

## RESEARCH ARTICLE

# Storage related changes in Pizza cheese as affected using *Saccharomyces boulardii* adjunct culture

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**Abstract:** The investigation examined the storage related changes in Pizza cheeses for a period of 28 days at 4°C. The physicochemical properties [i.e. moisture, titratable acidity (TA), pH, soluble nitrogen (SN), total volatile fatty acids (TVFA)] were markedly affected by temperature (T), period (P) and their interaction T×P. The TA, SN and TVFA exhibited marked increases with the advancement of the storage period. The sensory scores differed markedly for cheeses amongst each other with regard to T and P, except for stringiness. The stringiness score was markedly affected by T, P and their interaction T×P. Microbial analysis revealed the absence of coliforms in fresh as well as stored cheeses. The *S. boulardii* and LAB counts were markedly affected by T, P and their interaction T×P. The *S. boulardii* and LAB counts of all the cheeses tended to show a gradual decrease during the storage period. Cheese CBHM was associated with a higher *S. boulardii* count and elicited the desired baking and sensory characteristics when judged as topping on pizza pie. Since storage beyond 14 days led to a reasonable decline in the count of *S. boulardii*, only 2 weeks of refrigerated storage is advocated to reap the health benefits accrued from the consumption of such cheese containing probiotic microbe.

**Keywords:** Pizza cheese, *Saccharomyces boulardii*, Cheddaring, Homogenized milk, Refrigerated storage

## Abbreviations

|           |                                                                                         |
|-----------|-----------------------------------------------------------------------------------------|
| CBHM      | – Cheese from ‘milk blend’ comprising of homogenized and unhomogenized milks (1:1, w/w) |
| CFU       | – Colony Forming Unit                                                                   |
| CUM (C1)  | – Cheese from unhomogenized milk                                                        |
| CUMY (C2) | – Cheese from unhomogenized milk with <i>S. boulardii</i> (yeast) culture               |
| FCRD      | – Factorial Completely Randomized Design                                                |
| FDM       | – Fat-on-Dry Matter                                                                     |
| IMCU      | – International Milk Clotting Units                                                     |
| LAB       | – Lactic Acid Bacteria                                                                  |
| P         | – Period                                                                                |
| SC        | – Starter Culture                                                                       |
| SN        | – Soluble nitrogen                                                                      |
| T         | – Treatment                                                                             |
| T × P     | – Interaction of Treatment with Period                                                  |
| TA        | – Titratable Acidity                                                                    |
| TVFA      | – Total Volatile Fatty Acids                                                            |

## Introduction

Mozzarella cheese (particularly the Pizza cheese type, also known as low-moisture part-skim Mozzarella) is the preferred choice with regard to its application as a topping on pizza pie (Bihola et al. 2024a). Historically, the production of Mozzarella cheese utilized the traditional technique employing “Starter Culture” (SC). Various researchers have prepared Mozzarella/Pizza cheese employing the SC technique utilizing adjunct cultures such as *Lactobacillus acidophilus* and *Lactocaseibacillus rhamnosus* (Ortakci et al. 2012; Cuffia et al. 2017; Akarca and Yildirim 2022). There is lack of information on the manufacture of Pizza cheese using *Saccharomyces boulardii* (a proven probiotic yeast) as an adjunct culture. *Saccharomyces boulardii* is a well-known thermotolerant probiotic yeast that grows well at 37°C (Bihola et al. 2024c, Bihola et al. 2024d).

Clinical trials by Unique Biotech revealed that *S. boulardii* (unique 28 strain) could relieve the symptoms of diarrhoea and other intestinal problems, including traveller’s diarrhoea and Irritable Bowel Syndrome with constipation and even prevented gastrointestinal infections (Unique Biotech 2023). *S. boulardii*

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has found application in ice cream as well as in cultured dairy products such as yoghurt, kefir, buttermilk and cheese. When consumed at the recommended dose (minimum  $10^7$  CFU/g or mL), *S. boulardii* had a positive impact on the health of host. Improvement in the gut flora, immunological modulation, avoidance of enteric infections, diarrhoea and inflammatory bowel disease are some of the advantages gained when consuming food products containing *S. boulardii* (Staniszewski and Kordowska-Wiater 2021, Bihola et al. 2024b).

*S. boulardii* showed antidiarrheal activity by restoring the normal balance of microorganisms in the intestines. Such organism inhibited bacterial toxins from binding with the intestinal cells and neutralizing them prior to its absorption. *S. boulardii* improved the digestive enzyme activity and strengthened the integrity of epithelial cells of the intestine, preventing infection from spreading to other parts of the body. The immune system gets augmented in the presence of *S. boulardii* in the digestive tract. Such effect took place through stimulation of T-cells and macrophages as well as greater killer cell activity (Unique Biotech 2023).

Hence, the investigation was carried out to study the storage related changes in Pizza cheeses prepared using *S. boulardii* as an adjunct starter through technological interventions in order to obtain the desired viable counts. This research generated helpful data for cheesemakers who intend to produce Pizza cheese made using lactic acid bacteria (LAB) along with *S. boulardii* as an adjunct culture.

## Materials and Methods

### Materials

Chilled, mixed (i.e. buffalo and cow) milk was purchased from Vidya Dairy, Anand. The milk was separated at Anubhav Dairy, Anand to produce skim milk and cream. The cheese milk was standardized employing freshly separated skim milk. A fungal rennet from *Rhizomucor miehei* (strength of 2400 IMCU/g) was obtained from M/s. Caglifacio Clerici, Cadorago, Italy and used as the milk coagulant. *Saccharomyces boulardii* unique 28, sourced from M/s. Unique Biotech, Hyderabad was used as an adjunct culture in Pizza cheese making. Starter cultures (i.e. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) were obtained from M/s. DSM, Netherlands. Calcium chloride, dihydrate was purchased from M/s. Loba Chemie Pvt. Ltd., Mumbai and added to the cheese milk. Tata brand vacuum-evaporated common salt (NaCl) was used for salting the cheese.

### Equipment

Kenstar 3D Power OM-34ECR baking oven was used to bake the cheese-topped pizza pie. An Infrared Thermometer (model No.

GIS-500, Bosch, Bengaluru) was used to note the temperature of the plasticized cheese mass during cheese making.

### Pizza Cheese Making

Pizza cheese was prepared from a 'milk blend' [i.e. unhomogenized and homogenized (1.96 and 0.98 MPa pressure, 65°C temperature) milks - 1:1, w/w; 3.2% milk fat] following the SC method of Patel (2022) using *S. boulardii* as an adjunct culture (cheese designated as CBHM). CBHM was prepared by inoculating cheese milk with *S. boulardii* culture adjunct at a level of 3.5 g/100 kg milk and keeping salt (NaCl) levels at 1.75% by weight of the cheese curd during dry salting (Bihola et al. 2024c).

Control Pizza cheeses, with and without inclusion of *S. boulardii* adjunct culture, were prepared from standardized (3.2% milk fat), pasteurized (72°C/15 s) mixed milk as per the process of Patel et al. (1986) and Rajani et al. (2024a) employing the SC method (moulding water temperature was  $95 \pm 3^\circ\text{C}$ ).

The *S. boulardii* (unique 28) adjunct culture was incorporated into cheese milk during Pizza cheese making [in control (C2) and experimental] and after preincubation of such culture in a sufficient quantity of milk (i.e. ~ 500 mL) at 25°C for 6 h in a thermostatically controlled incubator (Bihola et al. 2024b, Bihola et al. 2024c).

### Analyses

The moisture content and titratable acidity (TA) of Pizza cheeses were determined employing standard procedures (AOAC 2023). The pH of cheese slurry made in distilled water was measured using a digital pH meter (M/s. Mettler Toledo AG, Schwerzenbach). The soluble nitrogen content of fresh and stored cheese samples was determined using the procedure of Mamo (2017). Total volatile fatty acids (TVFA) were measured using the procedure of Kosikowski and Dahlberg (1946). The LAB and *S. boulardii* counts of Pizza cheese were determined using the methods described by ISO (1998) and Niamah et al. (2017) respectively. The coliform count of the cheese samples was determined (BIS 1964).

