Feasibility Study of Utilizing Saccharomyces boulardii as an Adjunct Culture in Mozzarella Cheese Making and Quality Characterization of Cheese

Ankit Bihola

ankitbihola2111@gmail.com

National Dairy Research Institute

Atanu H. Jana

Kamdhenu University

Satish C. Parmar

Kamdhenu University

Shaikh Adil

Parul University

Research Article

Keywords: Mozzarella cheese, Plasticizing, Saccharomyces boulardii, Cheddaring, Homogenization, Adjunct culture

Posted Date: March 12th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4032926/v1

License: Creative Commons Attribution 4.0 International License.

Additional Declarations: No competing interests reported.
Abstract

Research pertained to investigate influence of utilizing *Saccharomyces boulardii* as an adjunct culture at two levels on the quality aspects of Mozzarella cheese. Two protocols were employed: Protocol I using unhomogenized milk, adopting cheddaring of curd before plasticizing (i.e. CCUM) and Protocol II using blend of homogenized and unhomogenized milks (1:1; i.e. CBHM) to evaluate which method lends led to more *S. boulardii* count. Control cheese (CUM) was prepared from unhomogenized milk involving stretching of curd, post whey draining. Cheese CBHM$_{4.2}$ showed the highest yield. The textural properties of cheeses CCUM$_{3.5}$ and CCUM$_{4.2}$ as well as CBHM$_{3.5}$ and CBHM$_{4.2}$ were at par. CUM and CCUM cheeses had excellent baking properties (viz., shred, melt, fat leakage, stretch), while CBHM demonstrated desirable baking properties. Cheese made using lower level of starter adjunct received higher sensory scores on pizza pie. Using adjunct culture at higher usage level led to cheese having increased *S. boulardii* and Lactic Acid Bacteria count. It is recommended to adopt Protocol II in order to have *S. boulardii* count of ~$10^7$/g product since such method enabled use of milder plasticizing condition.

INTRODUCTION

Mozzarella cheese (particularly the Pizza cheese type, also known as low-moisture part-skim Mozzarella) is the favoured choice when it comes to topping a pizza. Traditionally, the production of Mozzarella cheese utilized the traditional technique of using a “starter culture”. There is no information available on the manufacture of Mozzarella cheese using *Saccharomyces boulardii* (proven probiotic yeast) as an adjunct culture. *Saccharomyces boulardii* is a well-known thermotolerant probiotic yeast that grows well at 37°C.

Various researchers have prepared Mozzarella cheese employing Starter Culture (SC) technique utilizing adjunct cultures such as *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, etc. [6, 18, 20]. To produce satisfactory quality probiotic *Pasta filata* cheese, it is imperative to carefully adjust the technological variables to enable use of lower degree of plasticizing condition. Cuffia et al. [6] adjusted the technological variables [i.e. acidication of the curd to pH 5.25 and keeping plasticizing conditions of 81°C with 10 min. contact period] to produce Fior di latte cheese (*Pasta filata* cheese similar to Mozzarella) having probiotic *Lactobacillus rhamnosus* GG count to the tune of 7.55 log$_{10}$ cfu/g.

Clinical trials by Unique Biotech revealed that *S. boulardii* (unique 28 strain) relieved the symptoms of diarrhoea and other intestinal problems, including traveller’s diarrhoea and Irritable Bowel Syndrome with constipation and prevented gastrointestinal infections [28]. *S. boulardii* has been incorporated in fermented (kefir, yoghurt, cheese, buttermilk) and non-fermented (ice cream) dairy products as well. When consumed at the recommended dose (minimum $10^7$ cfu/g or mL), *S. boulardii* conferred positive impact on the host. Improvement in the gut flora, immunological modulation, avoiding enteric infections, diarrhoea and inflammatory bowel disease are few documented advantages when consuming products containing *S. boulardii* [3, 26].
Hence, the research was conducted to arrive at technological means utilizing *Saccharomyces boulardii* as an adjunct culture in Mozzarella cheese making, keeping survival of such culture as an aim. The repercussions of utilizing *S. boulardii* on the quality characteristics (i.e. proximate composition, texture and baking properties, sensory and microbial quality) of Mozzarella cheeses and cheese yield were studied. The outcome of this research will generate information that will help the cheesemakers to produce Mozzarella cheese having desirable count of *S. boulardii* adjunct culture.

**MATERIALS AND METHODS**

**Materials**

Mixed (i.e. buffalo and cow) milk was procured from Vidya dairy, Anand. The milk was separated at Anubhav Dairy, Anand to obtain skim milk and cream; milk was standardized using skim milk. A fungal rennet (source *Rhizomucor miehei*, 2400 IMCU/g, was strength was obtained from M/s. Caglicio Clerici, Cadorago, Italy. *Saccharomyces boulardii* strain unique 28 was sourced from M/s. Unique Biotech, Hyderabad. Starter culture (i.e. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) was obtained from M/s. DSM, Netherlands. Calcium chloride dihydrate was procured from M/s. Loba Chemie Pvt. Ltd., Mumbai. Tata brand common salt (NaCl) was used.

