Journal of
Dairy & Veterinary Sciences
ISSN: 2573-2196

Research Article
Volume 3 Issue 5 - September 2017
DOI: 10.19080/JDVS.2017.03.555625

Dairy and Vet Sci J


# Study on Proteolytic Potential of Buffalo Milk Using *Lactococcus Lactis Ssp. Cremoris* and *Lactococcus Lactis Ssp. Lactis*

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**Submission:** July 19, 2017; **Published:** September 27, 2017

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**Abstract**

The present investigation was designed to determine proteolytic potential of lactic acid bacteria (LAB) wiz. *Lactococcus lactis ssp. cremoris* and *Lactococcus lactis ssp. Lactis* at different time interval for a period of 12 hours. Study revealed that pH value and its rate of decrement was higher in samples, which are fermented with *Lactococcus lactis ssp. cremoris* comparing with *Lactococcus lactis ssp. lactis*, in buffalo milk samples.

## Introduction

Buffalo milk contains all the nutrients in higher proportions than cow milk as per the nutrient components. The compositional differences between buffalo and cow milk are reflected on their physico-chemical properties. Milk from buffalo preferred for preparing dairy products of western and traditional (indigenous) type and nutritionally superior. Buffalo milk contains less cholesterol (total cholesterol 275 mg and free cholesterol 212mg per 100g of fat) in compared to cow milk (total cholesterol 330 mg and free cholesterol 280mg per 100g of fat) and more tocopherol (334.21µg per kg for buffalo and 312.3µg per kg of cow milk). Due to high peroxidase activity, buffalo milk can be preserved naturally for a longer period. Buffalo milk contains more calcium, better calcium: phosphorous ratio and less sodium and potassium than cow milk which makes it a better nutritional supplement for infants.

Due to growth requirements, dairy starter cultures have developed highly sophisticated proteolytic system that capable of break down milk proteins, mainly α1 and β-caseins. The proteolytic structure of lactic acid bacteria (LAB) and their activities in dairy products including yogurt and cheese have been studied extensively [1-5].

*Lactococcus lactis* has two subspecies with few phenotype and genotype differences, *Lactococcus lactis ssp. lactis* and *ssp. cremoris*, where subsp. lactis is preferred for making soft cheese while subsp. *Cremoris* is for hard cheese.

## Material and methods

### Periodical evaluation of fermented buffalo milk

Fresh buffalo milk was skimmed to bring the fat contents to below 0.5% using cream separator. The samples were heated to boil at least for 5min to inactivate/kill the inherent microbial population present in milk. Then *Lactococcus lactis ssp. cremoris* and *Lactococcus lactis ssp. lactis* cultures were inoculated @ 1% and after proper mixing, the samples were inoculated at 30 °C .The samples were drawn at 0, 2, 4, 6, 8, 10, 12 hours and were subjected to analysis for change in pH.

### Bacterial cultures and their propagation

Glass ampoules containing Lyophilized powder of *Lactococcus lactis ssp. cremoris* NCDC 81 and *Lactococcus lactis ssp. lactis* NCDC 88 were obtained from the NCDC (National Collection of Dairy Cultures) Dairy Microbiology Division ICAR-National Dairy Research Institute, Karnal (INDIA). The organisms were stored at 4 °C. The propagation for each strain was performed according to Donker et al., [1] with slight modification. Sterile 5ml aliquots of reconstituted sterile skim milk (RSM) (Himedia Laboratories) were inoculated with each strain individually and incubated at 30 °C for 24h in BOD incubator. After incubation, the pre-inoculated cultures were prepared by transferring loop full of activated culture to 10ml aliquots of litmus milk (Himedia Laboratories) to determine the activation of culture

activity by observing change in color of litmus milk after 24 hour of inoculation Figure 1. The skim milk and litmus milk were autoclaved following the standard procedure (121°C for 15min @15lbs).

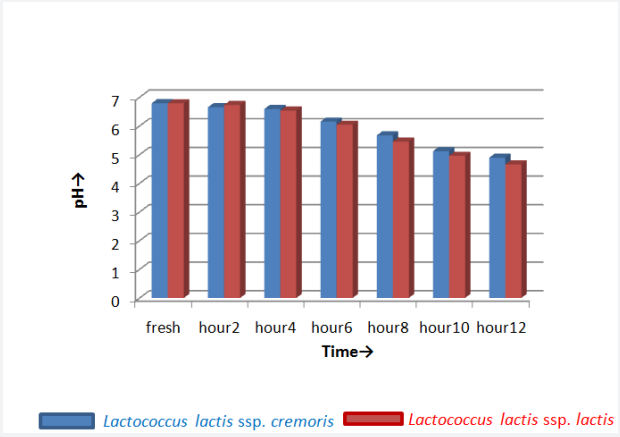


**Figure 1:** pH of buffalo milk during fermentation.

### pH measurement of milk samples:

The pH of samples was measured by using combined glass electrode of Milkoscan at camel milk research laboratory, ICAR-NRC on Camel, Bikaner.

