Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2020; 8(1): 1503-1508

Received: 25-11-2019
Accepted: 27-12-2019

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Shelf life estimation of probiotic buffalo milk Ricotta cheese

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Abstract
Probiotic Ricotta Cheese was packed in Polystyrene cups and stored at 7 ℃ for shelf life study. The various parameters like titratable acidity, water activity, overall acceptability, textural and color parameters were analysed throughout the storage. The probiotic count of LA-05 cells was checked over the storage time. The titratable acidity of PRC increased significantly ($p<0.05$) during 12 days of storage period. The water activity value remained stable ($p>0.05$) throughout the storage. The sensory quality of the product reduced during refrigerated storage as observed by significant ($p<0.05$) declined score of overall acceptability at the end of storage. The initial count of *Lactobacillus acidophilus* La 05 was 7.87 log CFU/g, which was reduced significantly ($p<0.05$) to 7.6 log CFU/g at the end of the 12 days of refrigerated storage. The instrumental firmness, stickiness, work of adhesion and work of shear of PRC increased significantly ($p<0.05$) throughout the 12 days of storage. The color values (L*, a* and b*) of the PRC remained stable throughout the refrigerated storage as revealed by instrumental color analysis.

Keywords: Probiotic ricotta cheese, *L. acidophilus* LA-05, polystyrene, refrigerated storage

1. Introduction

Among the whey cheeses, Ricotta is the most popular and oldest variety of cheese. It is characterized by its high moisture content. Ricotta can be characterized as a co-precipitate of protein [1]. Ricotta is a soft, mild flavoured unripened variety of cheese which is originally prepared from small ruminants' milk. Ricotta cheese preparation from small ruminant's milk restrict its availability. Factors such as higher price, lower availability and lower production of small ruminant's milk limits the production and availability of Ricotta cheese. India represents 56.5% of the world buffalo population. Buffalo milk shows 49% of India's total milk production. As the market of Mozzarella cheese is growing in India, the resultant volume of whey is also increasing leading to an interest in Ricotta cheese production from buffalo milk system.

Probiotics are 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host' [2]. Probiotic organisms are well-known for providing multiple health beneficial effects like: modulation of immune system, lower cholesterol level, prevention of cancer, improved lactose intolerance, and reduced effects of diarrhoea, constipation and urinary tract infections (UTI) etc. [2]. The effective dose of probiotic bacteria is varied between 10^6 CFU/g to 10^9 CFU/g of a product for assured health benefits [2]. Probiotics can be bacteria, molds or yeasts, though the most commonly used probiotics are Lactobacilli and Bifidobacteria [3]. Apart from health beneficial properties, probiotic organism are known for their antimicrobial activity against specific spoilage and pathogenic micro-organisms [4], which is mainly attributed to the production of organic acids, bacteriocins, hydrogen peroxide and other metabolites, etc. [5].

Ricotta, a soft whey cheese may provide several benefits over other foods in terms of delivery vehicle of probiotic microorganisms because of its intrinsic characteristics. Ricotta has relatively high moisture, high pH, low salt concentration and low-oxygen level; all these features in combination may offer an extra protection to probiotic organisms during storage and gastric transit. Probiotic incorporation in Ricotta cheese matrix may improve sensory quality, shelf life and functional properties of product. Moreover, fresh Ricotta has a limited shelf life mainly due to the availability of nutrients and high water activity, which support the growth of yeast, mold and other contaminants. Fresh ricotta is a product that represents a good medium for microbial contaminants also, mainly due to the availability of nutrients and high water activity which limits the shelf life of the product upto 5-10 days. The major spoilage flora of Ricotta cheese are yeast, mould, *E coli*, *Staphylococcus aureus* and *Psuedomonas spp*. etc. High microbial contamination in Ricotta cheese can be controlled by incorporation of bacteriocin producing probiotic organisms, which in turn may enhance the quality, safety and shelf -life of product [6].

The objective of this study was to estimate the shelf life of probiotic buffalo milk Ricotta cheese by analysing various physicochemical, sensory and textural parameters and to check the stability of probiotic count in buffalo milk Ricotta cheese matrix during the refrigerated storage of product in Polystyrene cups.