### Refrigerated storage of Pizza cheese

Pizza cheeses were vacuum-packed in polyethylene bags (~ 80.0 µm thick) and stored in a refrigerator maintained at  $7 \pm 1^\circ\text{C}$  until they were organoleptically acceptable (for up to 21 days). The storage stability of Pizza cheeses was evaluated in terms of the changes occurring in their physico-chemical properties (i.e. moisture, TA, pH, SN and TVFA); sensory evaluation of cheese as pizza topping (at 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day of storage) and microbial count [i.e. Lactic Acid Bacteria count (LAB), *S. boulardii* count] during refrigerated storage. Analyses were performed every 7 days for 28 days of refrigerated storage. Coliform count was

performed for freshly prepared cheese (i.e. 0 day) and cheese stored for 21 days only.

### Statistical Analysis

A factorial completely randomized design (FCRD) was applied to statistically evaluate the findings obtained in the investigation. The averages of the results of the investigation of duplicate samples of pizza cheese, obtained in four separate replications for three treatments were examined by statistical examination employing FCRD (Steel and Torrie 1980).

### Results and Discussion

#### Temperature of Plasticized Cheese Mass

It is obvious that the cheese produced from milk blend (unhomogenized: homogenized, 1:1 w/w), the homogenized milk portion possesses a greater amount of protein adsorbed onto the increased fat surface area. Additionally, the pH of curd at stretching (i.e. whey acidity at stretching stage of curd was 0.44% LA) was considerably lower than for control cheese (Jana and Upadhyay, 1993). Both these factors led to cheese curds getting plasticized at a much lower temperature for cheese CBHM.

Based on the plasticizing conditions adopted in preparing control cheeses (i.e. C1, C2) and cheese CBHM, the temperature of the plasticized cheese mass was 63.6°C and 59.5°C respectively. The plasticizing conditions for the cheeses, in the same order as specified above, were 93.5°C temperature with contact period with cheese curd of 4.5 min. (for both control cheeses) and 79.0°C with contact period of 2.5 min. respectively (Bihola et al. 2024b, Bihola et al. 2024c, Bihola et al. 2024c).

#### Changes Occurring in Pizza Cheeses During Refrigerated Storage

Fresh mozzarella cheese is not considered suitable for its application on pizza because it melts into a tough, rubbery and grainy consistency, exhibiting limited stretch (Jana and Mandal 2011). The refrigerated storage of Pizza cheese, over a period of approximately 2 weeks, is reported to bring about desirable changes (i.e. mellowness, melt and stretch), as well as simultaneous unwanted changes (i.e. shred, fat leakage and sliminess), most of which are dictated by proteolytic activity. Enzymes from milk, starter culture, non-starter flora and rennet all contribute to the proteolytic changes in pizza cheese during refrigerated storage (Jana and Tagalpallewar 2017). Storage changes can have a significant impact on the survival of desired microbes (such as LAB and probiotics) and the end-use applications of cheese, such as its application as a pizza topping.

The end use application of Pizza cheese (fresh or stored) on pizza pie was conducted only from the 7<sup>th</sup> day until two more times, at an interval of one week (i.e. till 21<sup>st</sup> day of storage). This was

followed deliberately, since freshly prepared Pizza cheese does not behave satisfactorily when used as a topping on pizza pie (Jana and Mandal 2011). Refrigerated storage of the cheese beyond 21 days posed problem related to ease of shredding; in some instances, an unpleasant flavour was perceived and subsequent storage sometimes showed visible mold on cheese surface. The pertinent results related to the changes in the quality characteristics of Pizza cheeses are described in this research investigation.

### Physicochemical Changes in Cheese

#### Moisture

The moisture content of the cheeses gradually decreased throughout the storage period of 28 days. The changes in the moisture content of cheeses were significantly ( $p < 0.05$ ) affected by the treatment (T), storage period (P) and their interaction i.e. T x P. All the three cheeses differed markedly ( $p < 0.05$ ) from each other. Cheeses CBHM and C1 were associated with the maximum and minimum moisture content respectively. During storage, the first significant ( $p < 0.05$ ) drop in the moisture content of cheeses was noted on the 14<sup>th</sup> day; a marked decline took place again on the 28<sup>th</sup> day of refrigerated storage (Table 1).

Moisture loss during refrigerated storage of Mozzarella cheese is a usual feature. A reduction in the moisture content (loss of 4.2%) of Mozzarella cheese has been reported during refrigerated storage (7°C) of one month (Felfoul et al. 2018). Rajani et al. (2021b) also noted a gradual decline in the moisture content of Pizza cheeses during refrigerated storage (7°C); the moisture content of cheeses was 51.75, 51.39 and 51.05% at storage periods of 0, 2 and 4 weeks, respectively.

#### Titrateable Acidity

There was a progressive increase in the titrateable acidity (TA) of all the cheeses during storage. The changes in the TA of cheeses were markedly affected by T, P and interaction T x P. All the cheeses differed markedly ( $p < 0.05$ ) from each other; maximum (i.e. 0.67% LA) and minimum (i.e. 0.48% LA) values were associated with cheeses CBHM and C1 respectively. There was a linear increase in the TA of cheeses during the advancement of storage; such a marked ( $p < 0.05$ ) increase in the TA of cheeses was first noted on the 7<sup>th</sup> day and subsequently on the 14<sup>th</sup> and 28<sup>th</sup> days of storage (Table 1).

The TA of Mozzarella cheeses prepared employing SC method was 0.78 and 0.90% LA at storage (4°C) periods of 2 and 4 weeks respectively; freshly prepared cheese had 0.68% LA (Ahmed et al. 2011). Abd El-Gawad et al. (2012) reported a progressive rise in the TA values of Mozzarella cheeses made using SC method from homogenized (2.45 MPa) and unhomogenized milks (3.0% fat for both) during storage at 4°C. The TA value (as % LA) of homogenized milk cheese was 0.67 and 0.76 when fresh and at 4

weeks of storage respectively; the TA value of cheese from unhomogenized milk was 0.64 and 0.72 respectively at the same period stated above.

## pH

There was a progressive decrease in the pH of all the cheeses throughout storage (i.e. up to 28<sup>th</sup> the day). The changes in the pH of cheeses differed significantly ( $p < 0.05$ ) with respect to T, P and interaction T x P. There was a marked drop in the pH of

cheeses, at each 7 days interval of storage, up to 28<sup>th</sup> day. The pH of all the cheeses differed significantly ( $p < 0.05$ ) from each other; maximum and minimum pH was associated with cheeses C1 (i.e. 5.26) and CBHM (i.e. 4.97) respectively (Table 1).

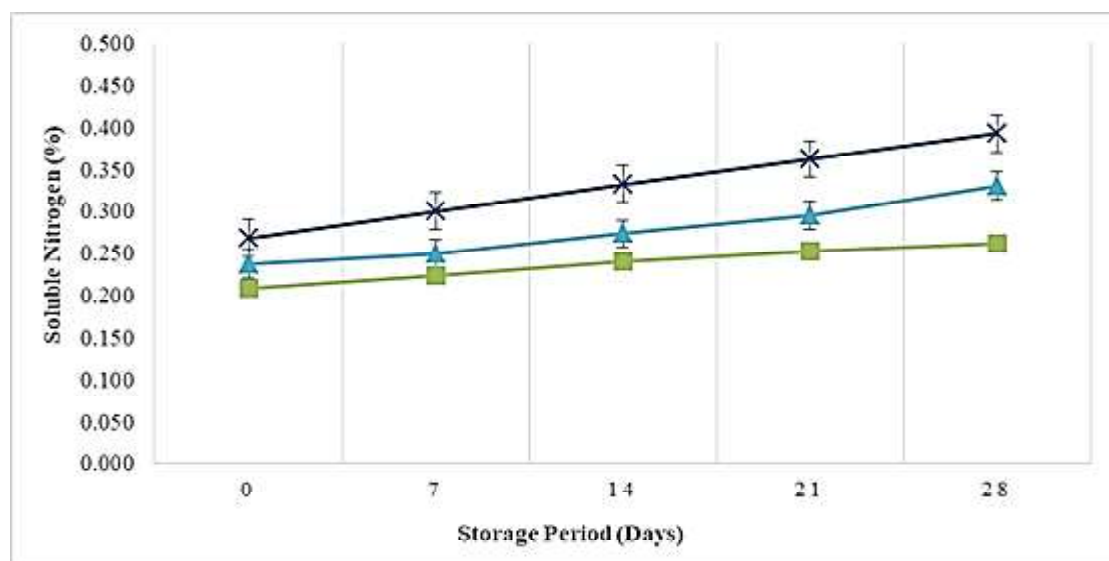
Several researchers have reported a steady decline in the pH of Mozzarella cheeses during their refrigerated storage of one month (Felfoul et al. 2018). During ageing, the cheese undergoes several types of microbiological and biochemical changes, involving proteolysis, lipolysis with simultaneous conversion of remaining

