scores of *Khoa* prepared from fresh milk had slightly higher scores than *Khoa* prepared from lactose hydrolyzed milk. The variation can be attributed to the development of a sweet flavour owing to the release of monosaccharides i.e. glucose and galactose released upon lactose hydrolysis in milk used for the preparation of *Khoa*. Similar results were obtained where higher sweetness in lactose hydrolyzed gulabjamun (a deep-fried *Khoa* Indian dessert) compared with control was observed (Harini and Rao 2011).

The body and texture scores of fresh milk *Khoa* samples were significantly lower than those of the *Khoa* prepared from lactose hydrolyzed milk (Fig. 1b). This may be due to the increased content of monosaccharides which are more soluble and hence imparted a soft body and a creamier texture to the *Khoa*. Similar results were obtained in yoghurt prepared from 50 to 70% lactose hydrolysed milk where significantly higher scores were observed for body and texture than those of the control (Nagaraj et al. 2009). Statistical analysis revealed significant changes ($p < 0.05$) in colour and appearance in *Khoa* prepared from lactose hydrolyzed milk compared with those of fresh milk *Khoa* samples (Fig. 1c). Fresh milk *Khoa* samples had

Table 1 Chemical comparison of buffalo milk and lactose hydrolyzed milk

	Milk constituents					
	Fat (%)	Protein (%)	Lactose (%)	Ash (%)	SNF (%)	pH
Fresh buffalo milk	6.1 $\pm$ 0.09 ^a	4.34 $\pm$ 0.05 ^a	4.95 $\pm$ 0.10 ^a	0.68 $\pm$ 0.02 ^a	9.2 $\pm$ 0.21 ^a	6.6 $\pm$ 0.14 ^a
Lactose hydrolyzed milk	6.1 $\pm$ 0.09 ^a	4.47 $\pm$ 0.05 ^a	0.23 $\pm$ 0.02 ^b	0.72 $\pm$ 0.02 ^b	9.5 $\pm$ 0.19 ^a	6.7 $\pm$ 0.14 ^a

Data is presented as mean $\pm$ SEM (n = 3)

^a, ^b Means within columns with different lowercase superscript letters are significantly different ($p < 0.05$) from each other

Table 2 Proximate composition of the control *khoa* and *khoa* prepared from lactose hydrolyzed milk

Component	Content (g/100 g)	
	<i>Khoa</i> prepared from buffalo milk	<i>Khoa</i> prepared from lactose hydrolyzed milk
Moisture	21.76 ± 0.32 ^a	22.13 ± 0.30 ^a
Fat	35.24 ± 0.41 ^a	35.02 ± 0.55 ^a
Protein	17.60 ± 0.10 ^a	17.96 ± 0.08 ^a
Lactose	21.13 ± 0.06 ^a	3.63 ± 0.05 ^b
Glucose	0.34 ± 0.22 ^a	9.60 ± 0.05 ^b
Galactose	0.01 ± 0.00 ^a	8.50 ± 0.05 ^b
Ash	2.74 ± 0.05 ^a	2.87 ± 0.21 ^a

Data is presented as mean ± SEM (n = 3)

^{a, b}Means within row with different lowercase superscript letters are significantly different ($p < 0.05$) from each other

Fig. 1 Effect of storage at 5–7 °C on the sensory attributes of *Khoa* samples **a** flavour **b** body and texture **c** colour and appearance **d** (i) control and (ii) low lactose *Khoa*. Error bars show the variations

of three determinations in terms of standard error of mean. Different lowercase letters denote significant difference ($p < 0.05$) between *Khoa* samples

significantly higher colour and appearance scores ($p < 0.05$) than those of low lactose *Khoa* throughout 28 days of storage. During heating, a slightly brown colour was attained due to increased Maillard reactions in low lactose *Khoa* (Fig. 1d(ii)) which is preferred by consumers compared with fresh *Khoa* (Fig. 1d(i)) which was white with a greenish shade. The results agreed with earlier findings where decreases in colour and appearance scores of *Khoa* stored at room as well as refrigerated temperatures were observed (Kulkarni and Hembade 2012).

Physico-chemical changes in low lactose *Khoa* during storage

The physico-chemical changes occurring in *Khoa* prepared from buffalo milk and lactose hydrolysed milk during storage are illustrated in Fig. 2a–e. An increase in milk acidity during preparation of *Khoa* from milk was generally observed caused by the breakage of phosphate bonds, lactose thermal decomposition and conversion of ionic form of calcium to colloidal form with the release of hydrogen ions leading to the heat coagulation of milk (Prakash and Sharma 1984). The acidity development was significantly ($p < 0.05$) lower in *Khoa* prepared from lactose hydrolyzed milk compared with fresh milk *Khoa* during storage (Fig. 2a). The glucose and galactose liberated in lactose hydrolyzed milk contributed to lowering the acidity compared with only lactose present in the control milk. A continuous increase in the acidity of *Khoa* samples was observed throughout the 28 days of storage which might be due to the action of microorganisms on the lactose (Ladkani and Mulay 1974). Breakdown of lactose during storage at 22 °C for 9–11 days and at 37 °C for 5–7 days was observed to range from 19 to 60% (Bansal 2011).