2. Materials and Methods

2.1 Materials

Fresh raw buffalo milk was procured from the Experimental Dairy, National Dairy Research Institute, Karnal, India. Salt and citric acid were purchased from standard manufacturers. Polystyrene tubs were purchased from local markets of Karnal city. The chemicals required for analysis were procured from Sigma Aldrich, USA. All microbiological media were purchased from Himedia, Mumbai.

Lactobacillus acidophilus (LA-05), the probiotic bacteria was taken from the depository, National Collection of Dairy Cultures (NCDC), Karnal, India.

2.2 Preparation of probiotic Ricotta cheese (PRC)

Probiotic Ricotta cheese was prepared following the protocol of Bhagwat *et al.* [7] as described below. The freshly prepared Mozzarella cheese whey was taken and heated to 70 ^{0}C/5 min for inactivation of rennet. Pasteurized buffalo milk with 1% fat was added to whey, so that final mixture would contain 80:20, whey and buffalo milk. The mixture was heat treated at 90 ^{0}C/15min and cooled to 75 ^{0}C, prior to coagulation. 1% citric acid solution was used to coagulate the mix (pH 5.4 obtained). The Ricotta curd was mixed with 1% salt and stored at 7 ^{0}C, prior to probiotic addition.

The freeze dried culture of *L. acidophilus* was activated in MRS broth and subsequently sub-cultured before adding to cheese. Three times sub-cultured LA-05 was centrifuged (REMI centrifuge) at 8000 rpm/15 min. at 4 ^{0}C. The obtained cell pellets were washed with 0.85% sterile saline solution. The count was adjusted (~8 log CFU/mL) by measurement of optical density at wavelength of 625 nm (OD_{625}) using a spectrophotometer (SHIMADZU UV-1800). The desired level of probiotic organism was inoculated into Ricotta cheese matrix by homogenous mixing.

2.3 Estimation of shelf life of RC

In order to determine shelf life of control RC, the product was stored in Polystyrene jars at 7 °C and the overall acceptability (Ricotta Cheese Score Card of total score 100) and% titratable acidity [8] were checked during refrigerated storage after every 4 days interval.

2.4 Estimation of shelf life of PRC

PRC was stored in Polystyrene jars at 7 °C and analysed at 4 days interval for several parameters. The change in sensory quality of PRC was estimated at every 4 days of interval by Ricotta Cheese Score Card (Total Score-100). The change in titratable acidity [8] of PRC estimated at every 4 days of interval, till end of the sensory shelf life. The change in textural quality of PRC was estimated at every 4 days of interval, till end of the shelf life using Texture Analyzer TAXT2i (Stable Micro Systems, Godalming, Surrey, UK). The colour of product was measured by reflectance spectroscopy technique employing reflectance meter, colour flex (Hunter lab, Reston, Virginia, USA) and the Universal Software (Version 4.10).

2.4.1 Viability of probiotic organism in Ricotta matrix

The stability of the probiotic organism in Ricotta matrix was estimated at refrigerated condition. Probiotic count was determined by pour plate method, at every 4 days of interval, till the end of the sensory shelf life of the product.

2.5 Statistical analysis

The obtained experimental data was subjected to suitable statistical analysis using MS-Excel 2010, SPSS 16.0 Software. Student's t-test and analysis of variance (ANOVA) with Tukey's post hoc test was applied for testing the significance of difference between two or more treatments respectively at 5% level of significance. Prism Graph-pad version 8.1.1 was used for graphical representation of data.

3. Results and Discussions

3.1 Chemical composition of RC and PRC

The total solids content of RC and PRC were 24.86% and 25.03% respectively, which were statistically non-significant ($p>0.05$). The respective values for fat, protein, lactose and ash were 6.67%, 11.67%, 4.54% and 1.98% for RC, and 6.41%, 11.88%, 4.88%, and 1.86% for PRC, all the compositional parameters were statistically non-significant ($p>0.05$) for both the samples. The water activity and titratable acidity of both the samples were 0.99 and 0.145%, respectively. The enumerated probiotic count in PRC was 7.8±0.2 log CFU/g of product. Incorporation of probiotic organism did not alter the composition of Ricotta cheese [6].