**Equipment**

A Kenstar 3D Power OM-34ECR baking oven was used for pizza making. A 5.0 kW muffle furnace (Model No. EIE-500, Erection and Instrumentation Engineers, Ahmedabad) was used in ash determination. An Infrared Thermometer (model No. GIS-500, Bosch, Bengaluru) sensed the temperature of the cheese mass.

**Mozzarella Cheese Making**

Mozzarella cheeses were prepared from standardized (3.2% milk fat), pasteurized (72°C/no hold) mixed milk employing two cheese making protocols. Protocol I involved following the process standardized by Ghosh and Singh [7] employing starter culture (SC) involving cheddaring of cheese curd prior to its plasticizing (cheese designated as CCUM). Cheese making protocol II involved the use of a ‘milk blend’ [i.e. unhomogenized and homogenized (1.96 and 0.98 MPa pressure, 65°C) milks – 1:1, w/w] standardized to 3.2% milk fat and following the SC method of Patel [22] (cheese designated as CBHM). *Saccharomyces boulardii* was used as an adjunct culture at two usage levels viz., 3.5 and 4.2 g/100 kg milk.

Figure 1

Control Mozzarella cheese was prepared from standardized (3.2% milk fat), pasteurized (72°C/15 s) mixed milk as per the process of Patel et al. [21] employing SC method involving whey draining (omitting cheddaring step) prior to the cheese curd plasticizing stage.
Analyses

The Mozzarella cheeses were analyzed for fat, protein, moisture, ash, salt, pH and titratable acidity (TA). The moisture, fat, salt content and TA were determined employing standard procedures [4]. The fat content of cheese also expressed as fat-on-dry matter (FDM). The protein content of cheese was determined using Kjeldahl method employing Kjel-Vac digestion system and Kjel-Plus distillation system (M/s. Pelican Instruments, Chennai) [4]. The ash content of cheese was determined using standard procedure [5]. The pH values of cheese slurry made in distilled water were recorded using a digital pH meter (M/s. Mettler Toledo AG, Schwerzenbach). The LAB and *S. boulardii* count of Mozzarella cheese were determined following the methods of ISO [9] and Niamah et al. [19] respectively.

Yield of cheese

The yield of Mozzarella cheese was calculated as kg cheese obtained from 100.0 kg standardized cheese milk.

Texture profile analysis

Food Texture Analyzer (M/s. Lloyd Instruments, LRX Plus, England, Sr. No. 160374) with a 50.0 Newton (N) load cell was used in compression analysis of cheese; speed was 20 mm/min. The trigger was set to 10 gf (1 gf = 0.00980665 N). The cheese samples were tempered at 23 ± 1°C for an hour in an air-conditioned room (23 ± 1°C, 55.0 ± 1.0% RH) and then subjected to texture evaluation. The cubic cheese samples (edges of 1.00 ± 0.06 cm) were compressed up to 70.0% of their initial size. For each cheese, seven cubic samples were subjected to texture analysis and the average of these readings was considered.

Baking characteristics of cheese

The shredability of cheese was assessed subjectively by manual shredding through a 2.0 mm stainless steel shredder. Meltability and fat leakage were determined by the procedure described by Rajani et al. [23]. The stretch length of pizza cheese was tested using the ‘Fork Test’ [7] with a 4-pronged fork.

Sensory evaluation of cheese as a pizza topping

The baked pizza, retrieved from the baking oven, were subjected to sensory evaluation by a panel of minimum eight judges [13]. A 10-point score card was used involving five sensory attributes viz., appearance (involving colour, melting, fat leakage and browning), flavour, melting, stringiness and chewiness; the total sensory score was out of 50.0. The triangular cut pieces of pizza, topped with melted cheese, were served to the judges in hot (≥ 80°C) condition. All the judges were well aware of the characteristics of Pizza cheese suited for pizza application.

Statistical Analysis
A factorial completely randomized design (FCRD) was applied to evaluate the findings obtained in the investigation. The averages of the results of the investigation of duplicate samples of pizza cheese, collected in four separate replications for three treatments were examined for statistical examination using IBM SPSS Statistics software (Version 27, IBM Corp., USA).

RESULTS AND DISCUSSION

Temperature of plasticized cheese mass

It is clearly understood that the use of the cheddaring step led to Mozzarella cheese having a lower pH (i.e. stretching of the curd at 0.75% LA) which led to more dissolution of colloidal calcium phosphate, making the curd prone to plasticizing at lower temperatures [11]. In the case of cheese made from blended milk (unhomogenized: homogenized, 1:1 w/w), the homogenized milk portion has a greater proportion of protein adsorbed onto the increased fat surface area. Moreover, the pH of curd at stretching (i.e. whey acidity at stretching was 0.44% LA) was lower than for control cheese [10]. Both of these factors led to cheese curds getting plasticized at a much lower temperature.

