

RESEARCH ARTICLE

Preparation and quality assessment of Mozzarella cheese from Buffalo milk with addition of specific type of LAB

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Abstract: This study investigates the chemical composition, microbiological attributes, and sensory properties of Mozzarella cheese produced from buffalo milk collected from selected farms in Haripur, Jaintapur Upazila at Sylhet district. The cheese was prepared using varying concentrations of lactic acid bacteria (LAB) starter cultures (0.0%, 0.5%, 1.0%, and 2.0%), followed by enzymatic coagulation with rennet. The physico-chemical analysis revealed that the addition of LAB significantly ($P < 0.05$) increased the dry matter (DM%) and ether extract (EE%) content, with the highest values observed in the T3 treatment (2.0% LAB). Conversely, nitrogen-free extract (NFE%) decreased significantly with increasing LAB concentration. The microbiological analysis demonstrated a significant increase ($P < 0.05$) in LAB count across treatments, while the standard plate count (SPC) showed an upward trend, though not statistically significant. Coliform bacteria were absent in all samples. Sensory evaluation indicated substantial improvements ($P < 0.05$) in color, taste, texture, flavor, and appearance with increasing LAB levels, particularly in T2 (1.0% LAB) and T3 (2.0% LAB). These findings suggest that the incorporation of LAB enhances both the quality and sensory attributes of Mozzarella cheese, making it a promising approach for cheese production.

Key words: Mozzarella cheese, Buffalo milk, quality and Lactic acid bacteria

Introduction

Buffalo is known as the black gold of South Asia. It plays a very important role in the South Asia along with cattle, which constitutes 73.77% of world buffalo population. The South Asian countries share 93.19% of world buffalo milk production where India and Pakistan contributes 67.99 and 23.96%, respectively (Hamid et al. 2016). In Bangladesh, recent year's dairying has been transforming from customary subsistence to market oriented and enterprise driven approach in the dairy production system (Uddin et al. 2021). The rapid growth of urbanization, poverty reduction, increase in middle class and their increased income have changed their food habit. These recent developments have major impacts on demand for animal derived products like as milk, meat, cheese, butter, ghee, ice-cream, yoghurt and other traditional sweetmeats which are merely dependent on milk. The major market players in the country are Milk vita, Pran Dairy Ltd, BRAC Dairy and Food (Arong) and Akij Dairy Ltd (Farm fresh) corresponding to only 5% share to the total milk production in the country. In Bangladesh, the contribution of buffalo in total milk production is more or less stagnant due to absence of any milk improvement program. Buffalo milk has much total solids than cow milk that is useful for making cheese, butter and other dairy products.

Cheese is the curd or substance formed by the coagulation of milk of certain mammals by rennet or similar enzymes in the presence of lactic acid produced by added or adventitious microorganisms (Mirsalami SM, Alihosseini A, 2023). The moisture has been removed by cutting, warming and pressing, which has been shaped in mould and then ripened (also unripened) by holding for sometime at suitable temperatures and humidity (Huang X et al. 2022). Cheese has high protein content and it is commonly known as milk meat. Mozzarella cheese is a soft, unripened cheese variety of the Pasta-filata family which had its origin in the Battipaglia region of Italy (Citro, 1981). Conventionally, mozzarella cheese was made from buffalo milk. The cheese is soft, white with a glossy surface is valued for its stretch property (Deshwal et al. 2023). Day by day it is becoming popular in Bangladesh for preparing many delicious food items like pizza, sandwich, salad and other items. Cheese would be one of the economically valuable dairy products in Bangladesh.

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If the buffalo farmers of haor areas get proper scientific support for the production of milk and cheese, then they will contribute in national economy through cheese marketing. Considering those facts, the objectives of this study were to investigate the effects of different concentration of lactic acid bacteria (LAB) starter cultures on the chemical composition, microbiological characteristics and sensory attributes of Mozzarella cheese produced from buffalo milk.

Materials and Methods

Sample collection

Milk samples were collected from selected farms of Haripur, Jaintapur Upazila for both manufacturing and quality analysis of Mozzarella cheese. For sample collection once in a week buffalo milk was collected in a large plastic bottle and kept it in a cool box for transportation. Samples were transported via CNG vehicle from Haripur to Sylhet Agricultural University.

Preparation of Mozzarella cheese

The cheese was produced by enzymatic coagulation of milk by following steps (Fig. 1)

Physico-chemical examination

Chemical composition of milk samples were analyzed by milk analyzer (Lactoscan, Bulgaria). The organoleptic tests of manufactured cheese sample were carried out by the panel of judges using score card. And chemical compositions of cheese samples were analyzed according to AOAC (2003).