3.2 Shelf life of control RC

The overall acceptability score of the product on 0th day was 87.60±1.14, which was reduced significantly ($p<0.05$) to 80.40±1.52 and 72.60±2.07, respectively on 4th and 8th days of storage. All the sensory scores decreased during storage leading to a reduced score of overall acceptability. The product was not acceptable by the panellists after 8th days of storage because of pronounced fruity flavour development. The body and texture score reduced during storage as the product became harder, which may be attributed to slow whey release from protein matrix, might be attributed to slow whey release from whey matrix making protein cluster stiffer.

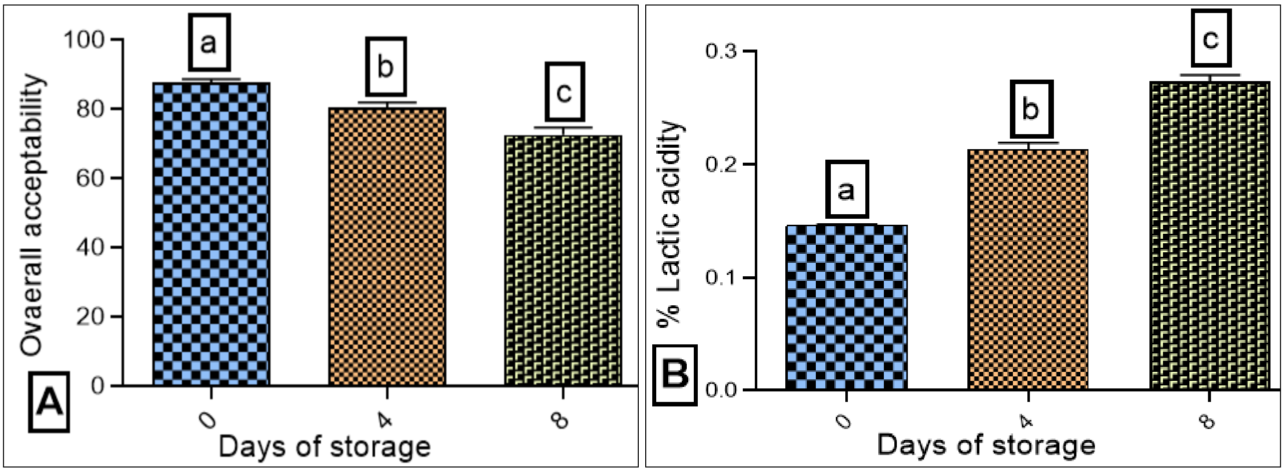


Fig 1: Changes in Ricotta cheese during storage

A: Change in overall acceptability; B: Change in acidity Values with different superscripted letters in graph differs significantly (*p*<0.05)

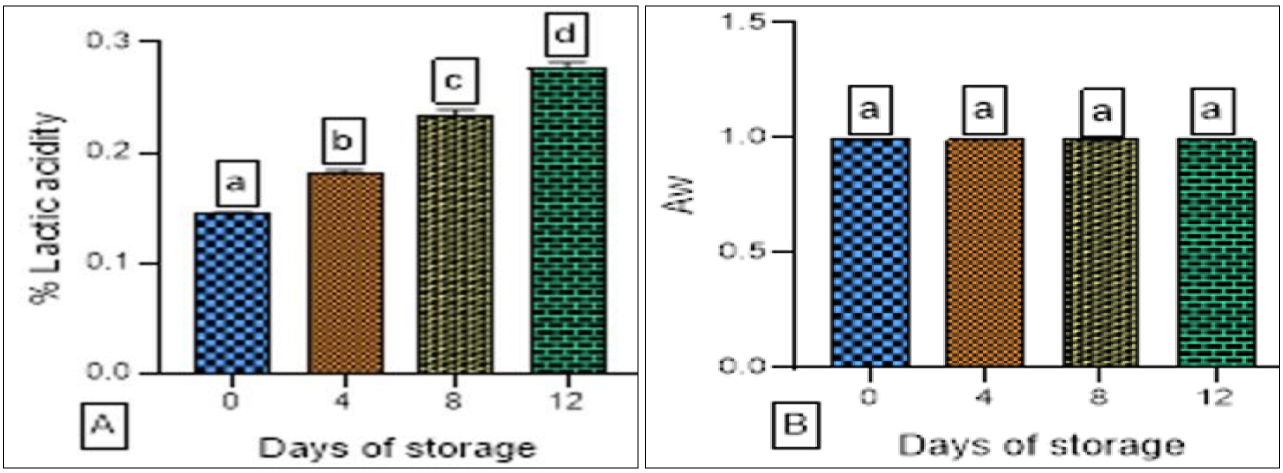
Additionally, yeasty and moldy flavour was detected due to growth of yeast, molds and psychrotrops after 8th days of storage, decreasing flavour score of the product, leading to deacrease overall acceptability. Similarly, an incidence of reduction in flavour score had been reported in creamy Ricotta cheese during storage [1]. A considerable decrease in sensory quality had been reported in functional Ricotta cheese due to excessive acidity [9].