Based on the plasticizing conditions adopted in preparing cheeses CUM, CCUM and CBHM, the temperature of the plasticized cheese mass was 63.6°C, 61.5°C and 59.5°C respectively. The plasticizing conditions for the three cheeses, in the same order as specified above, were 93.5°C for 4.5 min., 84.5°C for 2.5 min. and 79.0°C for 2.5 min. respectively.

The influence of varying the level of *S. boulardii* (@ 3.5 and 4.2 g/100 kg milk) as an adjunct culture in Mozzarella cheese making on the proximate composition, physico-chemical characteristics, yield, textural attributes, baking properties and sensory quality of cheese (i.e. as pizza topping) was studied. Any change in the cheese making protocol (i.e. use of adjunct culture) can affect the composition and even physico-chemical properties of the resultant product. The consumer preference of Mozzarella cheese is based on its meltability, elasticity, stretchability and fat leakage. Of these, elasticity, stretchability and meltability are related to textural properties. Stretch character is one significant aspect of this cheese variety, especially for its end use application [12].

EFFECTS ON THE PROXIMATE COMPOSITION, PHYSICO-CHEMICAL CHARACTERISTICS AND YIELD OF CHEESE

The proximate composition, physico-chemical characteristics and yield of Mozzarella cheeses as affected by the two inoculum levels of *S. boulardii* are shown in Table 1. These two levels were decided based on several preliminary trials (below and above such usage levels). Each cheese was assigned an abbreviated code as shown below.

**Designated code of Mozzarella cheeses**
Cheese making protocol employing SC method, with or without *S. boulardii* as adjunct | Designated code for cheese
---|---
Cheese from unhomogenized milk (control) following method of Patel et al. [21] | CUM
Cheese from unhomogenized milk involving cheddaring of cheese curd as per the method of Ghosh and Singh [7] | CCUM
Cheese from ‘milk blend’ (homogenized: unhomogenized milk, 1:1, w/w) using modified method as per Patel [22] | CBHM

The fat content of cheeses CUM and CCUM\textsubscript{3.5}, CCUM\textsubscript{3.5} and CCUM\textsubscript{4.2} as well as CBHM\textsubscript{3.5} and CBHM\textsubscript{4.2} was at par with each other. The moisture content of cheese CCUM\textsubscript{4.2} was significantly (p < 0.05) higher (i.e. 48.98%) than the value associated with cheese CCUM\textsubscript{3.5}; however, former cheese had a moisture content at par with that of cheese CUM. Except for cheese CBHM\textsubscript{3.5}, product CBHM\textsubscript{4.2} had significantly (P < 0.05) higher moisture (i.e. 52.58%) content when compared to that of CUM and two CCUM cheeses. The FDM content of cheese CBHM\textsubscript{4.2} (i.e. 47.13%) was at par with cheese CBHM\textsubscript{3.5}; the former cheeses had significantly (p < 0.05) higher FDM content compared to the CUM and two CCUM cheeses. The cheeses CCUM\textsubscript{3.5} and CCUM\textsubscript{4.2} as well as CBHM\textsubscript{3.5} and CBHM\textsubscript{4.2} had similar values of protein content; cheese CUM had an intermediate value for protein content (i.e. 22.89%). The higher and lower values of ash content were associated with cheeses CCUM\textsubscript{3.5} and CBHM\textsubscript{4.2} respectively. The cheeses CUM and CCUM\textsubscript{4.2} as well as CCUM\textsubscript{3.5}, CBHM\textsubscript{3.5} and CBHM\textsubscript{4.2} had similar values for salt content. The TA and pH of all cheeses differed significantly (p < 0.05) from each other, except for the acidity values of cheeses CCUM\textsubscript{3.5} and CCUM\textsubscript{4.2} was at par with each other. The maximum TA value was associated with cheese CBHM\textsubscript{4.2} (i.e. 0.68% LA). The maximum yield noted for cheese CBHM\textsubscript{4.2} (i.e. 12.83%) was significantly (p < 0.05) higher than the yield of the CUM cheese and two CCUM cheeses; cheese CBHM\textsubscript{3.5} had a similar yield (Table 1).
Table 1
Proximate composition, physico-chemical characteristics and yield of Mozzarella cheeses as influenced by the inclusion of adjunct culture at two levels