**Table 1:** Changes in the physico-chemical properties of Pizza cheeses during refrigerated storage (4°C)

| Cheeses                                                    | Storage Period (Days) |                    |                    |                     |                    | Mean<br>(Treatment) |
|------------------------------------------------------------|-----------------------|--------------------|--------------------|---------------------|--------------------|---------------------|
|                                                            | 0                     | 7                  | 14                 | 21                  | 28                 |                     |
| Moisture (%)                                               |                       |                    |                    |                     |                    |                     |
| CUM (C1)                                                   | 48.88 <sup>f</sup>    | 48.61 <sup>h</sup> | 48.41 <sup>i</sup> | 48.22 <sup>jk</sup> | 48.13 <sup>k</sup> | 48.45 <sup>c</sup>  |
| CUMY (C2)                                                  | 49.03 <sup>c</sup>    | 48.77 <sup>g</sup> | 48.56 <sup>h</sup> | 48.37 <sup>i</sup>  | 48.24 <sup>j</sup> | 48.60 <sup>b</sup>  |
| CBHM                                                       | 52.30 <sup>a</sup>    | 52.10 <sup>b</sup> | 51.95 <sup>c</sup> | 51.86 <sup>c</sup>  | 51.80 <sup>d</sup> | 52.00 <sup>a</sup>  |
| Mean (Period)                                              | 50.07 <sup>a</sup>    | 49.82 <sup>b</sup> | 49.64 <sup>c</sup> | 49.48 <sup>d</sup>  | 49.39 <sup>e</sup> | ---                 |
| Source of Variation                                        | Treatment (T)         |                    | Period (P)         |                     | T x P              |                     |
| SEm±                                                       | 0.018                 |                    | 0.023              |                     | 0.039              |                     |
| Titratable Acidity (% LA)                                  |                       |                    |                    |                     |                    |                     |
| CUM (C1)                                                   | 0.46 <sup>j</sup>     | 0.47 <sup>ij</sup> | 0.48 <sup>i</sup>  | 0.49 <sup>h</sup>   | 0.50 <sup>h</sup>  | 0.48 <sup>c</sup>   |
| CUMY (C2)                                                  | 0.56 <sup>g</sup>     | 0.57 <sup>g</sup>  | 0.57 <sup>g</sup>  | 0.59 <sup>f</sup>   | 0.61 <sup>e</sup>  | 0.58 <sup>b</sup>   |
| CBHM                                                       | 0.62 <sup>e</sup>     | 0.65 <sup>d</sup>  | 0.67 <sup>c</sup>  | 0.70 <sup>b</sup>   | 0.72 <sup>a</sup>  | 0.67 <sup>a</sup>   |
| Mean (Period)                                              | 0.55 <sup>d</sup>     | 0.56 <sup>d</sup>  | 0.58 <sup>c</sup>  | 0.59 <sup>bc</sup>  | 0.61 <sup>a</sup>  | ---                 |
| Source of Variation                                        | Treatment (T)         |                    | Period (P)         |                     | T x P              |                     |
| SEm±                                                       | 0.003                 |                    | 0.004              |                     | 0.007              |                     |
| pH                                                         |                       |                    |                    |                     |                    |                     |
| CUM (C1)                                                   | 5.31 <sup>a</sup>     | 5.27 <sup>b</sup>  | 5.26 <sup>b</sup>  | 5.23 <sup>c</sup>   | 5.21 <sup>d</sup>  | 5.26 <sup>a</sup>   |
| CUMY (C2)                                                  | 5.24 <sup>c</sup>     | 5.20 <sup>d</sup>  | 5.16 <sup>c</sup>  | 5.08 <sup>g</sup>   | 4.94 <sup>i</sup>  | 5.12 <sup>b</sup>   |
| CBHM                                                       | 5.13 <sup>f</sup>     | 5.05 <sup>h</sup>  | 4.95 <sup>i</sup>  | 4.90 <sup>j</sup>   | 4.83 <sup>k</sup>  | 4.97 <sup>c</sup>   |
| Mean (Period)                                              | 5.23 <sup>a</sup>     | 5.18 <sup>b</sup>  | 5.12 <sup>c</sup>  | 5.07 <sup>d</sup>   | 5.00 <sup>e</sup>  | ---                 |
| Source of Variation                                        | Treatment (T)         |                    | Period (P)         |                     | T x P              |                     |
| SEm±                                                       | 0.003                 |                    | 0.004              |                     | 0.006              |                     |
| Soluble Nitrogen (%)                                       |                       |                    |                    |                     |                    |                     |
| CUM (C1)                                                   | 0.208 <sup>j</sup>    | 0.224 <sup>i</sup> | 0.240 <sup>h</sup> | 0.253 <sup>g</sup>  | 0.261 <sup>f</sup> | 0.237 <sup>c</sup>  |
| CUMY (C2)                                                  | 0.237 <sup>h</sup>    | 0.250 <sup>g</sup> | 0.273 <sup>e</sup> | 0.295 <sup>d</sup>  | 0.330 <sup>c</sup> | 0.277 <sup>b</sup>  |
| CBHM                                                       | 0.268 <sup>e</sup>    | 0.300 <sup>d</sup> | 0.332 <sup>c</sup> | 0.362 <sup>b</sup>  | 0.392 <sup>a</sup> | 0.331 <sup>a</sup>  |
| Mean (Period)                                              | 0.238 <sup>e</sup>    | 0.258 <sup>d</sup> | 0.282 <sup>c</sup> | 0.304 <sup>b</sup>  | 0.328 <sup>a</sup> | ---                 |
| Source of Variation                                        | Treatment (T)         |                    | Period (P)         |                     | T x P              |                     |
| SEm±                                                       | 0.001                 |                    | 0.001              |                     | 0.002              |                     |
| Total Volatile Fatty Acids (ml of 0.1 N NaOH/100 g cheese) |                       |                    |                    |                     |                    |                     |
| CUM (C1)                                                   | 2.29 <sup>k</sup>     | 2.91 <sup>j</sup>  | 3.78 <sup>hi</sup> | 5.81 <sup>f</sup>   | 9.96 <sup>c</sup>  | 4.95 <sup>c</sup>   |
| CUMY (C2)                                                  | 2.92 <sup>j</sup>     | 3.62 <sup>i</sup>  | 4.83 <sup>g</sup>  | 7.55 <sup>e</sup>   | 12.69 <sup>b</sup> | 6.32 <sup>b</sup>   |
| CBHM                                                       | 3.16 <sup>j</sup>     | 4.07 <sup>h</sup>  | 5.46 <sup>f</sup>  | 8.47 <sup>d</sup>   | 14.09 <sup>a</sup> | 7.05 <sup>a</sup>   |
| Mean (Period)                                              | 2.79 <sup>e</sup>     | 3.54 <sup>d</sup>  | 4.69 <sup>c</sup>  | 7.28 <sup>b</sup>   | 12.25 <sup>a</sup> | ---                 |
| Source of Variation                                        | Treatment (T)         |                    | Period (P)         |                     | T x P              |                     |
| SEm±                                                       | 0.063                 |                    | 0.081              |                     | 0.140              |                     |

CUM (C1) – Cheese from unhomogenized milk; CUMY (C2) – Cheese from unhomogenized milk with *S. bouardii* culture; CBHM – Cheese from ‘milk blend’ comprising of homogenized and unhomogenized milks (1:1, w/w); Figures placed after ± indicates standard deviation, the values indicated row and column wise having differing superscripted alphabets differs significantly ( $p < 0.05$ ) from each other; n=4

**Fig. 1** Changes in the soluble nitrogen content of Pizza cheeses during storage



lactose to lactate and citrate. Residual lactose is quickly transformed into lactate during the initial phases of maturation. Lactate is a necessary precursor for a number of reactions that reduce the pH of cheese, comprising of racemization, oxidation, and microbial metabolism (Mc Sweeney and Fox 2004).