Microbial examination

The experimental procedure was followed for the determination of the number of total viable bacteria in a sample and the detection, LAB and enumeration of coliform bacteria as per recommendation of APHA (1998).

Statistical analysis

The differences between four groups (six replications in each group) among physical, chemical and microbial parameters were analyzed by ANOVA using SPSS version 28 software from IBM.

Results and Discussion

The chemical composition of mozzarella cheese, such as dry matter (DM%), ash (Ash%), crude protein (CP%), crude fiber (CF%), ether extract (EE%), and nitrogen-free extract (NFE%), is depicted in Table 1. The chemical analysis of the cheese revealed the mean DM% (50.94 ± 0.33) in the control group (T_0). After adding LAB, the DM% gradually increased in all treatment groups (53.01 ± 0.88) in T_1 , (54.35 ± 0.72) in T_2 , and the highest was found in T_3 (56.23 ± 0.44). However, the result showed a significant ($P < 0.05$) variation between the control group (T_0) and all treatment groups. This suggests that adding LAB to cheese enhances its solids content.

Further, the ash content was demonstrated to be (3.63 ± 0.13) in the control group (T_0), T_1 (3.67 ± 0.33), and T_2 (3.55 ± 0.16), respectively, and was relatively consistent across treatments except T_3 (3.02 ± 0.03). T_3 revealed a significant decrease ($P < 0.05$) in the ash content compared with the control groups (T_0), T_1 , and T_2 . Moreover, the crude protein (CP%) content was determined to be highest in T_2 (17.36 ± 0.27) and T_0 (17.25 ± 0.46), with significant differences ($P < 0.05$) observed in T_1 ($15.89 \pm$