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mozzarella cheeses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CUM</td>
</tr>
<tr>
<td>Proximate composition</td>
<td></td>
</tr>
<tr>
<td>Moisture, %</td>
<td>48.59\textsuperscript{b} ± 0.38</td>
</tr>
<tr>
<td>Fat, %</td>
<td>23.10\textsuperscript{a} ± 0.14</td>
</tr>
<tr>
<td>FDM\textsuperscript{1}, %</td>
<td>44.93\textsuperscript{c} ± 0.40</td>
</tr>
<tr>
<td>Protein, %</td>
<td>22.89\textsuperscript{b} ± 0.21</td>
</tr>
<tr>
<td>Salt, %</td>
<td>1.03\textsuperscript{a} ± 0.03</td>
</tr>
<tr>
<td>Ash, %</td>
<td>2.34\textsuperscript{a} ± 0.06</td>
</tr>
<tr>
<td>Physico-chemical properties</td>
<td></td>
</tr>
<tr>
<td>Acidity, %</td>
<td>0.48\textsuperscript{d} ± 0.02</td>
</tr>
<tr>
<td>pH</td>
<td>5.29\textsuperscript{a} ± 0.01</td>
</tr>
<tr>
<td>Yield</td>
<td></td>
</tr>
<tr>
<td>kg cheese/100 kg milk</td>
<td>12.08\textsuperscript{b} ± 0.27</td>
</tr>
</tbody>
</table>

The subscripts 3.5 and 4.2 represents two usage rates (g/100 kg milk) of \textit{S. boulardii}; CUM = Cheese from un-homogenized milk (control); CCUM, CBHM = Cheeses employing Protocol I and II respectively; Figures after ± indicates standard deviation, \textsuperscript{1} - Fat-on-dry matter; the values indicated row wise having differing superscripted alphabets differs significantly (p < 0.05) from each other; \textit{n} = 5

The Mozzarella cheese made from a milk blend [unhomogenized: homogenized (2.6 MPa) milk; 1:1, w/w] had higher moisture (i.e. 49.6%) and FDM (i.e. 47.60%), but lower protein content (i.e. 21.6%) compared to the control cheese made from untreated milk; the latter cheese had 45.8% moisture, 23.7% protein and 45.80% FDM [24]. Abd El-Gawad et al. [1] reported an increase in the moisture (61.52 vs. 54.78% for control), FDM (38.20 vs. 37.88% for control) and acidity (0.67 vs. 0.64% LA for control) of Mozzarella cheese when made from homogenized milk; the protein content of such cheese was lower (19.02 vs. 23.26% for control) than that of control cheese.
Jana et al. [14] reported the proximate composition of Mozzarella cheeses made from homogenized (2.45 and 0.98 MPa pressure in 2-stage homogenizer) and un-homogenized milks; both made from standardized milk (3% fat). The respective values (%) for such cheeses were 51.57 and 48.94 for moisture, 50.37 and 48.63 for FDM, 21.43 and 23.37 for protein; the TA was 0.362 and 0.344% LA, while the pH of the cheeses was 5.51 and 5.55 respectively.

The yield of Mozzarella cheese was markedly higher (i.e. 11.24 vs. 10.20% for control from unhomogenized milk) when made from homogenized (2.45 and 0.98 MPa pressure) milk; both cheeses were made from milk standardized to 3.0% fat [14].

**EFFECTS ON THE TEXTURAL PROPERTIES OF CHEESE**

Texture is an indispensable factor in determining cheese quality, ultimately affecting its functional characteristics during end-use application. Cheese texture is regarded as an important factor that impacts the overall sensory acceptance of the product. Numerous studies have concluded that the instrumental texture profile correlates well with the sensory score relating to texture [17].

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mozzarella cheeses</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CUM</td>
<td>CCUM_3.5</td>
<td>CBHM_3.5</td>
<td>CCUM_4.2</td>
<td>CBHM_4.2</td>
</tr>
<tr>
<td>Hardness (N)</td>
<td>19.54^b ± 0.63</td>
<td>21.80^a ± 1.12</td>
<td>16.96^c ± 0.42</td>
<td>21.22^a ± 1.33</td>
<td>16.53^c ± 0.83</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>0.237^b ± 0.007</td>
<td>0.258^a ± 0.012</td>
<td>0.226^b ± 0.008</td>
<td>0.262^a ± 0.027</td>
<td>0.222^b ± 0.010</td>
</tr>
<tr>
<td>Springiness (mm)</td>
<td>4.50^bc ± 0.10</td>
<td>4.77^ab ± 0.08</td>
<td>4.27^c ± 0.15</td>
<td>4.84^a ± 0.43</td>
<td>4.26^c ± 0.29</td>
</tr>
<tr>
<td>Gumminess (N)</td>
<td>462.39^b ± 22.18</td>
<td>562.07^a ± 22.11</td>
<td>382.50^c ± 16.10</td>
<td>553.94^a ± 33.75</td>
<td>367.27^c ± 34.59</td>
</tr>
<tr>
<td>Chewiness (N-mm)</td>
<td>20.82^b ± 1.08</td>
<td>26.78^a ± 1.00</td>
<td>16.34^c ± 1.13</td>
<td>26.75^a ± 1.99</td>
<td>15.62^c ± 1.36</td>
</tr>
<tr>
<td>Adhesiveness (N-mm)</td>
<td>0.194^c ± 0.007</td>
<td>0.312^b ± 0.007</td>
<td>0.429^a ± 0.005</td>
<td>0.305^b ± 0.014</td>
<td>0.423^a ± 0.014</td>
</tr>
</tbody>
</table>