The pH of Pizza cheeses prepared employing SC method was 4.80 and 4.00 as noted during 2 and 4 weeks of refrigerated (4°C) storage respectively; freshly prepared cheese had pH of 5.30 (Ahmed et al. 2011). Likewise, the pH of freshly prepared Pizza cheese made using SC method was 5.30; the values were 4.90 and 4.60 respectively as noted at 2 and 4 weeks of storage (4°C) (Abd El-Gawad et al. 2012).

### Soluble nitrogen

The proteolytic and peptidolytic activities of starter bacteria have been held responsible as key factors in determining proteolytic breakdown during Pizza cheese ripening; plasmin has also been implicated. There is a consequential release of amino groups as a result of proteolysis. As a result of proteolytic breakdown, the soluble nitrogen (SN) content of cheese tended to increase linearly with the age of the product (Costabel et al. 2007). Such proteolytic changes occurring in Pizza cheeses during refrigerated storage improves the water binding capacity of casein and brings about desirable or undesirable changes in the functionality (melt, flow and stretch) of the product (Jana and Mandal 2011). Hence, it was necessary to monitor the changes in SN during the refrigerated storage of Pizza cheeses.

The SN content of all the cheeses increased markedly throughout the storage period; such an increase in SN was found to be significant ( $p < 0.05$ ) at each 7 days interval of storage, until the 28<sup>th</sup> day (Figure 1). The changes noted in the SN content of all three cheeses were significantly ( $p < 0.05$ ) influenced by T, P and the interaction T x P. The experimental cheese CBHM had a

markedly higher SN content (i.e. 0.331%) compared to that of the other two cheeses; control cheese C1 had the least SN value (i.e. 0.237%). The minimum and maximum values of SN were noted for fresh control cheese C1 (0.208%) and 28 days-old experimental cheese CBHM (0.392%) respectively (Table 1).

The SN content (at pH 4.6, expressed as % of total nitrogen) of Fior di Latte (similar to high moisture Mozzarella) cheese prepared employing the SC method using adjunct cultures (i.e. *L. rhamnosus* GG, *L. acidophilus* LA5 and their combination) showed an increase during refrigerated storage (4°C) of up to 15 days. The SN content of cheeses containing *L. rhamnosus* GG exhibited an increase from 2.47% when fresh to 3.68% as noted on the 15<sup>th</sup> day of refrigerated storage (Cuffia et al. 2019).

Abd El-Gawad et al. (2012) also noted an increase in the SN content during storage (4°C) of Mozzarella cheeses for up to 4 weeks; the cheeses were prepared using SC method from homogenized (2.45 MPa) and unhomogenized milks. The values of SN reported were 0.166 and 0.162% for freshly prepared cheeses from homogenized and unhomogenized milks respectively; the pertinent values noted on the 28<sup>th</sup> day of storage were 0.325 and 0.274% respectively. The SN content of Pizza cheese made using thermophilic SC increased from 0.173 to 0.228% over a span of 28 days; storage was at 7°C (Rajani et al. 2021b).

### Total Volatile Fatty Acids

The determination of free volatile fatty acids (VFAs) is of interest with respect to the lipolytic changes taking place in cheese during ageing and the resultant flavour profile of aged cheese. These VFAs are components of taste and flavour, and their concentration gives an indication of the metabolic reactions that took place during cheese ageing. Hence, the TVFA content of cheese is used to monitor the lipolytic changes occurring in the product during ageing (Collins et al. 2003).

The TVFA content of Pizza cheeses was significantly ( $P < 0.05$ ) affected by T, P and the interaction T x P. Such an increase in the TVFA of Pizza cheeses was found to be statistically significant ( $p < 0.05$ ) at each 7 days interval of storage, up to 28<sup>th</sup> day (Figure 2). All the cheeses differed markedly ( $p < 0.05$ ) from each other with regard to the TVFA content; the maximum (7.05) and minimum (4.95) values (expressed as ml of 0.1 N NaOH/100 g cheese) were associated with cheeses CBHM and C1 respectively (Table 1).

The linear increase in the TVFA content of Mozzarella cheese during refrigerated storage is documented in the literature. Ahmed et al. (2011) reported TVFA values (ml 0.1 N NaOH/100 g cheese) of 6.46 and 12.55 for Mozzarella cheeses prepared using SC Method stored for 2 and 4 weeks respectively; freshly prepared cheese had TVFA value of 3.10. The TVFA content of Mozzarella cheese, made utilizing *L. rhamnosus* and *L. paracasei* as an adjunct culture, increased from 2.0 to 13.0 mg/kg during refrigerated (4 °C) storage of 20 days (Huang et al. 2022).

### Changes in the sensory scores of cheeses as Pizza topping

The sensory scores of Pizza cheeses, judged as pizza topping, are depicted in Table 2. The pertinent findings related to the sensory scores of Pizza cheeses are discussed in the following paragraphs.

#### Appearance

There was an increase in the appearance score of all the cheeses in a span of 14 days (i.e. 7<sup>th</sup> to 21<sup>st</sup> day of storage). The changes in the appearance scores of cheeses were significantly ( $p < 0.05$ ) affected by T and P; the interaction T x P remained unaffected. The data shown in Table 2 revealed that the appearance score of the cheeses was in the order: C1 > C2 > CBHM; such difference in the appearance score was found to be significant ( $p < 0.05$ ). The overall improvement in the appearance score of cheeses, judged as pizza topping, could be attributed to the improvement in the melting property (uniform melt, devoid of any unmelted shred particles) and improved glossy appeal of the cheese as a result of ageing. The improvement in glossiness of melted cheese might be attributed to the increased water binding capacity of the cheese proteins as a consequence of ageing (Jana and Tagalpallewar 2017).

#### Flavour

The changes in the flavour scores of cheeses were significantly ( $p < 0.05$ ) affected by T and P; interaction T x P remained unaffected. An increase in the flavour scores of all the cheeses was noted during the initial 7 days (i.e. 7<sup>th</sup> to 14<sup>th</sup> day) of storage. The mean values of flavour scores (out of 10.0) for cheeses C1, C2 and CBHM were 8.12, 7.93 and 7.47 respectively. The difference in the flavour scores of the three cheeses was found to be significant ( $p < 0.05$ ); the maximum score was associated with cheese C1. Initial 7 days of storage (from the 7<sup>th</sup> day onward)

exhibited a significant ( $p < 0.05$ ) increase in the flavour scores of the cheeses, judged on pizza pie. However, subsequent storage for a week (i.e. 7 days) led to a significant ( $p < 0.05$ ) decline in the flavour score of cheeses (Table 2).

An improvement in the flavour score of Mozzarella/Pizza cheeses prepared using SC method, especially those prepared using SC, up to a certain period of refrigerated storage has been reported in the literature (Rajani 2021; Patel 2022).

#### Melting

The changes in the melting scores of Pizza cheeses, judged as pizza topping, were significantly ( $p < 0.05$ ) affected by T and P; the interaction T x P remained unaffected. The superiority in the melting scores of Pizza cheese C2 over cheeses C1 and CBHM was in consonance with the increasing trend noted for Schreiber meltability values of Pizza cheeses (Table 2). Cheese C2 had a markedly ( $p < 0.05$ ) higher melting score compared to the remaining two cheeses; CBHM had the least melting score. The melting scores of C1 and CBHM were also significantly ( $p < 0.05$ ) different; C1 had superior score (Table 2).