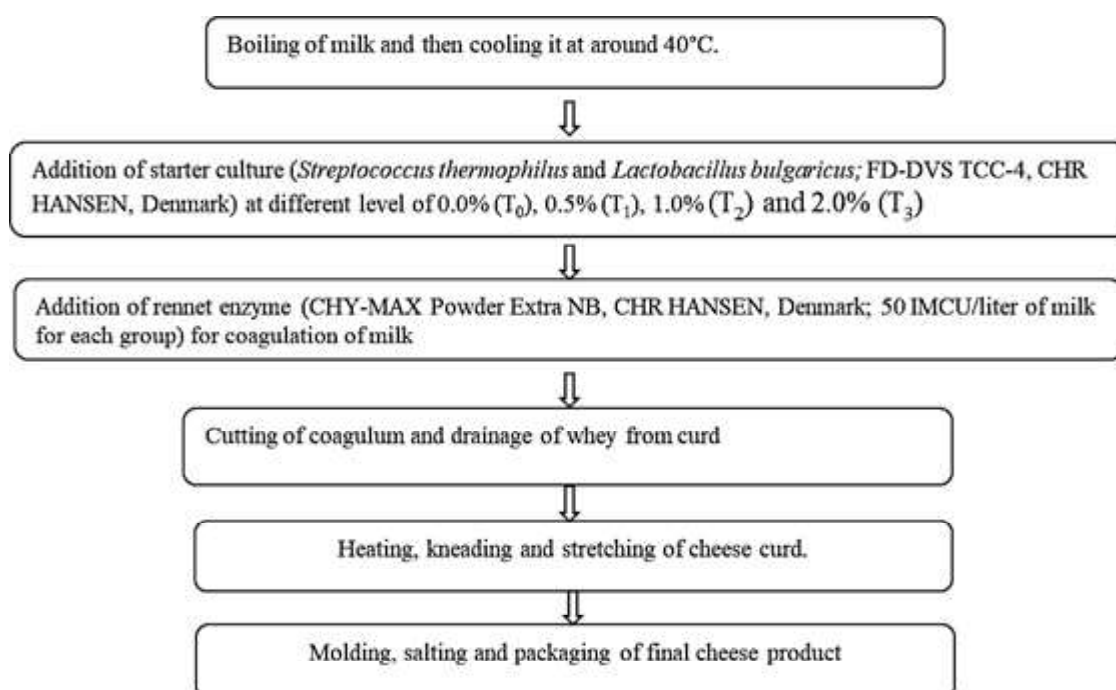


Fig. 1

0.40) and T_3 (16.38 ± 0.10). The crude fiber (CF%) content exposed slight variations across treatments, with the maximum value in T_2 (0.032 ± 0.004) and the minimum in T_3 (0.022 ± 0.004). However, these changes were not statistically significant ($P > 0.05$). In addition, the chemical analysis of cheese revealed a notable increase in ether extract percentage after the addition of lactic acid bacteria (LAB) from (6.54 ± 0.41) in the control (T_0) to (17.50 ± 0.14) in T_1 , (17.75 ± 0.06) in T_2 , and (21.26 ± 0.29) in T_3 respectively, which indicated substantial changes ($P < 0.05$) among treatments. Finally, the nitrogen-free extract (NFE) was reduced significantly following the inclusion of LAB from (72.78 ± 0.35) in the control T_0 to (62.82 ± 0.24) in T_1 , (61.26 ± 0.34) in T_2 and (59.32 ± 0.54) in T_3 consecutively. Even so, there were significant differences ($P < 0.05$) observed between the treatments.

Table 2, provides insightful data on the microbiological properties of mozzarella cheese as well as changes after the addition of LAB. First of all, throughout all treatments (T_0 , T_1 , T_2 , and T_3), the coliform count was continuously found to be nil. In terms of the standard plate count (SPC), which measures the total number of viable bacteria present in cheese, there was an apparent increase from the control to the maximum treatment level. The SPC in the control (T_0) was found to be (6.28 ± 14.82), while it increased to (7.24 ± 17.49) in T_1 , (7.82 ± 24.35) in T_2 , and reached (8.90 ± 14.92) in T_3 . Although this difference was not statistically significant ($P > 0.05$). In the end, the most significant alterations were noticed in the lactic acid bacteria (LAB) count, which increased significantly with the addition of more lactic acid bacteria. The LAB count was found at (2.18 ± 8.07) in T_0 , which was raised to (2.34 ± 11.37) in T_1 , (5.58 ± 25.67) in T_2 , and elevated to (8.20 ± 16.02) in T_3 . This increase was statistically significant ($P < 0.05$).

By incorporating lactic acid bacteria (LAB), the sensory quality of the mozzarella cheese significantly improved, as illustrated in Table 3.

At first, the color scores were observed (5.20 ± 0.84) in T_0 and gradually increased in all treatments, with the maximum observed in T_2 (6.60 ± 0.55). This study revealed a significant variation

($p < 0.05$) in color among different treatments. Repeatedly, a similar result was found in case of taste (5.00 ± 0.71) in T_0 , and the highest improvement was seen in T_3 (6.60 ± 0.55) that was significantly ($p < 0.05$) different between treatments while compared with T_0 . Notably, texture and flavor had the most significant ($p < 0.05$) improvements, with texture scoring (7.60 ± 0.55) and flavor scoring (7.40 ± 0.55) in T_3 , compared to the control (T_0) with scores of (4.60 ± 0.55) and (4.80 ± 0.84), respectively. Finally, the appearance score was noted (5.40 ± 0.55) in T_0 and progressively elevated (6.00 ± 0.71) in T_1 , (7.00 ± 0.71) in T_2 , and T_3 had the highest score (7.40 ± 0.55) and exhibited substantial ($p < 0.05$) variation among treatments.

The present study emphasized the substantial effects on the chemical composition, microbiological characteristics, and sensory qualities of mozzarella cheese by incorporating lactic acid bacteria (LAB). The chemical composition of mozzarella cheese, as illustrated in Table 1, revealed the significant ($P < 0.05$) differences among different treatments with the incorporation of LAB. Initially, the DM% increased significantly from (50.94 ± 0.33) in T_0 to (56.23 ± 0.44) in T_3 . This increase indicates that LAB enhances the cheese solids content, maybe as a result of better fermentation and retention of moisture accordance with the findings of (McSweeney et al. 2013) and Parvez et al. (2006). This result is consistent with earlier studies by Settanni and Moschetti, (2010), who noticed that adding LAB to cheese manufacturing resulted in substantial increases in DM%. A similar trend was observed in the case of ether extract, which significantly increased from 6.54% in T_0 to 21.26% in T_3 , demonstrating the substantial improvement of fat% in cheese. However, the NFE% decreased significantly across the treatments, from 72.78% in T_0 to 59.32% in T_3 , which showed a negative relationship between fat content and the NFE% of cheese. The existing study correlated with the findings Kondyli et al. (2022), who reported that LAB can impact lipid metabolism in cheese, resulting in increased ether extract values. In line with studies by Beresford et al. (2001), that show LAB fermentation results in decreased NFE concentrations in dairy products, the decrease in NFE% suggests that LAB uses more nitrogen-free substances.