The subscripts 3.5 and 4.2 represents the usage rates (g/100 kg milk) of *S. boulardii*; CUM = Cheese from unhomogenized milk (control); CCUM, CBHM = Cheeses employing Protocol I and II respectively; Figures placed after ± indicates standard deviation; Values indicated row wise having differing superscripted alphabets differs significantly (p < 0.05) from each other; n = 5

Table 2

An increase in the values of textural attributes up to a certain level (viz., hardness, cohesiveness, springiness) of Mozzarella cheese is construed to be a positive feature; excessive adhesiveness and
chewiness are undesirable. A firm cheese would contribute to the desired shred quality, while the springiness of cheese is usually construed to relate to elasticity [12].

The textural properties of Mozzarella cheeses as affected by the two inoculum levels of *S. boulardii* are shown in Table 2. All the textural attributes of the cheeses CCUM$_{3.5}$ and CCUM$_{4.2}$ as well as CBHM$_{3.5}$ and CBHM$_{4.2}$ were at par with each other. Cheese CUM had textural values that lie intermediate within the values shown by cheeses CCUM and CBHM, except for adhesiveness. The adhesiveness value of such cheese was the least (i.e. 0.194 N·mm). Cheese CBHM$_{4.2}$ exhibited least values of literally all of the textural properties, except for adhesiveness (Table 2). This implied that the textural properties of Mozzarella cheeses prepared using a specific manufacturing protocol (i.e. Protocol I or Protocol II) remained unaffected by the level of adjunct culture.

Jana and Upadhyay [10] reported textural properties of Mozzarella cheese made from unhomogenized and homogenized (2.45 MPa pressure) milks; the respective values were 3.05 and 2.61 kg for hardness, 5.30 and 5.01 mm for springiness and 0.475 and 0.337 for cohesiveness. The authors noted lower values for all these textural attributes in Mozzarella cheese made from homogenized milk.

Kwak et al. [15] reported a reduction in the hardness (2.20 vs. 5.46 kg for control) and an increase in the cohesiveness (1.05 vs. 0.96 for control) of Mozzarella cheese when made from milk (3.5% fat) homogenized at 6.86 MPa pressure; control cheese was prepared from unhomogenized milk.

**EFFECTS OF PRESENCE OR ABSENCE OF ADJUNCT STARTER ON THE BAKING CHARACTERISTICS OF CHEESE**

The baking properties of cheese studied were shredability, melt, fat leakage and stretch. The baking characteristics of Mozzarella cheeses as affected by the cheesemaking protocols (protocol I and II) and the two levels of adjunct culture are shown in Table 3.
Table 3

<table>
<thead>
<tr>
<th>Baking characteristics</th>
<th>Mozzarella cheeses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CUM</td>
</tr>
<tr>
<td>Shredability</td>
<td>Excellent</td>
</tr>
<tr>
<td>Melting time in oven at 230°C (s)</td>
<td>419.00&lt;sup&gt;c&lt;/sup&gt; ± 8.94</td>
</tr>
<tr>
<td>Schreiber meltability (Arbitrary value)</td>
<td>4.17&lt;sup&gt;a&lt;/sup&gt; ± 0.12</td>
</tr>
<tr>
<td>Fat leakage (cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>5.59&lt;sup&gt;a&lt;/sup&gt; ± 0.67</td>
</tr>
<tr>
<td>Stretch value (cm)</td>
<td>50.20&lt;sup&gt;b&lt;/sup&gt; ± 1.30</td>
</tr>
</tbody>
</table>

The subscripts 3.5 and 4.2 represent the usage rates (g/100 kg milk) of <i>S. boulardii</i>; CUM = Cheese from unhomogenized milk (control); CCUM, CBHM = Cheeses employing Protocol I and II respectively; Figures placed after ± indicates standard deviation; Values indicated row wise having differing superscripted alphabets differs significantly (p < 0.05) from each other; n = 5

Table 3

**Shredability**

To facilitate melting of cheese on pizza pie, the cheese needs to be sliced, diced, or shredded into separate entities. Hence, shredability and resistance to clumping, post shredding are of significance [12]. The cheese should be easy to shred into thin and long shreds.

Shredded cheeses are classified in some categories viz., ‘Excellent’, ‘Very Good’, ‘Good’ and ‘Fair’. ‘Excellent’ shredded quality is for cheese that enables ease of shred sand form thin, long shreds, without any tendency to mat with each other. ‘Good’ category denotes cheese that offers some resistance to shred and forms short and thick shreds with slight matting tendency. The cheeses that behaved midway between the previous two categories were categorized as ‘Very Good’. Cheese posing difficulty in shredding and showing more tendency to mat after shredding was categorized as ‘Fair’. Mozzarella cheeses possessing requisite firmness yielded a greater proportion of longer shreds [12].