#### Stringiness

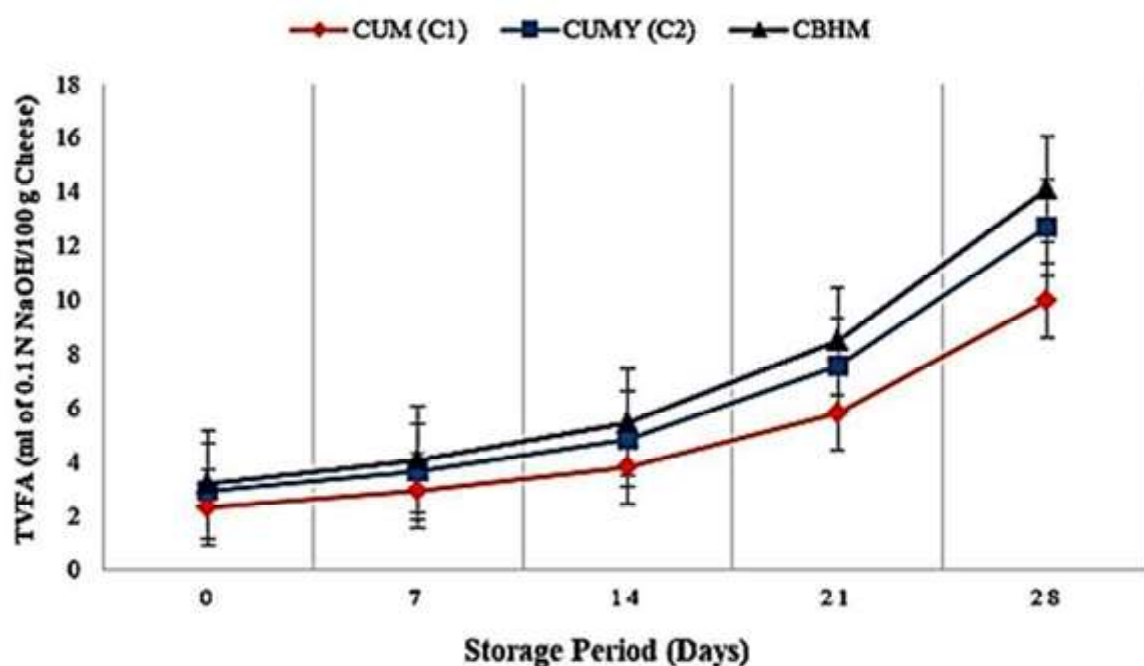
The changes in the stringiness score of cheeses, assessed on pizza pie, were significantly ( $p < 0.05$ ) affected by T, P and their interaction T x P. Cheese C1 was associated with the highest stringiness score; such score was significantly ( $p < 0.05$ ) superior when compared with the scores of the other two cheeses. Cheese CBHM had the least stringiness score; such score was significantly ( $p < 0.05$ ) lower than the score allotted to cheese C2. There was a linear decrease in the stringiness scores of all the cheeses with the advancement of the storage period; such change was significant ( $p < 0.05$ ) at each 7 days interval of storage, until the 21<sup>st</sup> day (Table 2).

Such a marked decrease in the stringiness score of cheeses upon refrigerated storage was due to a decrease in the stretch character of the product upon ageing; attributed to the proteolytic changes. With regard to interaction T x P, the highest and least scores for stringiness (out of 10.00) were noted for 7 days old cheese C2 (i.e. score of 8.30) and 21 days old cheese CBHM (i.e. score of 7.24) respectively (Table 2).

Mozzarella cheese made utilizing SC method is associated with poor stretch and melt when freshly prepared; such properties improve during the initial stage of ageing and become optimal within 2 to 3 weeks of refrigerated storage. Nevertheless, such functional traits tend to deteriorate upon further storage/ageing (Rajani 2021; Patel 2022).

#### Chewiness

**Fig. 2** Changes in the total volatile fatty acids content of Pizza cheeses during storage



**Table 2** Changes in the sensory scores of Pizza cheeses, evaluated as a pizza topping, during refrigerated storage (4°C)

| Cheeses                           | Storage Period (Days) |                   |                   | Mean<br>(Treatment) |
|-----------------------------------|-----------------------|-------------------|-------------------|---------------------|
|                                   | 7                     | 14                | 21                |                     |
| Appearance Scores (out of 10.00)  |                       |                   |                   |                     |
| CUM (C1)                          | 8.30                  | 8.66              | 8.98              | 8.64 <sup>a</sup>   |
| CUMY (C2)                         | 8.28                  | 8.52              | 8.65              | 8.49 <sup>b</sup>   |
| CBHM                              | 7.81                  | 8.30              | 8.61              | 8.24 <sup>c</sup>   |
| Mean (Period)                     | 8.13 <sup>c</sup>     | 8.49 <sup>b</sup> | 8.74 <sup>a</sup> | ---                 |
| Source of Variation               | Treatment (T)         | Period (P)        | T x P             |                     |
| SEm±                              | 0.061                 | 0.061             | 0.0106            |                     |
| Flavour Scores (out of 10.00)     |                       |                   |                   |                     |
| CUM (C1)                          | 8.16                  | 8.34              | 7.87              | 8.12 <sup>a</sup>   |
| CUMY (C2)                         | 7.91                  | 8.10              | 7.78              | 7.93 <sup>b</sup>   |
| CBHM                              | 7.45                  | 7.78              | 7.18              | 7.47 <sup>c</sup>   |
| Mean (Period)                     | 7.84 <sup>b</sup>     | 8.07 <sup>a</sup> | 7.61 <sup>c</sup> | ---                 |
| Source of Variation               | Treatment (T)         | Period (P)        | T x P             |                     |
| SEm±                              | 0.028                 | 0.028             | 0.048             |                     |
| Melting Scores (out of 10.00)     |                       |                   |                   |                     |
| CUM (C1)                          | 7.66                  | 8.01              | 8.32              | 8.00 <sup>b</sup>   |
| CUMY (C2)                         | 8.15                  | 8.26              | 8.55              | 8.32 <sup>a</sup>   |
| CBHM                              | 7.48                  | 7.74              | 8.05              | 7.75 <sup>c</sup>   |
| Mean (Period)                     | 7.76 <sup>c</sup>     | 8.00 <sup>b</sup> | 8.30 <sup>a</sup> | ---                 |
| Source of Variation               | Treatment (T)         | Period (P)        | T x P             |                     |
| SEm±                              | 0.028                 | 0.028             | 0.048             |                     |
| Stringiness Scores (out of 10.00) |                       |                   |                   |                     |
| CUM (C1)                          | 8.22 <sup>a</sup>     | 8.07 <sup>b</sup> | 7.52 <sup>c</sup> | 7.94 <sup>a</sup>   |
| CUMY (C2)                         | 8.30 <sup>a</sup>     | 7.94 <sup>c</sup> | 7.26 <sup>f</sup> | 7.83 <sup>b</sup>   |
| CBHM                              | 8.00 <sup>bc</sup>    | 7.63 <sup>d</sup> | 7.24 <sup>g</sup> | 7.62 <sup>c</sup>   |
| Mean (Period)                     | 8.18 <sup>a</sup>     | 7.88 <sup>b</sup> | 7.34 <sup>c</sup> | ---                 |