Table 1. Chemical composition of mozzarella cheese

Parameters	T_0	T_1	T_2	T_3	<i>P</i> -value
DM%	50.94 ± 0.33^d	53.01 ± 0.88^c	54.35 ± 0.72^b	56.23 ± 0.44^a	< 0.05
ASH%	3.63 ± 0.13^a	3.67 ± 0.33^a	3.55 ± 0.16^a	3.02 ± 0.03^b	< 0.05
CP%	17.25 ± 0.46^a	15.89 ± 0.40^c	17.36 ± 0.27^a	16.38 ± 0.10^b	< 0.05
CF%	0.026 ± 0.011^{ab}	0.028 ± 0.004^{ab}	0.032 ± 0.004^a	0.022 ± 0.004^b	0.183
EE%	6.54 ± 0.41^c	17.50 ± 0.14^b	17.75 ± 0.06^b	21.26 ± 0.29^a	< 0.05
NFE%	72.78 ± 0.35^a	62.82 ± 0.24^b	61.26 ± 0.34^c	59.32 ± 0.54^d	< 0.05

Parameters' values were shown as Mean $\pm$ Standard Deviation

^{a,b,c,d}Means in the same row with different superscript letters differ significantly ($P < 0.05$), DM= Dry Matter, CP= Crude Protein, CF= Crude Fiber, EE= Ether Extract, NFE= Nitrogen Free Extract, T_0 = contain no lactic acid bacteria, T_1 = contain 0.5% lactic acid bacteria, T_2 = contain 1% lactic acid bacteria, T_3 = contain 2% lactic acid bacteria.

Table 2. Microbiological quality of mozzarella cheese

Parameters (cfu/ml)	T ₀ (Mean±SD×10 ⁸)	T ₁ (Mean ±SD×10 ⁸)	T ₂ (Mean ±SD×10 ⁸)	T ₃ (Mean ±SD×10 ⁸)	P-value
Coliform	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	-
SPC	6.28 ± 14.82 ^b	7.24 ± 17.49 ^{ab}	7.82 ± 24.35 ^{ab}	8.90 ± 14.92 ^a	0.189
LAB	2.18 ± 8.07 ^c	2.34 ± 11.37 ^c	5.58 ± 25.67 ^b	8.22 ± 16.02 ^a	< 0.05

Parameters' values were shown as Mean ± Standard Deviation

^{a,b,c,d}Means in the same row with different superscript letters differ significantly ($P < 0.05$), T₀= no lactic acid bacteria, T₁= contain 0.5% lactic acid bacteria, T₂= contain 1% lactic acid bacteria, T₃= contain 2% lactic acid bacteria, cfu= Colony Forming Unit, SPC= Standard Plate Count, LAB= Lactic Acid Bacteria.

Table 3. Sensory quality of mozzarella cheese

Parameters	T ₀	T ₁	T ₂	T ₃	P-value
Color	5.20 ± 0.84 ^b	6.40 ± 0.55 ^a	6.60 ± 0.55 ^a	6.20 ± 0.45 ^a	0.011
Taste	5.00 ± 0.71 ^c	5.80 ± 0.84 ^{bc}	6.40 ± 0.55 ^{ab}	6.60 ± 0.55 ^{ab}	0.007
Texture	4.60 ± 0.55 ^c	5.80 ± 0.84 ^b	6.20 ± 0.84 ^b	7.60 ± 0.55 ^a	< 0.05
Flavor	4.80 ± 0.84 ^c	5.20 ± 0.84 ^{bc}	6.20 ± 0.84 ^b	7.40 ± 0.55 ^a	< 0.05
Appearance	5.40 ± 0.55 ^b	6.00 ± 0.71 ^b	7.00 ± 0.71 ^a	7.40 ± 0.55 ^a	< 0.05

Parameters' values were shown as Mean ± Standard Deviation

^{a,b,c}Means in the same row with different superscript letters differ significantly ($P < 0.05$), T₀= no lactic acid bacteria, T₁= contain 0.5% lactic acid bacteria, T₂= contain 1% lactic acid bacteria, T₃= contain 2% lactic acid bacteria