The shredability of the CUM and two CCUM cheeses was graded as ‘Excellent’ while the other two CBHM cheeses received ‘Very good’ grade (Table 3). The shred quality of Mozzarella cheeses made from homogenized (2.45 and 0.98 MPa pressure) and unhomogenized milks were categorized as ‘Fair’ and ‘Very Good’ respectively [14].
'Meltability' is the ability of cheese shreds to coalesce to a uniform, continuous layer when subjected to the heat of baking oven; ease of melt is desirable [12]. Cheese meltability occurs as a consequence of disintegration of the protein matrix upon heating, with consequent melting of entrapped fat and weakening of protein-protein linkages paving the way to ‘flow’ of the product [16]. Delayed melting of cheese leads to undesirable blistering or browning [12].

The Schreiber meltability values of the two CCUM and CBHM cheeses were at par with each other; the meltability of CUM cheese was also at par with two CCUM cheeses. The melting time of all cheeses differed significantly (p < 0.05) from each other, except when comparing cheeses CUM and CCUM. The Schreiber meltability value of cheeses tended to improve when a higher usage level of S. boulardii (i.e. 4.2 g/100 kg milk) was used in Mozzarella cheese making (Table 3).

Tunick et al. [27] reported an impairment in the meltability (i.e. 1.00 vs. 2.60 for control, expressed as arbitrary value) of Mozzarella cheese made from homogenized milk (6.87 and 3.43 MPa pressure in 2-stages) compared to that made from an unhomogenized milk counterpart.

Mozzarella cheeses prepared from homogenized (2.45 and 0.98 MPa pressure) milk exhibited lower meltability; the Schreiber meltability values (as arbitrary value) of such cheeses were 3.66 and 6.07 when made from homogenized and unhomogenized milks respectively [14].

**Fat leakage**

The tendency of liquid fat to separate from the melted cheese and forming oil pockets at the surface of baked cheese is termed ‘oiling-off’ or ‘free oil formation’. Moderate quantum of free fat is desirable in Mozzarella cheese, excessive free fat detracts from its appearance, palatability and crispness [25].

The fat leakage values of cheeses made using a similar cheesemaking protocol (i.e. CCUM and CBHM, dealt solely) were at par with each other. The fat leakage values of the two CCUM cheeses were at par with the fat leakage value of cheese CUM (Table 3).

Rowney et al. [24] reported that fat leakage values of Mozzarella cheeses made from unhomogenized milk and blended milk [homogenized (2.6 MPa pressure): unhomogenized, 1:1 w/w] were 9.0 and 5.5 respectively, expressed as per cent of cheese.

The reduction in the fat leakage of Mozzarella cheeses was noted when made from homogenized (2.45 and 0.98 MPa pressure) milk; the fat leakage values were 3.26 and 5.61 cm$^2$ for cheeses made from milk homogenized and unhomogenized milks respectively [14].

**Stretch length**

The ability of melted Mozzarella cheese to form long, thin strands that do not break easily when stretched is known as stretchability. According to United States Department of Agriculture, Mozzarella cheese should stretch to minimum 3.0 inches (7.62 cm) of continuous strand measured from top surface of pizza base [29].
The stretch length of Mozzarella cheeses prepared employing similar cheese making protocols were at par with each other. Cheese CUM exhibited a stretch length that was intermediate between the stretch lengths exhibited by cheeses CCUM and CBHM (Table 3).

The stretch length of control cheese made from unhomogenized milk was > 30.0 cm, whereas the homogenized (pressures ranging from 5.14 to 8.92 MPa) milk cheeses exhibited stretch values ranging between 5.0 and 10.0 cm [15].

The stretch length, determined by the ‘Fork test’, of Mozzarella cheeses made from unhomogenized and homogenized (2.45 and 0.98 MPa pressure) milks were reported to be 24.10 and 25.10 cm respectively [14].

**SENSORY SCORES OF CHEESES EVALUATED AS PIZZA TOPPING**

Since Mozzarella cheese is consumed preferentially as a pizza topping, the sensory quality of the heated cheese (in melted state) on pizza pie was assessed with regard to appearance (i.e. colour, uniformity of cheese melt, fat leakage), flavour, melting, stringiness, and chewiness. Cheese exhibiting glossiness, uniform melting and limited fat leakage is desirable. The stretch character in cheese and moderate degree of chewiness are desirable [12].
Table 4
Sensory scores of Mozzarella cheeses evaluated as pizza topping as affected by cheese manufacturing protocols and usage levels of *S. boulardii*