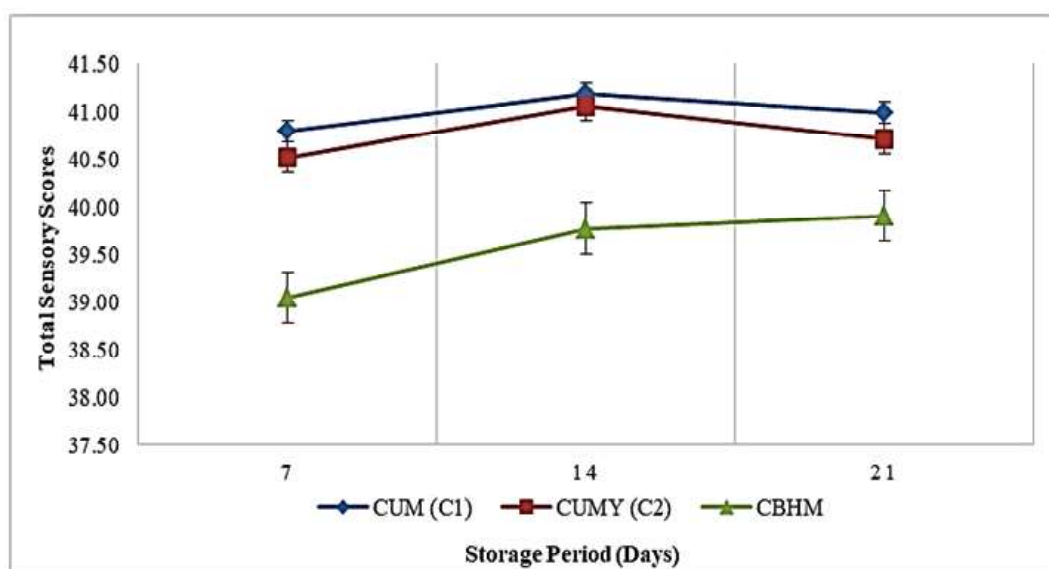
It is important to note that Pizza cheese with moderate chewiness, tends to obtain a higher score than its counterpart cheese exhibiting greater chewiness. The changes in the chewiness

scores of cheeses, assessed on pizza pie, were significantly ( $p < 0.05$ ) affected by T and P; the interaction T x P remained unaffected. Cheese CBHM had significantly ( $p < 0.05$ ) superior

| Chewiness Scores (out of 10.00)     |                    |                    |                    |                    |
|-------------------------------------|--------------------|--------------------|--------------------|--------------------|
| CUM (C1)                            | 7.85               | 8.10               | 8.30               | 8.08 <sup>b</sup>  |
| CUMY (C2)                           | 7.88               | 8.22               | 8.48               | 8.19 <sup>b</sup>  |
| CBHM                                | 8.30               | 8.65               | 8.82               | 8.59 <sup>a</sup>  |
| Mean (Period)                       | 8.00 <sup>c</sup>  | 8.30 <sup>b</sup>  | 8.53 <sup>a</sup>  | ---                |
| Source of Variation                 | Treatment (T)      | Period (P)         | T x P              |                    |
| SEm±                                | 0.041              | 0.041              | 0.072              |                    |
| Total Sensory Scores (out of 50.00) |                    |                    |                    |                    |
| CUM (C1)                            | 40.19              | 41.18              | 40.99              | 40.78 <sup>a</sup> |
| CUMY (C2)                           | 40.52              | 41.06              | 40.71              | 40.76 <sup>a</sup> |
| CBHM                                | 39.04              | 39.77              | 39.90              | 39.68 <sup>b</sup> |
| Mean (Period)                       | 39.92 <sup>b</sup> | 40.10 <sup>a</sup> | 40.54 <sup>a</sup> | ---                |
| Source of Variation                 | Treatment (T)      | Period (P)         | T x P              |                    |
| SEm±                                | 0.112              | 0.112              | 0.194              |                    |

CUM (C1) – Cheese from unhomogenized milk; CUMY (C2) – Cheese from unhomogenized milk with *S. boulardii* culture; CBHM – Cheese from ‘milk blend’ comprising of homogenized and unhomogenized milks (1:1, w/w); Figures placed after ± indicates standard deviation, the values indicated row and column wise having differing superscripted alphabets differs significantly ( $p < 0.05$ ) from each other;  $n=4$

**Fig. 3** Changes in the total sensory scores of Pizza cheeses as pizza topping as affected by storage



(i.e. 8.59 out of 10.00) chewiness score (i.e. since cheese had moderate chewiness) when compared to the scores associated with the other two cheeses; the chewiness scores of the latter two control cheeses (i.e. C1, C2) were at par with each other. The chewiness scores of all the cheeses improved with advancement in storage; such improvement was noticed at each 7 days interval of storage, until the 21<sup>st</sup> day (Table 2). The mellowing of Mozzarella cheese (i.e. decrease in chewiness) during its refrigerated storage is documented in the literature (Rajani 2021).

#### Total sensory score

The changes in the total sensory scores of cheeses, judged as pizza topping, were significantly ( $p < 0.05$ ) affected by T and P; the interaction T x P remained unaffected. Both the control cheeses (i.e. C1, C2) had total scores that were at par with each other (Figure 3). Cheese CBHM had the least total score (i.e. 39.68 out

of 50.0). During storage, the total sensory scores of cheeses remained fairly constant up to the 14<sup>th</sup> day (sensory evaluation started on the 7<sup>th</sup> day). Further storage (i.e. up to 21<sup>st</sup> day) did not influence the total sensory score of the resultant cheeses markedly (Table 2). The incorporation of *S. boulardii* as an adjunct culture in experimental cheeses (i.e. CBHM and C2) led to greater flavour impairment during storage when compared to control cheese (i.e. cheese C1) containing only yogurt starter.

The appearance, flavour, body and texture and overall acceptability score (each attribute out of 5.00) of Mozzarella cheese prepared using SC, assessed on baked pizza pie, were 5.00, 4.90, 4.60 and 4.70 respectively for freshly prepared products. The aforementioned pertinent scores were 2.80, 1.30, 1.30 and 1.00 as noted on the 10<sup>th</sup> day of refrigerated (7°C) storage (Singh and Goyal 2010).

### Changes in the Microbial Count of Cheeses as Influenced by Storage

The microbial count of cheeses is depicted in Table 3. The relevant findings relating to such aspects are discussed herein. Based on the plasticizing conditions adopted in preparing cheeses CUM (C1), CUMY (C2) and CBHM, the temperatures of the cheese mass during plasticizing were 63.6°C (for both C1 and C2) and 59.5°C respectively. The plasticizing conditions for the cheeses, in the same order as specified above, were 93.5°C for 4.5 min. (for C1, C2) and 79°C for 2.5 min. respectively.

#### *S. boulardii* count

The changes in the *S. boulardii* count of cheeses were significantly ( $p < 0.05$ ) affected by T, P and the interaction T x P. The experimental cheese CBHM had significantly ( $p < 0.05$ ) higher *S. boulardii* count compared to its counterpart control cheese (i.e. C2). This implied that the less severe plasticizing conditions employed during stretching of the cheese curd (79°C for 2.5 min.) and the lower pH (5.13) of cheese CBHM led to greater survivability of the inoculated *S. boulardii*, even during storage (Table 3, Figure 4). The salting rate was, however, kept constant at 1.75% by weight of cheese curd (plasticizing in hot moulding water followed) in both the cheese making protocols.

A progressive but significant ( $p < 0.05$ ) decline in the count of *S. boulardii* for all the cheeses was noted during refrigerated storage of up to 21 days; the changes in the *S. boulardii* count were marked ( $p < 0.05$ ) at each 7 days interval of storage, up to 21<sup>st</sup> day. In context to the interaction T x P, the highest and least counts of *S. boulardii* were noted in fresh cheese CBHM (i.e.

6.08 log<sub>10</sub> cfu/g) and 21 days aged control cheese CUMY (i.e. C2) (i.e. 2.46 log<sub>10</sub> cfu/g) respectively (Table 3).

The *Lactobacillus rhamnosus* GG (an adjunct culture) count of Fior di Latte cheese was 7.76 log<sub>10</sub> cfu/g when freshly prepared; the count decreased to 7.55 log<sub>10</sub> cfu/g on the 15<sup>th</sup> day of refrigerated (4°C) storage. A viability loss of 0.21 log<sub>10</sub> cfu/g was observed for *L. rhamnosus* GG after 15 days of vacuum storage at 4°C. As per their findings, adjusting the technological variables [i.e. acidification of the curd to pH 5.25 and keeping plasticizing conditions (81°C with contact period of 10 min.)] in the manufacture of Fior di Latte cheese enabled the *L. rhamnosus* GG population to exceed 7.5 log<sub>10</sub> cfu/g in the resultant cheese, till its use-by-date (Cuffia et al. 2017). Such count satisfied the requirement of probiotic food in such cheese.