Interestingly, the ash content was found to range from (3.63 ± 0.13 to 3.02 ± 0.03) relatively consistent in all treatments, following a significant decrease in (3.02 ± 0.03) in T₃. This finding is aligned with the result of (Bintsis et al. 2002) who observed variations in ash content with different microbial cultures of cheese. In contrast with the result of Bhat et al. (2022), who found the ash content of buffalo milk mozzarella cheese ranged from 5.30 and 7.80%, which are higher than the current study. The substantial decrease in T₃ indicates that increased levels of LAB could potentially impact the mineral composition of mozzarella cheese. Moreover, the CP content exhibited a significant decrease. Significant differences ($P < 0.05$) were observed in T₁ (15.89 ± 0.40%) and T₃ (16.38 ± 0.10%) due to the proteolytic function of LAB. According to Martinez-Martinez & Velez-Ruiz, (2019), who noted the CP% of mozzarella cheese was 13.2–25.2%, higher than a recent study. Finally, a non-significant variation in CF% was observed across the treatments, which was in harmony with the result of Awad et al. (2005), reported slight variation in crude fiber with LAB inclusion cheese.

Table 2 presents the microbiological characteristics of mozzarella cheese and the effects of introducing lactic acid bacteria (LAB). The coliform count remained zero in all treatments (T₀, T₁, T₂, and T₃), exhibiting appropriate hygiene practices and efficient microbial control during cheese manufacture. This is in line with the results of several studies on dairy hygiene standards Giraffa et al. (2010); Quigley et al. (2013); Mirsalami et al. (2024) and Rehman et al. (2017), who observed no *E. coli* in cheese. The

current study revealed that the standard plate count (SPC) was increased from T₀ (6.28 ± 14.82) to T₃ (8.90 ± 14.92), although the increase was not statistically significant ($P > 0.05$). This trend suggests that the addition of LAB may have increased microbial quantity. In this study, the most significant ($P < 0.05$) change was observed in the LAB count, which increased significantly from 2.18 ± 8.07 in T₀ to 8.22 ± 16.02 in T₃. This results in coherence with the findings of Rehman et al. (2017) and Fontana et al. (2013), who claimed substantial increases in LAB counts in dairy products fortified with probiotics.

The sensory evaluation of mozzarella cheese is depicted in Table 3. This study revealed that the sensory qualities, including color, taste, texture, flavor, and appearance of the mozzarella cheese, were significantly ($P < 0.05$) increased by the inclusion of LAB. All sensory parameters showed enhanced scores with an increasing concentration of LAB. Notably, the most significant ($P < 0.05$) increases were found in texture and flavor, scoring 7.60 ± 0.55 and 7.40 ± 0.55 in T₃ compared to 4.60 ± 0.55 and 4.80 ± 0.84 in T₀, respectively. The study conveyed by (Mijan et al. 1970), who determined the color score, were 8.2 ± 0.2 and 8.2 ± 0.1, manufactured from buffalo and cow milk, which is somewhat higher than recent studies. A study was carried out by (Bhattarai et al. 2013), who exhibited the sensory scores of mozzarella cheese made from buffalo milk for flavor (7.00), appearance (7.00), taste (7.00), and texture (7.00), which was in accordance with the current study. Cheese quality can be measured mostly by its texture. Additionally, the texture and overall acceptance of a cheese are

more important than its taste. Consumers should prioritize these components Aday & Yuceer, (2014). According to Cosentino et al. (2016), there is a considerable difference in the odour and flavour of mozzarella cheeses depending on the variety of milk utilized during the manufacturing process. The colour scores of mozzarella cheeses made from water buffalo milk were shown to be greater than those made from cow's milk by Fasale et al. (2017). Finally, the existing study emphasizes the importance of LAB strains on the quality of Mozzarella cheese, which not only impacts microbiological and nutritional qualities but also maintains or improves the sensory quality of the Mozzarella cheese.

Conclusion

This study highlights that the incorporation of varying levels of lactic acid bacteria (LAB) starter cultures significantly enhances the chemical composition, microbiological safety, and sensory qualities of Mozzarella cheese made from buffalo milk. The addition of LAB improved the dry matter and ether extract content while reducing nitrogen-free extract, leading to a more concentrated cheese. Microbiological assessments confirmed a notable increase in LAB counts without the detection of coliform bacteria, ensuring product safety. Sensory analysis showed that Mozzarella cheese with 1.0% and 2.0% LAB concentrations achieved the highest scores in parameters such as color, taste, texture, flavor, and appearance, reflecting its superior quality. These findings underscore the potential of LAB incorporation as an effective approach to enhance the overall quality and marketability of Mozzarella cheese.

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