The initial acidity of the product was 0.146%, which increased significantly (p<0.05) to 0.21% and 0.27%, respectively on 4th and 8th days of storage. This might be due increment in the total plate count leading to enhancement of acidity in high moisture and pH environment. The increment in titratable acidity upto 0.26% was reported during 7 days of storage for control goat Ricotta cheese under refrigerated storage [6]. The changes in RC are represented in Fig. 1 A and Fig. 2 B.

3.3 Estimation of shelf life and stability of PRC

The changes in PRC during storage are represented in Fig. 2. The initial Titratable acidity of RC was 0.145% LA which increased gradually during whole storage period. On 4th day of storage the titratable acidity increased significantly (p<0.05) to 0.182% LA. Similarly, the respective values for acidity on 8th and 12th days of storage were 0.233% LA and 0.277% LA, which were statistically significant (p<0.05). The increase in acidity was most likely due to increase in content of organic acids produced by metabolism of probiotic cells [10]. The increment in acidity and consequently decrease in pH during refrigerated storage had been reported in Minas fresh cheese containing *L. acidophilus* La 05 [11]. The Aw value remained stable throughout the storage, which was 0.99. Same trend was reported for goat Ricotta cheese during 7 days of refrigerated storage [6].

The sensory quality of the product deteriorated during refrigerated storage as observed by declined score of overall acceptability. The initial overall acceptability score of PRC was 88.70±0.84 which decreased non-significantly (p>0.05) to 86.50±1.12 on 4th day of storage. Further, overall acceptability score decreased significantly (p<0.05) to 82.20±1.92 and 76.40±1.34 respectively on 8th and 12th day of storage. No difference in sensory score had been reported for creamy Ricotta cheese for 7 days of refrigerated storage [1]. A considerable decrease in taste, pleasantness had been reported in functional Ricotta cheese due to excessive acidity [9]. Acidic flavour development was observed after 12th day of storage in our case, which might be attributed to considerable increase in acidity of product. Lower scores for all the sensory parameters had been reported during storage of Brazilian probiotic Coalho cheese [12]. Further decrease in body and texture score was also observed in acidic product at the end of the storage. Additionally, yeasty and mouldy flavour was detected due to growth of yeasts and psychrotrops at the end of the refrigerated storage, decreasing flavour score of the product, leading to decreased overall acceptability of product.