Akarca and Yildirim (2022) prepared Pizza cheeses from cow and buffalo milks utilizing the *L. acidophilus* adjunct culture. The probiotic bacterial count increased during storage (initial count was 5.0 log<sub>10</sub> cfu/g). However, in the case of *Bifidobacterium lactis* subsp. *animalis*, the count showed a rise during the initial 14 days of refrigerated storage and subsequent storage led to a decrease in their count. Cow milk Pizza cheese tended to show higher count of probiotic bacteria (i.e. 5.00 log<sub>10</sub> cfu/g) compared to the counterpart buffalo product (i.e. 4.71 log<sub>10</sub> cfu/g).

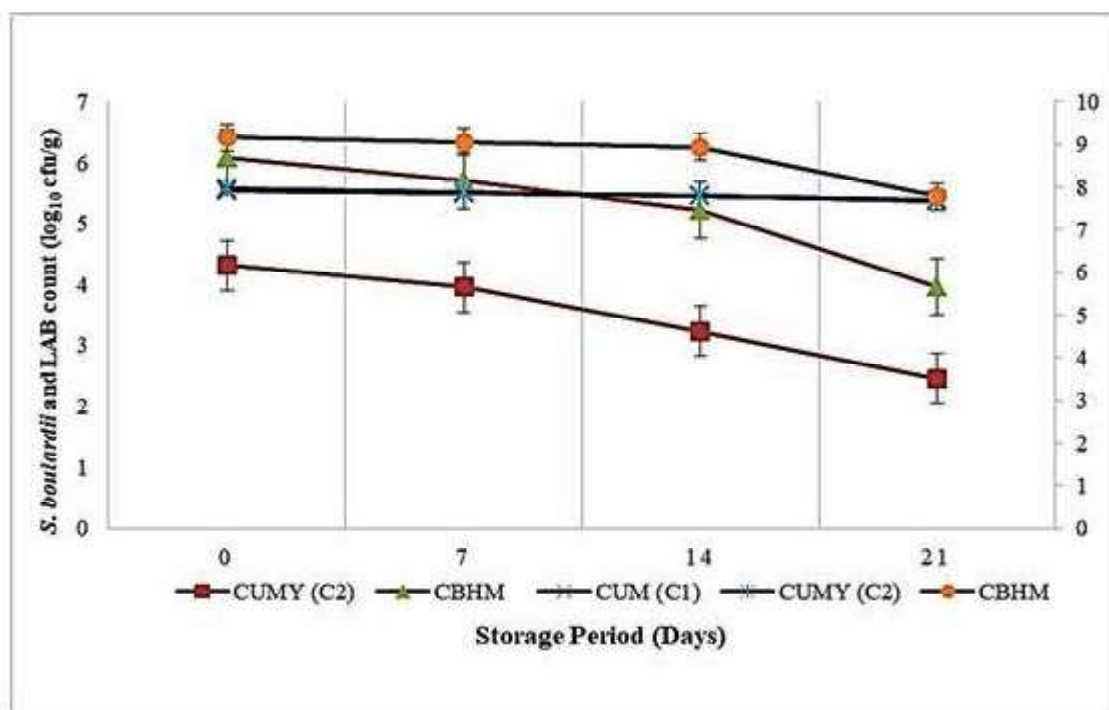
The probiotic *L. paracasei* ssp. *paracasei* LBC-1 count of freshly prepared Mozzarella cheese was 8.73 log<sub>10</sub> cfu/g; the count declined to 8.40 log<sub>10</sub> cfu/g on the 42<sup>nd</sup> day of refrigerated (4°C) storage (Ortakci et al. 2012).

**Table 3** *S. boulardii* and LAB count of Pizza cheeses during refrigerated storage (4°C)

| Cheeses             | Storage Period (Days)                               |                    |                    |                   | Mean<br>(Treatment) |
|---------------------|-----------------------------------------------------|--------------------|--------------------|-------------------|---------------------|
|                     | 0                                                   | 7                  | 14                 | 21                |                     |
|                     | <i>S. boulardii</i> Count (log <sub>10</sub> cfu/g) |                    |                    |                   |                     |
| CUMY (C2)           | 4.31 <sup>d</sup>                                   | 3.96 <sup>e</sup>  | 3.23 <sup>f</sup>  | 2.46 <sup>g</sup> | 3.48 <sup>b</sup>   |
| CBHM                | 6.08 <sup>a</sup>                                   | 5.70 <sup>b</sup>  | 5.22 <sup>c</sup>  | 3.96 <sup>c</sup> | 5.24 <sup>a</sup>   |
| Mean (Period)       | 5.20 <sup>a</sup>                                   | 4.83 <sup>b</sup>  | 4.23 <sup>c</sup>  | 3.21 <sup>d</sup> | ---                 |
| Source of Variation | Treatment (T)                                       |                    | Period (P)         |                   | T x P               |
| SEm±                | 0.008                                               |                    | 0.011              |                   | 0.016               |
|                     | LAB Count (log <sub>10</sub> cfu/g)                 |                    |                    |                   |                     |
| CUM (C1)            | 7.90 <sup>c</sup>                                   | 7.84 <sup>fg</sup> | 7.80 <sup>gh</sup> | 7.68 <sup>i</sup> | 7.81 <sup>b</sup>   |
| CUMY (C2)           | 7.96 <sup>d</sup>                                   | 7.88 <sup>ef</sup> | 7.80 <sup>gh</sup> | 7.70 <sup>i</sup> | 7.83 <sup>b</sup>   |
| CBHM                | 9.16 <sup>a</sup>                                   | 9.06 <sup>b</sup>  | 8.94 <sup>c</sup>  | 7.78 <sup>h</sup> | 8.74 <sup>a</sup>   |
| Mean (Period)       | 8.34 <sup>a</sup>                                   | 8.26 <sup>b</sup>  | 8.18 <sup>c</sup>  | 7.72 <sup>d</sup> | ---                 |
| Source of Variation | Treatment (T)                                       |                    | Period (P)         |                   | T x P               |
| SEm±                | 0.008                                               |                    | 0.010              |                   | 0.017               |

CUM (C1) – Cheese from unhomogenized milk; CUMY (C2) – Cheese from unhomogenized milk with *S. boulardii* culture; CBHM – Cheese from ‘milk blend’ comprising of homogenized and unhomogenized milks (1:1, w/w); Figures placed after ± indicates standard deviation, the values indicated row and column wise having differing superscripted alphabets differs significantly ( $p < 0.05$ ) from each other; n=4

**Fig. 4** *S. boulardii* and LAB count of Pizza cheeses during storage



#### LAB count

The changes in the LAB count of cheeses were significantly ( $p < 0.05$ ) affected by T, P, and interaction T x P. The experimental cheese CBHM had significantly ( $p < 0.05$ ) higher LAB count when compared with the count of other two control cheeses. The two control cheeses had LAB counts that were statistically at par ( $p > 0.05$ ) with each other. It is clearly evident that the use of adjunct *S. boulardii* culture helped in boosting the LAB count of Pizza cheese (Table 3). In addition to the probiotic count, the large number of LAB cells in fermented dairy products (including cheese) is beneficial to the well-being of humans (Staniszewski and Kordowska-Wiater 2021).

A progressive decrease in the LAB count of all the three cheeses was observed during storage for up to 21 days. Such a decrease in LAB count was significant ( $p < 0.05$ ) at each 7 days interval of storage, until the 21<sup>st</sup> day. The highest and least counts of LAB were noted for freshly prepared CBHM cheese (i.e.  $9.16 \log_{10}$  cfu/g) and 21 days aged C1 cheese (i.e.  $7.68 \log_{10}$  cfu/g) respectively (Table 3, Figure 4).