<table>
<thead>
<tr>
<th>Sensory attributes</th>
<th>Mozzarella cheeses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CUM</td>
</tr>
<tr>
<td>Appearance (Max. 10)</td>
<td>8.13 ± 0.36</td>
</tr>
<tr>
<td>Flavour (Max. 10)</td>
<td>8.04&lt;sup&gt;a&lt;/sup&gt; ± 0.26</td>
</tr>
<tr>
<td>Melting (Max. 10)</td>
<td>8.14&lt;sup&gt;a&lt;/sup&gt; ± 0.22</td>
</tr>
<tr>
<td>Stringiness (Max. 10)</td>
<td>8.28&lt;sup&gt;a&lt;/sup&gt; ± 0.23</td>
</tr>
<tr>
<td>Chewiness (Max. 10)</td>
<td>7.70&lt;sup&gt;b&lt;/sup&gt; ± 0.12</td>
</tr>
<tr>
<td>Total score (Max. 50)</td>
<td>40.29&lt;sup&gt;a&lt;/sup&gt; ± 0.73</td>
</tr>
</tbody>
</table>

The subscripts 3.5 and 4.2 represents the usage rates (g/100 kg milk) of *S. boulardii*; CUM = Cheese from unhomogenized milk (control); CCUM, CBHM = Cheeses employing Protocol I and II respectively; Figures placed after ± indicates standard deviation; Values indicated row wise having differing superscripted alphabets differs significantly (p < 0.05) from each other; n = 5

Cheeses CCUM<sub>3.5</sub> and CCUM<sub>4.2</sub> were rated at par with each other with regard to appearance, melting and chewiness attributes. Both CBHM cheeses had superior chewiness scores as compared to the two counterpart CCUM cheeses. The total sensory scores of the two Mozzarella cheeses using a lower level of adjunct starter employing two cheese making protocols (i.e. CCUM and CBHM) were seemingly superior over the pertinent scores of the Mozzarella cheeses incorporating a higher usage level of adjunct starter (Table 4). The cheese CUM had similar total sensory scores as the cheese CCUM<sub>3.5</sub>; such scores were significantly (p < 0.05) higher than the rest of the three cheeses.

The appearance, melting and chewiness scores of Mozzarella cheeses, judged as pizza topping, made from unhomogenized milk and homogenized (2.45 and 0.98 MPa pressure) milk were 8.60 and 7.98; 8.89 and 8.36, and 7.85 and 8.48 respectively [14].

**MICROBIAL QUALITY OF MOZZARELLA CHEESES**

The microbial count [i.e. Lactic acid bacteria (LAB) and *S. boulardii* count] of Mozzarella cheeses as influenced by two levels of *S. boulardii* are shown in Table 5.
Table 5

<table>
<thead>
<tr>
<th>Microbial count (log_{10} cfu/g)</th>
<th>Mozzarella cheeses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CUM</td>
</tr>
<tr>
<td>Lactic Acid Bacteria</td>
<td>8.30\textsuperscript{d} ± 0.04</td>
</tr>
<tr>
<td>S. boulardii</td>
<td>-</td>
</tr>
</tbody>
</table>

The subscripts 3.5 and 4.2 represents the usage rates (g/100 kg milk) of \textit{S. boulardii}; CUM = Cheese from unhomogenized milk (control); CCUM, CBHM = Cheeses employing Protocol I and II respectively; Figures placed after ± indicates standard deviation; Values indicated row wise having differing superscripted alphabets differs significantly (p < 0.05) from each other; n = 5

Table 5

The \textit{S. boulardii} count of the experimental cheeses was significantly (p < 0.05) higher when the usage level of such culture was raised from 3.5 to 4.2 g/100 kg milk. The maximum and minimum count of \textit{S. boulardii} were associated with cheeses CBHM\textsubscript{4.2} (i.e. 6.16 log\textsubscript{10} cfu/g) and CCUM\textsubscript{3.5} (i.e. 4.91 log\textsubscript{10} cfu/g) respectively. However, considering the sensory quality of cheese, cheese CBHM\textsubscript{3.5} was recommended. The LAB count of the experimental cheeses was significantly (p < 0.05) influenced by the presence of \textit{S. boulardii} adjunct culture. The maximum and minimum LAB count were associated with cheeses CBHM\textsubscript{4.2} (i.e. 9.31 log\textsubscript{10} cfu/g) and CCUM\textsubscript{3.5} (i.e. 8.40 log\textsubscript{10} cfu/g) respectively. The LAB count of cheese CBHM reached 9.31 log cfu/g when \textit{S. boulardii} was used @ 4.2 g/100 kg milk; the count of LAB was 9.02 log cfu/g at \textit{S. boulardii} usage rate of 3.5 g/100 kg milk (Table 5). The lowest LAB count (i.e. 8.3 log\textsubscript{10} cfu/g) was associated with control cheese CUM. This clearly indicates that the presence of \textit{S. boulardii} had a positive influence on the growth of LAB used as starter culture in the preparation of Mozzarella cheese.

Since \textit{S. boulardii} is a probiotic culture, a count exceeding 10\textsuperscript{6} cfu/g of cheese would have been ideal. Nevertheless, work needs to be carried out to ascertain whether the \textit{S. boulardii} count in Mozzarella cheese at ~ 10\textsuperscript{5} cfu/g can confer beneficial effects on human health.

