Influence of salt levels on the quality characteristics and microbial count of Pizza cheese prepared employing a starter culture technique through technological interventions using *Saccharomyces boulardii* as an adjunct

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Abstract

Research was carried out to study the influence of two levels of salt on the quality characteristics of Pizza cheeses prepared using *Saccharomyces boulardii* adjunct culture. Two protocols were employed: Protocol I using unhomogenized milk and cheddaring of cheese curd before plasticizing (CCUM) and Protocol II using a milk blend of homogenized and unhomogenized milks (CBHM) to determine the *S. boulardii* count as affected by salt levels. All the cheeses conformed to the FSSR standard. The cheese CBHM$_{2.25}$ was associated with higher moisture, FDM content and yield. The hardness, cohesiveness and springiness of CCUM$_{1.75}$ and CCUM$_{2.25}$ as well as CBHM$_{1.75}$ and CBHM$_{2.25}$ were similar. Judging cheese as a pizza topping revealed that the highest flavour and total scores were assigned to cheeses CCUM$_{1.75}$ and CCUM$_{2.25}$ respectively. Maximum stretch length was associated with cheese CCUM$_{1.75}$. The LAB count of cheeses CCUM and CBHM was favoured in the presence of *S. boulardii*. The highest LAB and *S. boulardii* counts were associated with cheese CBHM$_{1.75}$. The counts of *S. boulardii* and LAB in Pizza cheese tended to be lower when using higher salt levels (i.e. 2.25%). It is recommended to use salt at a level of 1.75% when employing *S. boulardii* culture as an adjunct starter.

INTRODUCTION

Mozzarella is a type of soft cheese that is white, fresh, and has a smooth surface. When it is cut open, the cheese is usually round or pear-shaped. It has a slightly wet and milky look and a mild, slightly tangy taste. One notable characteristic of mozzarella is its ability to stretch. Pizza cheese (i.e. low-moisture part-skim Mozzarella cheese) is often the top choice for pizza due to its ability to shred and stretch well. Salting played a crucial role in making Pizza cheese, as it enhanced the cheese flavour and had an impact on its texture, ultimately influencing the baking characteristics (Jana and Mandal 2011).

There is no information available on the manufacture of Pizza cheese using *Saccharomyces boulardii* (a proven probiotic yeast) as adjunct culture and/or the influence of salt levels on the *S. boulardii* count. *S. boulardii* is a well-known thermostolerant probiotic yeast that grows at 37°C. 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 (Unique Biotech 2023). *S. boulardii* has been used in cheese, ice cream and cultured dairy products such as yoghurt, kefir and buttermilk. When consumed at the recommended dose (minimum $10^7$ cfu/g or mL), *S. boulardii* had such a positive impact on the 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* (Ansari *et al.* 2021; Staniszewski and Kordowska-Wiater 2021).

Saltiness is considered to be one of the most essential flavour attributes of cheese and has a strong association with the overall enticement of cheese by consumers. Another function of salt in cheese manufacturing encompasses the control of moisture, improvement of texture, suppression of microorganisms and enhancement of flavour (Ganesan *et al.* 2014). Salt contributes to circumventing the
undesired proliferation of bacteria in cheese throughout cold storage and provides the desired flavour and performance characteristics. Combining salt with low temperature, acidic pH and low moisture inhibits acid production and shifts the metabolism of starter cultures toward breaking down amino acids to generate flavour compounds (Guinee 2004).

The salt in cheese remains in aqueous solution, so its level in solution (salt-in-moisture content) has become an important hindrance against the proliferation of microbial organisms. The antimicrobial effect caused by NaCl results from the reducing effects on the water activity of the cheese (Johnson et al. 2009).

When salt is added to cheese, it changes the composition of the cheese. This, in turn, has a direct impact on the properties of the cheese, such as syneresis. Salt controls various factors such as microbial growth, enzyme activities, texture and even the baking properties of cheeses. Hence, this research was conducted to study the influence of salt levels on \textit{S. boulardii} count and quality characteristics of Pizza cheese prepared using \textit{Saccharomyces boulardii} adjunct culture through technological interventions so as to obtain the desired viable counts.

**MATERIALS AND METHODS**

The research work was carried out in the Department of Dairy Technology at S.M.C. College of Dairy Science, Anand, Gujarat, India for a period of one year and completed in the month of June 2023.