The LAB count of control and probiotic Pizza cheese containing *L. rhamnosus* GG was 9.23 and  $9.38 \log_{10}$  cfu/g respectively when freshly prepared; the respective counts were 9.14 and  $9.37 \log_{10}$  cfu/g on the 15<sup>th</sup> day of refrigerated (4°C) storage. Earlier during the manufacturing stage of Pizza cheese, post-plasticizing of cheese curd with hot water (82°C) for 10 min. contact period, the LAB count of both cheeses got reduced by  $0.16 \log_{10}$  cfu/g (Cuffia et al. 2017).

#### Coliform count

Coliform bacteria were absent in the freshly prepared and stored (up to 21<sup>st</sup> day) Pizza cheeses; control as well as in experimental cheese. This implied that the manufacturing protocol, handling of the product and packaging of cheeses were performed under strict hygienic conditions. Rajani (2021) did not detect any coliforms either in fresh cheese or in aged (21 days at 7°C temperature) Pizza cheeses made using SC method.

#### Conclusion

For the production of health-promoting Pizza cheese featuring the presence of *S. boulardii* as an adjunct starter, it is recommended that cheese makers follow the standardized cheesemaking process indicated for product CBHM. Cheese CBHM prepared using SC method bearing *S. boulardii* as an adjunct culture was preferred over cheese CUMY containing the adjunct culture in the same amount for being associated with higher count of both *S. boulardii* and LAB. The moisture content of cheese CBHM complied with the FSSR specification as well as performed satisfactorily in sensory aspects for their end-use application as a topping on pizza pie. The incorporation of *S. boulardii* as an adjunct starter led to an increased LAB count in the resultant Pizza cheese. Cheese CBHM registered counts of *S. boulardii* and LAB that was higher by 20.20% and 5.49% respectively when compared to the count associated with control cheese C2. Since refrigerated storage beyond 14 days resulted in perceptible decline in the count of *S. boulardii*, only 2 weeks of refrigerated storage has been recommended to the cheesemakers.

in order to reap the health benefits associated with consumption of such cheese containing probiotic microbes.

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## References

- Abd El-Gawad MA, Ahmed NS, El-Abd MM, El-Rafee SA (2012) Effect of homogenization on the properties and microstructure of Mozzarella cheese from buffalo milk. *Acta Sci Pol Technol Aliment* 11(2):121-135
- Ahmed NS, El-Gawad MA, El-Abd MM, Abd-Rabou NS (2011) Properties of buffalo Mozzarella cheese as affected by type of coagulant. *Acta Sci Pol Technol Aliment* 10(3):339-357
- Akarca G, Yildirim G (2022) Effects of the probiotic bacteria on the quality properties of Mozzarella cheese produced from different milk. *J Food Sci Technol* 59(9):3408-3418
- AOAC (2023) Official Methods of Analysis of AOAC International. Oxford University Press, UK
- Bihola A, Adil S, Kumar D (2024a) Mozzarella mastery: Exploring the factors influencing stretching characteristics. *Indian Food Ind Mag* 6(2):19-26
- Bihola A, Jana AH, Parmar SC, Adil S (2024b) Functionality of pizza cheese as affected using *Saccharomyces boulardii* adjunct culture during refrigerated storage. *Asian J Dairy Food Res* (In press)
- Bihola A, Jana AH, Parmar SC, Shaikh A (2024c) Feasibility study of utilizing *Saccharomyces boulardii* as an adjunct culture in Mozzarella-type cheese and its quality characterization. *Discov Food* 4:105
- Bihola A, Sharma H, Chaudhary MB, Bumbadiya MR, Kumar D, Shaikh A (2024d) Recent developments in cheese technologies. *Food Rev Int* 1-35
- BIS (1964) Specification for cheese BIS 2802. Bureau of Indian Standards, Manak Bhavan, New Delhi, pp14-15
- Collins YF, Mc Sweeney PL, Wilkinson MG (2003) Lipolysis and free fatty acid catabolism in cheese: a review of current knowledge. *Int Dairy J* 13(11):841-866
- Costabel L, Pauletti MS, Hynes E (2007) Proteolysis in Mozzarella cheeses manufactured by different industrial processes. *J Dairy Sci* 90(5):2103-2112
- Cuffia F, George G, Godoy L, Vinderola G, Reinheimer J, Burns P (2019). *In vivo* study of the immunomodulatory capacity and the impact of probiotic strains on physicochemical and sensory characteristics: Case of pasta filata soft cheeses. *Food Res Int* 125:108606
- Cuffia F, George G, Renzulli P, Reinheimer J, Meinardi C, Burns P (2017) Technological challenges in the production of a probiotic Pasta filata soft cheese. *LWT – Food Sci Technol* 81:111-117
- Felfoul I, Attia H, Bornaz S (2018) Shelf life determination of fresh cheese subjected to different modified atmospheres packaging. *J Agric Sci Technol* 19(7):847-860
- Huang X, Nzekoue FK, Renzi S, Alesi A, Coman MM, Pucciarelli S, ... Silvi S (2022) Influence of modified governing liquid on shelf-life parameters of high-moisture Mozzarella cheese. *Food Res Int* 159:111627
- ISO (1998) Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of mesophilic lactic acid bacteria – Colony-count technique at 30°C. ISO 15214:1998, Geneva, Switzerland
- Jana AH, Mandal PK (2011) Manufacturing and quality of Mozzarella cheese: A review. *Int J Dairy Sci* 6(4):199-226
- Jana AH, Tagalpallewar GP (2017) Functional properties of Mozzarella cheese for its end use application. *J Food Sci Technol* 54(12):3766-3778
- Kosikowski FV, Dahlberg AC (1946) A rapid direct-distillation method for determining the volatile fatty acids of cheese. *J Dairy Sci* 29(12):861-871
- Mamo A (2017) Cheddar cheese characterization and its biochemical change during ripening. *Int J Adv Sci Res Manag* 2(5):53-59
- Mc Sweeney, P.L.H., & Fox, P.F. (2004). Metabolism of residual lactose and of lactate and citrate. In P.F. Fox (ed.) *Cheese: chemistry, physics and microbiology* (pp. 361-371), Springer: Boston
- Niamah AK (2017) Physicochemical and microbial characteristics of yogurt with added *Saccharomyces boulardii*. *Curr Res Nutr Food Sci* 5(3):300-307
- Ortakci F, Broadbent, JR, McManus, WR, Mc Mahon DJ (2012) Survival of microencapsulated probiotic *Lactobacillus paracasei* LBC-1e during manufacture of Mozzarella cheese and simulated gastric digestion. *J Dairy Sci* 95(11):6274-6281
- Patel GC, Vyas SH, Upadhyay KG (1986) Evaluation of Mozzarella cheese made from buffalo milk using direct acidification technique. *Ind J Dairy Sci* 39(4):394-403
- Patel HR (2022) Quality improvement of Mozzarella cheese by admixing homogenized milk with unhomogenized milk. MTech Thesis, Kamdhenu University, Gandhinagar, Gujarat
- Rajani B, Jana AH, Bihola A, Parmar SC, Shaikh A (2024a) Process standardization and characterization of pizza cheeses prepared employing ‘dual acidification’ method. *J Food Sci Technol* (In press)
- Rajani B, Jana AH, Bihola A, Shaikh A (2024b) Changes in physico-chemical and functional properties of pizza cheeses made using ‘dual acidification’ method during refrigerated storage. *Discov Food* 4:157
- Rajani BM (2021) Pizza cheese making employing starter culture technique using GDL as an adjunct. MTech Thesis, Anand Agricultural University, Anand, Gujarat
- Singh P, Goyal GK (2010) Modified atmosphere packaging and storage on sensory characteristics of ready to bake pizza *Nutr Food Sci* 40(3):299-304
- Staniszewski A, Kordowska-Wiater M (2021) Probiotic and potentially probiotic yeasts – Characteristics and food application. *Foods* 10(6):1306
- Steel RGD, Torrie JH (1980) Analysis of Variance - I: The one-way classification. *Principles and Procedure of Statistics – A Biometrical Approach*, 2<sup>nd</sup> edn Mc Graw Hill, Kogakusha Ltd, Japan, pp 137-167
- Unique Biotech (2023) *Saccharomyces boulardii* Unique 28. Retrieved from <https://www.uniquebiotech.com/probiotic-strains/saccharomyces-boulardii>