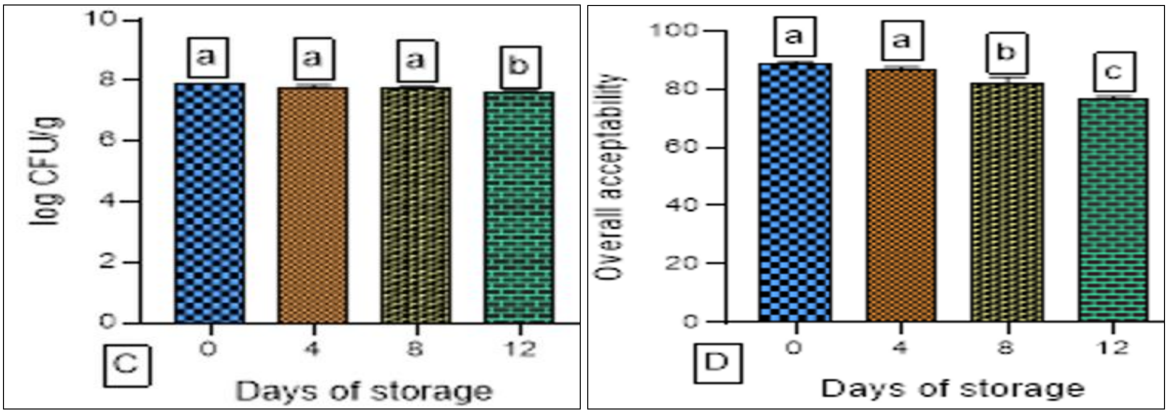


Fig 2: Changes in probiotic Ricotta cheese during storage
A: Change in acidity; B: Change in water activity; C: Change in probiotic count; D: Change in overall acceptability. Values with different superscripted letters in graph differs significantly (p<0.05)

The initial count of *L. acidophilus* La 05 was 7.87 log CFU/g, which reduced significantly (p<0.05) to 7.6 log CFU/g at the end of the 12 days of refrigerated storage. The respective count of *L. acidophilus* La 05 on 4th and 8th day of storage was 7.78 log CFU/g and 7.75 log CFU/g, which was statistically non-significant (p>0.05). The percent log reduction of organism during refrigerated storage was 3.43% from initial count. The *L. acidophilus* La 05 had maintained its viable count >7.5 log CFU/g, throughout the storage. The refrigerated storage did not have any effect on viability of probiotic cells and the count of *L. acidophilus* was almost stable throughout the refrigerated storage. Similar result had been reported in frozen storage of probiotic ice cream [13]. However, other researchers had reported an increase in probiotic count during refrigerated storage of probiotic Ricotta cheese [4, 6], this might be due to use of inoculum instead of cell pellets in the preparation of PRC.

The instrumental firmness of PRC increased significantly (p<0.05) throughout the storage period (Fig. 3). The initial value for firmness was 993.9 g which increased to 1063 g, 1172 g and 1456 g respectively at 4th, 8th and 12th day of storage. The stickiness values of PRC was -447.8 g on 0th day, which increased significantly (p<0.05) to -152.3 g on 12th day of refrigerated storage. Similarly, the initial values of work of adhesion and work of shear were -86.04 g. s and 3350.15 g. s on 0th day of storage respectively, which were increased significantly to -26.41 g. s and 4132 g. s respectively on 12th day of storage. Similar incidents of increased hardness during storage were reported in probiotic goat Ricotta cheese [6] and in probiotic whey cheese [4].