The initial HMF content of the control and the lactose hydrolyzed *Khoa* was $29.623 \pm 1.336 \mu\text{M/g}$ and $41.356 \pm 1.16 \mu\text{M/g}$, respectively. A significant difference ($p < 0.05$) was observed in HMF content in low lactose *Khoa* compared with that prepared from buffalo milk throughout the 28 days of storage period (Fig. 2b). The high content of monosaccharides (glucose and galactose) produced during lactose hydrolysis interacts with the milk protein resulting in a higher HMF content (Choudhary et al. 2017a, 2017b). Glucose and galactose participate in the Maillard reaction at higher temperature during *Khoa* preparation and also during storage at 5–7 °C. It was evident from Fig. 2b that there was a gradual increase in the HMF content in *Khoa* prepared from lactose hydrolyzed milk and control during storage. Our results are in agreement with the earlier findings of Harini and Rao (2011), where higher HMF content in lactose hydrolyzed gulabjamun was reported compared with control. Bansal (2011)

also reported that 5-hydroxymethyl furfural (HMF) gradually increased in *Khoa* stored at 30 °C.

The changes in the colour of fresh *Khoa* and *Khoa* prepared from buffalo milk stored (5–7 °C) in terms of lightness (L^*), yellowness (b^*) and redness (a^*) values were observed (Fig. 2c–e). The initial lightness (L^*) values of the control and of *Khoa* prepared by using lactose hydrolyzed milk were 59.05 and 39.50, respectively (Fig. 2c). The lightness value of *Khoa* prepared from lactose hydrolyzed milk was lower compared with that made from control milk which further decreased during storage which may be due to the enhanced Maillard reaction during storage (Fig. 2c). Acquistucci (2000) also reported decrease in the lightness index (L) as a result of Maillard reaction with the consequent increase of the brown index ($100 - L$) because of the temperature applied during the preparation of pasta.

Khoa prepared from lactose hydrolyzed milk showed no significant difference ($p > 0.05$) in yellowness (b^*) value when compared with that prepared from fresh milk throughout the storage period (Fig. 2d). The initial redness values of the control and of *Khoa* from lactose hydrolyzed milk were observed to be -0.39 and 7.15 , respectively. Significant differences ($p < 0.05$) were observed in redness value between *Khoa* prepared from lactose hydrolyzed milk and that of fresh milk *Khoa* throughout the storage period (Fig. 2e). This may be due to the enhanced Maillard reaction of the generated monosaccharides on lactose hydrolysis with the protein which might have increased the redness (a^*) value.

The initial color intensity of *Khoa* prepared from lactose hydrolyzed milk was higher compared with that made from control milk which decreased during storage which may be due to the enhanced Maillard reactions (Fig. 2f).

Texture profile analysis of *Khoa* samples during storage

Rheological parameters in terms of cohesiveness, gumminess, hardness, springiness and chewiness were used to determine the rate and extent of textural changes occurring during storage. Figure 3 depicts the textural changes occurring in *Khoa* prepared from buffalo milk and lactose hydrolysed milk during storage at refrigeration temperature.

In the present study, *Khoa* prepared from lactose hydrolysed milk had a significantly ($p < 0.05$) lower hardness value compared with the control *Khoa* (Fig. 3a). This may be due to less lactose protein interaction during preparation of low lactose *Khoa* thereby affecting the water holding capacity of protein and decreasing the hardness. Our results are in agreement with the findings of Grimbleby (1954) who also reported the denaturation of proteins

Fig. 2 Effect of storage at 5–7 °C on the physico-chemical attributes of *Khoa* samples **a** acidity **b** HMF **c** lightness **d** yellowness **e** redness **f** color intensity. Error bars show the variations of three determinations in terms of standard error of mean. ^{a–b}Different lowercase letters

denote significant difference ($p < 0.05$) between *Khoa* samples. ^{A–E}Different uppercase letters denote significant difference ($p < 0.05$) between storage times

in milk above 90 °C thereby reducing the interaction between amino groups and sugar affecting hardness value.

A significant difference ($p < 0.05$) was observed in the chewiness value of *Khoa* prepared from buffalo milk compared with low lactose *Khoa* during storage (Fig. 3b). Chewiness increased gradually throughout the storage period of 28 days for both the samples.

The changes in springiness (mm) during storage are shown in Fig. 3c. Initially, there was a significant difference ($p < 0.05$) in the springiness between *Khoa* prepared from buffalo milk and that prepared from lactose hydrolyzed milk. A continuous decrease in the springiness value was observed for both the *Khoa* samples because of the increase in total solid content of *Khoa* during storage.