According to Akarca et al. [2], the LAB count of control and experimental (\textit{L. rhamnosus} as an adjunct) bovine milk Mozzarella cheeses were 5.13 and 6.14 log\textsubscript{10} cfu/g respectively. In their study, the \textit{Lactococcus} counts of bovine milk cheeses were 5.79 and 5.26 log\textsubscript{10} cfu/g respectively.

The LAB count of freshly prepared control and experimental Fior di Latte (similar to high-moisture Mozzarella) cheese made utilizing \textit{Lactobacillus rhamnosus GG} was 9.23 and 9.38 log\textsubscript{10}cfu/g respectively. The LAB count of control as well as experimental cheese decreased (i.e. 0.16 log\textsubscript{10} cfu/g) upon stretching of the cheese curd for 10 min. in moulding water. The \textit{Lactobacillus rhamnosus GG} count of experimental cheese was 7.76 log\textsubscript{10} cfu/g [6].
The probiotic *Lactobacillus paracasei* ssp. *paracasei* LBC-1 count of fresh Mozzarella cheese was $5.37 \times 10^8 /g$ [20]. *L. acidophilus* cells encapsulated in sodium alginate media survived better ($3.41 \times 10^8$ cfu/g) in Mozzarella cheese than free cells ($1.10 \times 10^7$ cfu/g cheese) [18].

Similar to our findings, the LAB count of cow milk Scamorza cheese (similar to low-moisture Mozzarella cheese) utilizing an adjunct culture (i.e. *Lactococcus lactis, Lactobacillus paracasei* and *Lactobacillus helveticus*) was higher ($9.0 \log_{10}$ cfu/g) than that of control cheese (i.e. $8.6 \log_{10}$ cfu/g) (Guidone et al. 2015). In addition, the *Bifidobacteria bifidum* of probiotic Mozzarella cheese prepared using whey protein concentrate (added at a rate of 1.5%) was reported to be $6.94 \log$ cfu/g [8].

**CONCLUSIONS**

In order to produce health-promoting Mozzarella cheese involving the use of the probiotic culture *S. boulardii* as an adjunct starter, it is recommended that cheese makers adopt a specific cheese making protocol that affords better viability to such culture. Such cheese making protocol involves the use of a ‘milk blend’ (i.e. unhomogenized and homogenized (1.96 and 0.98 MPa pressure) as starting material, inoculating milk with *S. boulardii* @ 3.5 g/100 kg milk in addition to yoghurt culture and plasticizing the cheese curd using hot water of 80°C and holding period of 3 min. (i.e. CBHM). Cheese CBHM$_{3.5}$ had a higher count of both *S. boulardii* and LAB compared to cheese made using another protocol involving cheddaring of cheese curd (i.e. CCUM); the count of *S. boulardii* was somewhat below the level recommended for probiotic cultured dairy products. The recommended cheese CBHM$_{3.5}$ conformed to the compositional standard prescribed for Mozzarella cheese. Such cheese exhibited the desired baking properties and had acceptable sensory scores suitable for their end use application on pizza pie. Further research is warranted in this direction, utilizing encapsulated probiotic culture or using heat-tolerant strain of probiotic culture to achieve the required count of probiotic yeast, post plasticizing step in Mozzarella cheese making.

**Abbreviations**

CBHM – Cheese from ‘milk blend’ comprising homogenized and unhomogenized milks (1:1, w/w)

CCUM – Cheese from unhomogenized milk following the cheddaring step prior to plasticizing operation

CRD – Completely randomized design

FDM – Fat-on-Dry Matter

IMCU – International Milk Clotting Units

LAB – Lactic Acid Bacteria

SC – Starter Culture
Declarations

Acknowledgements
Authors are highly thankful to **Dr. N. Jayanthi**, Head – Scientific Affairs, Unique Biotech, Hyderabad and **Mr. Pravin Singh**, Key Account Manager, DSM Food Specialties Ltd., Anand for providing ‘Saccharomyces boulardii unique 28 strain’ and ‘Delvo DSL Direct Set Lyophilized Starter Cultures RST-776’ respectively for the research work.

Funding
Funding was not availed in conducting the research.

ETHICS DECLARATIONS

Conflicts of interest/Competing interests
The authors declare that they have no conflicts of interest/no competing interests.

Ethics approval
Not applicable

Consent to participate
Not applicable

Consent for publication
All authors agree to publish.

Availability of data and material
It is hereby acknowledged that the authors will make all data available.

Code availability
Not applicable

Authors contributions

**Ankit Bihola**: Investigation, Methodology, Writing – Original draft

**Atanu H. Jana**: Conceptualization, Supervision, Writing – Review and editing
Satish C. Parmar: Data analysis, Methodology, Validation

Shaikh Adil: Data curation, Statistical analysis

References


Figures
Figure 1

Mozzarella cheese making by starter culture method using adjunct *S. boulardii*