**Materials**

Chilled, mixed (i.e. buffalo and cow) milk was purchased from Vidya Dairy, Anand. The milk was separated at Anubhav Dairy to produce skim milk and cream. The cheese milk was standardized using freshly separated skim milk. A fungal rennet from \textit{Rhizomucor miehei} with a strength of 2400 IMCU/g, was obtained from M/s. Caglicio Clerici, Cadorago, Italy, was used as the milk coagulant. \textit{Saccharomyces boulardii} unique 28, sourced from M/s. Unique Biotech, Hyderabad, was used as an adjunct culture in Pizza cheese making. A starter culture (i.e. \textit{Streptococcus thermophilus} and \textit{Lactobacillus delbrueckii} subsp. \textit{bulgaricus}) was obtained from M/s. DSM, Netherlands. Calcium chloride dihydrate was added to the cheese milk. Such an additive was purchased from M/s. Loba Chemie Pvt. Ltd., Mumbai, AR grade. Vacuum-evaporated common salt (NaCl) from the Tata brand was used to salt the cheese.

**Equipment**

A Kenstar 3D Power OM-34ECR baking oven was used to bake cheese-topped pizza pie. A 5.0 kW muffle furnace (Model No. EIE-500, Erection and Instrumentation Engineers, Ahmedabad) was used to determine the ash content of cheese. An Infrared Thermometer (model No. GIS-500, Bosch, Bengaluru) was used to measure the temperature of the plasticized cheese mass during cheese making.
Pizza Cheese Making

Pizza cheese was prepared from standardized (3.2% milk fat), pasteurized (72°C/no hold) mixed milk as per the process standardized by Ghosh and Singh (1996) employing the SC method involving cheddaring of cheese curd prior to its plasticizing. *Saccharomyces boulardii* was used as an adjunct culture (cheese designated as CCUM; Protocol I). Another lot of Pizza cheese was prepared from ‘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; Protocol II). The *S. boulardii* (unique 28) adjunct culture was incorporated into cheese milk during Pizza cheese making by pre-incubation of such culture in a sufficient quantity of milk at 25°C for 6 h in a thermostatically controlled incubator.

The research explored the quality characteristics of Pizza cheeses made using the two cheese making protocols [i.e. Protocol (i) or Protocol (ii)] as influenced by two levels of salting (i.e. 1.75% and 2.25% w/w of curd) that afford better survivability of the incorporated *S. boulardii* culture (@ 3.5 g/100 kg milk).

**Analyses**

The Pizza cheeses were analyzed for fat, protein, moisture, ash, salt (NaCl), pH and TA. The moisture, fat and salt contents as well as the titratable acidity of the cheese samples were determined employing standard procedures (AOAC 2023). The fat content of cheese was also expressed as fat-on-dry matter (FDM). The protein content of cheese was determined using the Kjeldahl method (AOAC 2023). The ash content of cheese was determined using the standard method (BIS 1981). The pH readings of cheese slurry prepared in distilled water were taken on a digital pH meter (M/s. Mettler Toledo AG, Schwerzenbach).

The LAB and *S. boulardii* counts of Pizza cheese were determined using the methods described by ISO (1998) and Niamah et al (2017) respectively.

**Yield of cheese**

The yield of pizza cheese was calculated as the percentage of kg of cheese obtained from 100.0 kg of standardized cheese milk.

**Analysis of Texture profile**

Food Texture Analyzer (M/s. Lloyd Instruments, LRX Plus, England, Sr. No. 160374) with a 50.0 Newton (N) load cell was used to conduct compression analysis of cheese samples. The samples were compressed at a speed of 20 mm/min and the trigger was set to 10 gf (1 gf = 0.00980665 N). The cheese samples were tempered at 23 ± 1°C for one hour in an air-conditioned room (23 ± 1°C, 55.0 ± 1.0% RH) before being evaluated for their textural attributes. Cubic cheese samples (edges of 1.00 ± 0.06 cm) were placed in the compression support plate in a uniform direction. The samples were compressed up to
70.0% from their initial size. Seven cubic samples were tested for each cheese, and the average of the readings was recorded (Pande 2016).

**Baking characteristics of cheese**

The shredability of cheese was evaluated by hand shredding it through a 2.0 mm stainless steel grater. Schreiber meltability and fat leakage were determined using the using the procedure described by Rajani (Rajani et al. 2021). The stretch length of pizza cheese was tested using the Fork Test (USDA 2013) 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 (Jana et al. 2010). 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 four treatments, were examined by SPSS (Version 27, IBM Corp., USA) statistical examination software.

**RESULTS AND DISCUSSION**

The impact of varying the level of salt (@ 1.75 and 2.25% w/w of cued) with the exclusive focus on the survivability of *S. boulardii* adjunct culture (incorporated @ 3.5 g/100 kg milk) during Pizza cheese making and on the proximate composition, physico-chemical characteristics, yield, textural attributes, baking properties and sensory quality of cheese (i.e. as pizza topping) were explored in this investigation.