## Result and Discussion

### Change in pH during hydrolysis:

**Table 1:** pH (Mean ± SE) of buffalo milk during fermentation.

| Treatment | Lactococcus lactis ssp. cremoris | Lactococcus lactis ssp. lactis |
|---|---|---|
| Fresh | 6.79±0.007 | 6.79±0.004 |
| Hour 2 | 6.73±0.003 | 6.65±0.005 |
| Hour 4 | 6.53±0.003 | 6.59±0.006 |
| Hour 6 | 6.04±0.009 | 6.15±0.005 |
| Hour 8 | 5.45±0.010 | 5.67±0.006 |
| Hour 10 | 4.96±0.007 | 5.12±0.005 |
| Hour 12 | 4.66±0.011 | 4.89±0.004 |
| Overall | 5.88a±0.126 | 5.98b±0.111 |

Note - Means bearing different superscripts within a row differ significantly.

The data related to pH of buffalo milk has been shown in Table 1 .The pH of fresh milk was found to be 6.79±0.004 for *Lactococcus lactis ssp. cremoris* and 6.79±0.007 for *Lactococcus lactis ssp. lactis* before inoculation of treated bacteria.

The value of pH was dropped significantly as the fermentation hour were increased and at 12 hour of fermentation and it was observed to be 4.89±0.004 and 4.66±0.011 for *Lactococcus lactis ssp. cremoris* and *Lactococcus lactis ssp. lactis* respectively whereas the overall pH was 5.98±0.111 and 5.88±0.126 for *Lactococcus lactis ssp. cremoris* and *Lactococcus lactis ssp. lactis* respectively. The pH value demonstrated in Table 1 reveals that the rate of decrement was higher in samples, which are fermented with *Lactococcus lactis ssp. cremoris* comparing with *Lactococcus lactis ssp. lactis*, in buffalo milk samples. [6,7].

Process of fermentation is affected by several factors including the structure of the protein, temperature, enzyme/protein ratio, enzyme concentration and pH. In the present study, almost linear drop in pH was observed during the fermentation process in buffalo milk samples. The release of protons (H+ ion) and/or production of acidic amino acids into the surrounding medium results in reduction in the pH of the reaction mixture.

**Table 2:** Analysis of variance for pH of buffalo milk during fermentation.

| Source of variation | D.F. | Mean Square | Level of sig. |
|---|---|---|---|
| Treated bacteria | 1 | 0.207 | ** |
| Hour | 6 | 8.034 | ** |
| Reminder | 76 | 0.003 | |

** = Significant at 1% (P<0.01).

The statistical analysis of data as shown in Table 2, revealed that there was a highly significant (P<0.01) decrease in the pH value of buffalo milk samples with advancement of fermentation hours as well as between the treated bacteria that is *Lactococcus lactis ssp. cremoris* and *Lactococcus lactis ssp. lactis*. [8,9].

## References

1. Shah NP, Ravula RR (2000) Micro encapsulation of probiotic bacteria and their survival in frozen fermented dairy desserts. Australian Journal of Dairy Technology 55(3): 139-144.
2. Shalash MR (1979) Utilization of camel meat and milk in human nourishment. Provisional Report, International Foundation for Science 6: pp.285-306.
3. Hernandez D, Cardell E, Zarate V (2005) Antimicrobial activity of lactic acid bacteria isolated from Tenerife cheese: initial characterization of plantaricin TF711, a bacteriocin-like substance produced by Lactobacillus plantarum TF711. J Appl Micro 99(1): 77-84.
4. Lourens HA, Viljoen BC (2001) Yogurt as probiotic carrier food. Int dairy j 11(1): 1-17.
5. Donkor ON, Henriksson A, Vasiljevic T, Shah NP (2007) Proteolytic activity of dairy lactic acid bacteria and probiotics as determinant of growth and in vitro angiotensin-converting enzyme inhibitory activity in fermented milk. Lait 86: 21-38.
6. Rao MB, Gupta RC, Dastur NN (1970) Camels' milk and milk products. Ind J Dairy Sci 23: 71-78.
7. Minieri L, De Franciscis G, Intrieri F (1965) On behavior of pH value in relation to acidity and chemical constituents of milk: research in Bubalus buffalus. Acta Med Vet (Napoli) 11(1): 107-114.
8. Pan D, Luo Y, Tanokura M (2005) Antihypertensive peptides from skimmed milk hydrolysate digested by cell-free extract of *Lactobacillus helveticus* JCM1004. Food Chemistry 91(1): 123-129.
9. Gomes AMP, Malcata FX (1999) *Bifidobacterium* spp. and *Lactobacillus acidophilus*: biological, biochemical, technological and therapeutically properties relevant for use as probiotics. Trends in Food Science & Technology 10: 139-157.



**How to cite this article:** Lokesh T, Basant B, Raghvendar S, C. S. Dhaka, Sanjay S. Study on Proteolytic Potential of Buffalo Milk Using *Lactococcus Lactis Ssp. Cremoris* and *Lactococcus Lactis Ssp. Lactis*. Dairy and Vet Sci J. 2017; 3(5): 555625. DOI: 10.19080/JDVS.2017.03.555625.

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