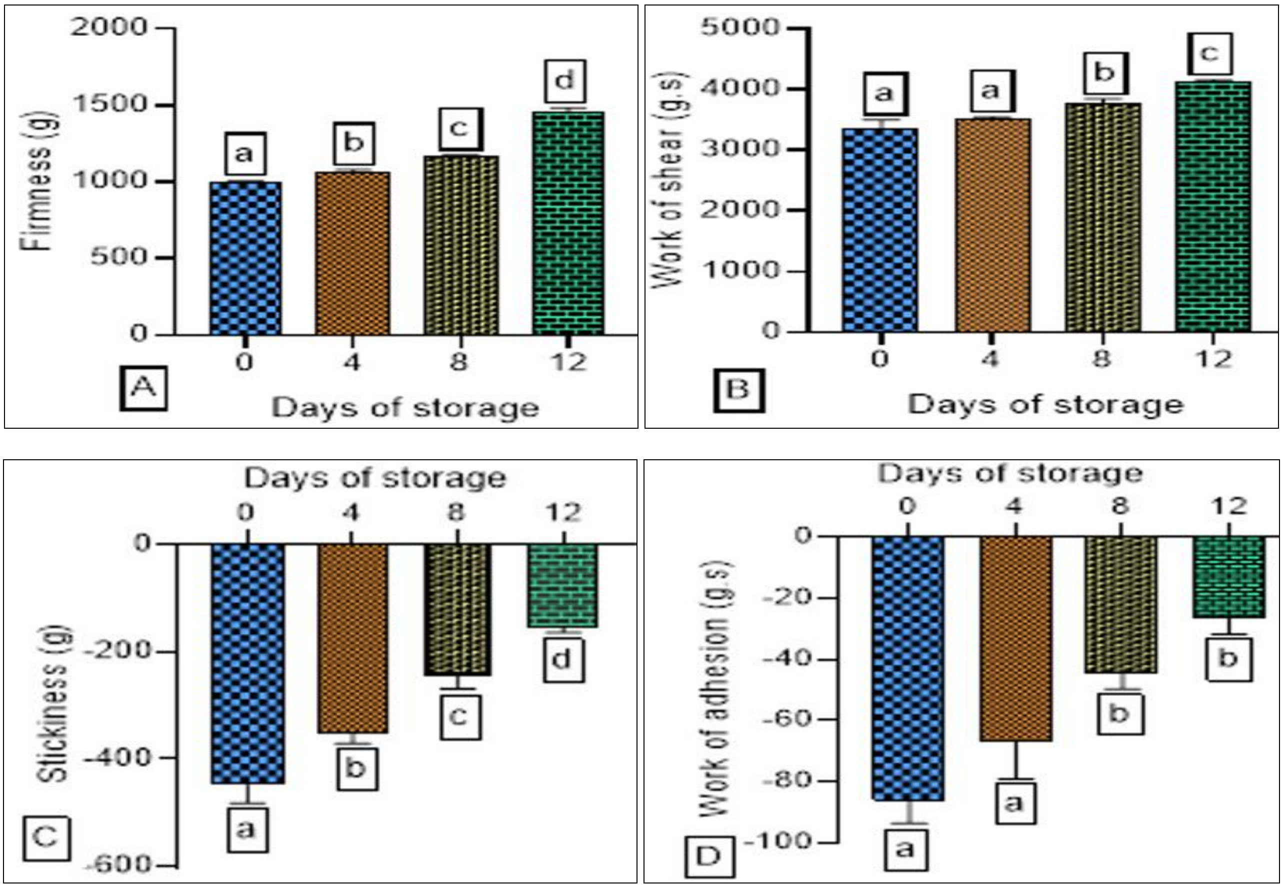


Fig 3: Change in textural attributes of probiotic Ricotta cheese during storage.
A: Firmness; B: Work of shear; C: Stickiness; D: Work of adhesion Values with different superscripted letters in a graph differs significantly (p<0.05)

Similarly, in previous study Minas-frescal cheese containing *L. acidophilus* became harder during storage, which was associated with an increment in acidity [11]. The degree of cross linking in between the proteins and formation of three-dimensional network increased the hardness of product during the storage [12, 6]. The Firmer matrices formed by acid generation during storage can be correlated to hardness of product, pH affects reactivity of the binding site if the casein molecule and therefore influences the matrix structure [14]. The lactic acid production enhances the protein coagulation turning matrices into harder entities and making more susceptible to fracture, which aid in filling spaces with water or residual whey, for this reason RC matrices exhibited higher values for hardness beside being more adhesive in texture [4].

Color of the probiotic RC remained stable throughout the 12 days of refrigerated storage as revealed by instrumental color analysis (Fig. 4). The L* value of the product decreased non-significantly ($p>0.05$) from 81.49 to 81.11 during 12 days of storage. The initial a* value of the product was -1.71, which increased non-significantly ($p>0.05$) to -1.63 on 12 days of storage. However, b* increased significantly ($p<0.05$) during storage period, the respective b*value at 0th and 12th day were 9.24 and 9.74. Similar incidents of lowering of L* and increase in a* and b* values during storage had been reported in control and Minas-frescal cheese [15]; buffalo probiotic Minas-frescal cheese [16]. Similarly, the decrease of lightness value during storage is probably due to higher hydration of proteins, leading to a reduced degree of light scattering.

Similar incidents had been observed in probiotic goat Ricotta cheese where, brightness (L*) reduced, green (a*) and yellow (b*) color increased during storage. Increment in green color might be associated with production of certain nutrients particularly B vitamins (riboflavin), which contributes to green pigment in product [17]. Further, the increase in a* value could be associated with biliverdin-IX-a, a blue green pigment present in buffalo milk and associated with caseins [18]. A significant ($p<0.05$) increase in b* value during storage is probably due to higher concentration of yellow color compounds formed due to mallard reaction and non-enzymatic browning, as preparation of RC is associated with high heat treatment [19, 20].