**Temperature of plasticized mass**

Based on the plasticizing conditions adopted in preparing cheeses CCUM and CBHM, the temperatures of the cheese mass during plasticizing were 61.5°C and 59.5°C respectively. The plasticizing conditions for the two cheeses, in the same order as specified above, were 84.5°C for 2.5 min. and 79°C for 2.5 min. Pizza cheese was able to have a lower pH as a result of cheddaring (i.e. stretching the curd at 0.75% LA), leading to colloidal calcium phosphate dissolution, which made the curd susceptible to plasticization at lower temperatures (Jana and Upadhyay, 1997). Cheese made from a blend of milk (unhomogenized: homogenized, 1:1 w/w) has a higher protein content in the homogenized milk fraction, which is due to the greater fat surface area. Additionally, the pH of the curd during stretching (where the acidity of the whey
was 0.44% LA) was lower than in the control cheese. These two factors caused the cheese curds to become more pliable at a lower temperature (Jana and Upadhyay, 1993).

**Effects on the proximate composition, physicochemical characteristics and yield of cheese as influenced by two levels of salt**

The proximate composition and physicochemical characteristics of experimental Pizza cheeses (i.e. CCUM\textsubscript{1.75}, CBHM\textsubscript{1.75}, CCUM\textsubscript{2.25} and CBHM\textsubscript{2.25}) are shown in Table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CCUM\textsubscript{1.75}</th>
<th>CBHM\textsubscript{1.75}</th>
<th>CCUM\textsubscript{2.25}</th>
<th>CBHM\textsubscript{2.25}</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proximate composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moisture, %</td>
<td>47.99 ± 0.19\textsuperscript{d}</td>
<td>52.20 ± 0.23\textsuperscript{b}</td>
<td>48.95 ± 0.25\textsuperscript{c}</td>
<td>52.95 ± 0.47\textsuperscript{a}</td>
</tr>
<tr>
<td>Fat, %</td>
<td>22.90 ± 0.14\textsuperscript{a}</td>
<td>22.20 ± 0.27\textsuperscript{b}</td>
<td>22.62 ± 0.24\textsuperscript{a}</td>
<td>22.35 ± 0.22\textsuperscript{b}</td>
</tr>
<tr>
<td>FDM\textsuperscript{1}, %</td>
<td>44.03 ± 0.27\textsuperscript{c}</td>
<td>46.45 ± 0.72\textsuperscript{b}</td>
<td>44.31 ± 0.44\textsuperscript{c}</td>
<td>47.50 ± 0.82\textsuperscript{a}</td>
</tr>
<tr>
<td>Protein, %</td>
<td>23.35 ± 0.14 \textsuperscript{a}</td>
<td>22.46 ± 0.60\textsuperscript{c}</td>
<td>22.87 ± 0.08\textsuperscript{b}</td>
<td>22.44 ± 0.11\textsuperscript{c}</td>
</tr>
<tr>
<td>Salt, %</td>
<td>0.96 ± 0.03\textsuperscript{b}</td>
<td>0.95 ± 0.02\textsuperscript{b}</td>
<td>1.22 ± 0.05\textsuperscript{a}</td>
<td>1.19 ± 0.02\textsuperscript{a}</td>
</tr>
<tr>
<td>Ash, %</td>
<td>2.37 ± 0.07\textsuperscript{a}</td>
<td>2.22 ± 0.08\textsuperscript{b}</td>
<td>2.35 ± 0.01\textsuperscript{a}</td>
<td>2.21 ± 0.06\textsuperscript{b}</td>
</tr>
<tr>
<td><strong>Physico-chemical properties</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acidity, (% LA)</td>
<td>0.56 ± 0.03\textsuperscript{b}</td>
<td>0.64 ± 0.03\textsuperscript{a}</td>
<td>0.49 ± 0.01\textsuperscript{c}</td>
<td>0.56 ± 0.01\textsuperscript{b}</td>
</tr>
<tr>
<td>pH</td>
<td>5.23 ± 0.01\textsuperscript{a}</td>
<td>5.14 ± 0.01\textsuperscript{b}</td>
<td>5.25 ± 0.05\textsuperscript{a}</td>
<td>5.15 ± 0.04\textsuperscript{b}</td>
</tr>
<tr>
<td><strong>Yield</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kg cheese/100 kg milk</td>
<td>12.20 ± 0.10\textsuperscript{d}</td>
<td>12.62 ± 0.09\textsuperscript{b}</td>
<td>12.43 ± 0.23\textsuperscript{c}</td>
<td>13.11 ± 0.18\textsuperscript{a}</td>
</tr>
</tbody>
</table>

The subscripts 1.75 and 2.25 represent the salt addition rate; CCUM and CBHM = Cheese made employing Protocol I and Protocol II respectively; Figures placed after ± indicate standard deviation, the values in each row that have different superscript letters are significantly (p < 0.05) different from each other; n = 5.