Fig. 3 Effect of storage at 5–7 °C on the texture profile analysis of *Khoa* samples **a** hardness **b** chewiness **c** springiness **d** gumminess. Error bars show the variations of three determinations in terms of standard error of mean. ^{a–b}Different lowercase letters denote

significant difference ($p < 0.05$) between *Khoa* samples. ^{A–E}Different uppercase letters denote significant difference ($p < 0.05$) between storage times

No significant difference ($p > 0.05$) was observed in gumminess value of the *Khoa* prepared from buffalo milk and that prepared from lactose hydrolyzed milk initially and on the 7th day. Thereafter, a significant difference ($p < 0.05$) in gumminess value was observed in *Khoa* prepared from lactose hydrolyzed fresh milk compared with that of low lactose *Khoa* up to the 28th day of storage (Fig. 3d).

Microbiological changes in *Khoa* samples during storage

The SPC, coliform and yeast and mould counts obtained at refrigeration temperature (5–7 °C) during storage for 28 days are depicted in Fig. 4a–c. Similar values for the coliforms, yeast and mould and SPC count were also observed by Karthikeyan and Pandiyan (2013) for the fresh *Khoa* samples.

SPC count

No significant difference ($p > 0.05$) was observed in SPC count between *Khoa* from lactose hydrolyzed milk and the control prepared from buffalo milk up to 21 days but a significant difference ($p < 0.05$) was observed in SPC content on the 28th day. *Khoa* from lactose hydrolyzed milk showed lower SPC (4.865 log₁₀ cfu/g) compared with *Khoa* from fresh milk (4.59 log₁₀ cfu/g) on the 28th day which was also within the limit for the microbial count set by FSSAI (4.8 log₁₀ cfu/g) for *Khoa* (Fig. 4a).

Yeast and mould

It is evident from Fig. 4b that the yeast and mould (log₁₀ cfu/g) counts of control *Khoa* and low lactose *Khoa* continuously increased during storage for 28 days at refrigeration temperature. There was an increase from initially not detectable for both the samples to 2.3 log₁₀ cfu/g and 1.72 log₁₀ cfu/g, respectively for control and low lactose *Khoa* on 28th day at 4 °C (Fig. 4b). No significant difference

Fig. 4 Microbiological changes in *Khoa* samples during storage at 5–7 °C **a** standard plate count **b** yeast and mould **c** coliform. Error bars show the variations of three determinations in terms of standard error of mean

($p > 0.05$) was observed between yeast and mould content in lactose hydrolyzed *Khoa* and control *Khoa* prepared from buffalo milk till 14 days but significant difference ($p < 0.05$) was observed after 14 days. Low lactose *Khoa* on the 21st day had a slightly lower log₁₀ cfu/g (1.38) compared with control *Khoa* (1.57 log₁₀ cfu/g) which was also below the limit of yeast and mould count as set by FSSAI (1.6 log₁₀cfu/g) for *Khoa*, indicating a higher shelf-life of the low lactose *Khoa* sample (Fig. 4b).

Coliform

It was evident from Fig. 4c that the coliform (log₁₀ cfu/g) counts of the control and *Khoa* prepared from lactose hydrolyzed milk increased continuously throughout the storage period of 28 days. The counts increased from a level of ND to 3.01 log₁₀ cfu/g and 2.27 log₁₀ cfu/g in control and low lactose *Khoa*, respectively at refrigeration storage temperature (4 °C) (Fig. 4c). The Lower initial coliform count might be due to the injury caused to the bacterial cell by high heat treatment during *Khoa* preparation (Bansal 2011). No significant difference ($p > 0.05$) was observed in coliform counts in lactose hydrolyzed *Khoa* compared with control *Khoa* prepared from buffalo milk until 14 days but a significant difference ($p < 0.05$) was observed in coliform counts after 14 days. In terms of coliform count, low lactose *Khoa* on the 21st day had lower log value (1.87 log₁₀ cfu/g) compared with control *Khoa*

(2.5 log₁₀ cfu/g) which was within the limit of coliform count set by FSSAI (2 log₁₀ cfu/g) for *Khoa*, indicating a higher shelf-life for the low lactose *Khoa* than the control. This might be due to the injury caused to bacterial cells by the high heat treatment and the degradation of lactose during low lactose *Khoa* preparation.

Conclusion

On the basis of the results obtained in the present investigation, it was concluded that hydrolysis of lactose in milk affected ($p < 0.05$) the physicochemical properties of the resulted *Khoa*. Chemical constituents of lactose hydrolyzed *Khoa* were significant higher in terms of glucose, galactose, HMF, lightness and redness, but lower in acidity, hardness and chewiness. The low lactose *Khoa* had better shelf-life as revealed by microbiological study. Thus, low lactose *Khoa* could be consumed by lactose intolerant people without any complications, supplying essential nutrients in concentrated form.

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