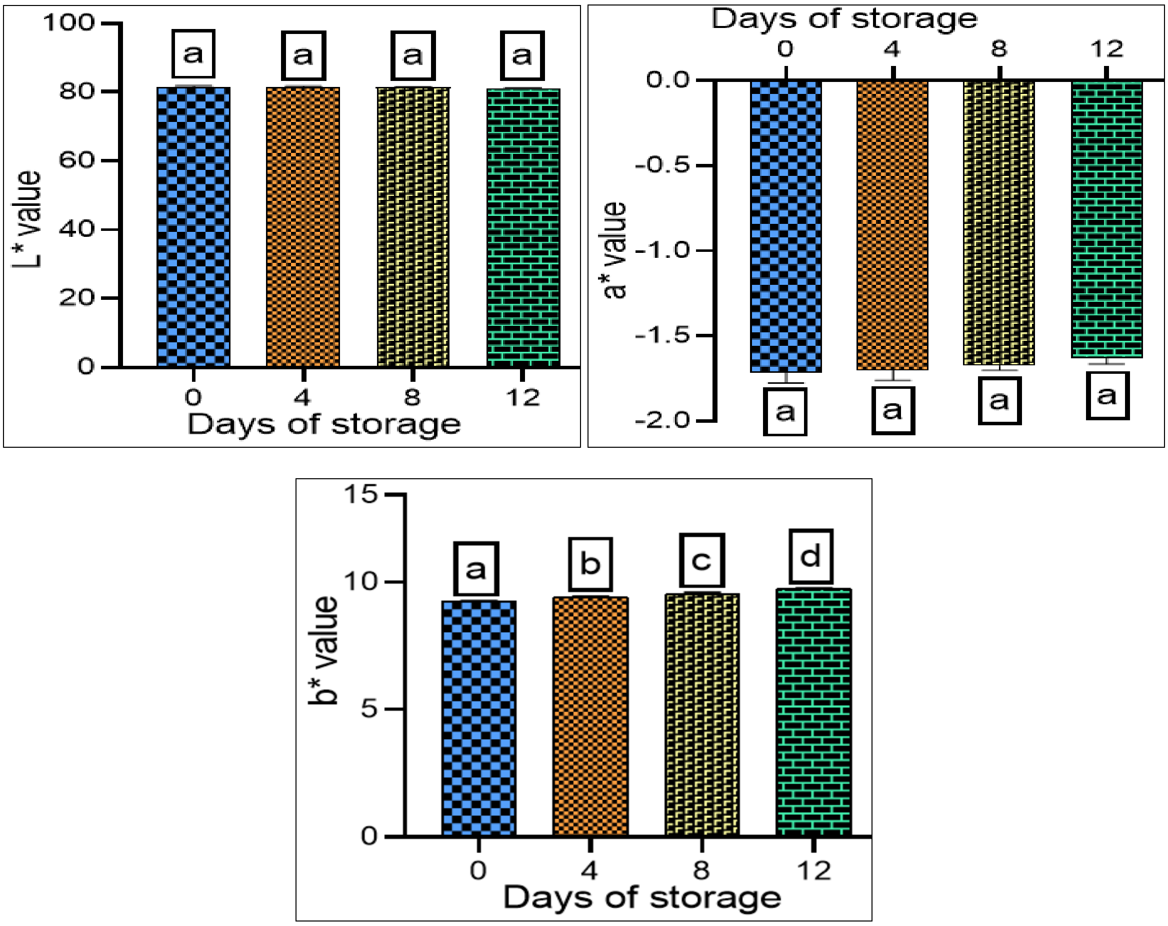


Fig 4: Change in colour profile of probiotic Ricotta cheese during storage
Values with different superscripted letters (a-d) in the graph differs significantly ($p<0.05$)

Conclusion

The current study estimated that, the control Ricotta cheese was remained stable for 8 days of refrigerated storage, whereas, probiotic Ricotta cheese was stable till 12 days of refrigerated storage. The product had maintained >7.5 log CFU/g probiotic count, over the 12 of days of refrigerated storage. The PRC showed significant change in titratable acidity, overall acceptability, textural properties and color in during storage. The water activity remained stable throughout the refrigerated storage.

Acknowledgement

The authors are thankful to ICAR- National Dairy Research Institute, Karnal, Haryana (India) for providing all necessary support for the project

Conflict of interest

The authors declare no conflict of interest.

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