All four Pizza cheeses complied with the FSSR compositional standard for Pizza cheese (FSSR 2023). The values shown in Table 1 revealed a significant (p < 0.05) difference with respect to all the constituents studied for the Pizza cheeses. The moisture content of CBHM\textsubscript{2.25} was markedly higher than that of the other three cheeses. The sequence of cheeses in decreasing order of moisture content was as follows: CBHM\textsubscript{2.25} > CBHM\textsubscript{1.75} > CCUM\textsubscript{2.25} > CCUM\textsubscript{1.75} (Table 1).
With respect to fat and ash contents as well as pH, cheeses CCUM\textsubscript{1.75} and CCUM\textsubscript{2.25} as well as CBHM\textsubscript{1.75} and CBHM\textsubscript{2.25} were statistically similar. The salt content of cheeses CCUM\textsubscript{1.75} and CBHM\textsubscript{1.75} as well as CCUM\textsubscript{1.75} and CBHM\textsubscript{2.25} were also statistically similar. With regard to FDM content, cheeses CCUM\textsubscript{1.75} and CCUM\textsubscript{2.25} were statistically similar. However, cheese CBHM\textsubscript{2.25} had a markedly higher FDM content compared to the remaining three cheeses. Both CBHM cheeses, irrespective of the salting rate, were at par with regard to the protein content. The highest values of protein and acidity (% LA) were associated with the cheeses CCUM\textsubscript{1.75} and CBHM\textsubscript{1.75} respectively (Table 1).

Table 1

The lowest moisture level (i.e., 47.99\%) of cheese CCUM\textsubscript{1.75} could be the reason for its noticeably greater fat, ash and protein content. Likewise, the minimum fat, protein, salt content and pH associated with cheese CBHM\textsubscript{1.75} as well as CBHM\textsubscript{2.25} were due to the markedly higher moisture content (i.e. 52.20 and 52.95\% respectively). The lowest acidity value was associated with cheese CCUM\textsubscript{2.25}; this value was markedly lower in comparison to the other remaining three cheeses (Table 1).

Mozzarella cheese that was dry salted at a higher level (1.0\% vs. 0.5\%) had a higher moisture content (Paulson et al. 1998). The decrease in the fat and protein content of Pizza cheeses when salting was carried out at a higher level (i.e. 1.75\%) could be attributed to the markedly higher moisture content associated with such cheeses. Pizza cheeses testing higher moisture content had marginally lower fat content (18.5\%) but had similar protein content when compared with cheeses testing lower (46.4\%) moisture content (Rowney et al. 2004).

The maximum yield was noted for cheese CBHM\textsubscript{2.25} (i.e. 13.11\%) among all cheeses. Likewise, cheese CCUM\textsubscript{2.25} had a markedly higher yield than cheese CCUM\textsubscript{1.75}. The plausible reason for the enhanced yield of cheeses made using a higher salting rate (i.e. 2.25\%), irrespective of the method adopted (i.e. protocols CCUM and CBHM), was due to the greater moisture content associated with such products; possibly the losses of milk constituents were also lower. The rate at which cheese is salted is known to affect the water-holding capacity of casein, which in turn can affect the cheese yield (Guinee and Fox 2004).

Pande (2016) found that the yield of Mozzarella cheese was not considerably affected by salting the cheese curd at different levels (2.0\%, 2.5\%, and 3.0\%).

**Effect of salting rate on the textural properties of cheese as influenced by two levels of salt**

The textural properties of Pizza cheeses as influenced by the salting rate are shown in Table 2. The hardness, cohesiveness and springiness values of cheeses CCUM\textsubscript{1.75} and CCUM\textsubscript{2.25} as well as of cheeses CBHM\textsubscript{1.75} and CBHM\textsubscript{2.25} were statistically similar. All four cheeses differed significantly (p < 0.05) from each other with regard to gumminess, chewiness and adhesiveness. Cheese CCUM\textsubscript{1.75} was associated with the maximum values for hardness (i.e. 21.80 N), cohesiveness (i.e. 0.258), springiness (i.e. 4.77 mm), gumminess (i.e. 562.07 N) and chewiness (i.e. 26.78 N-mm). The minimum hardness, cohesiveness
and springiness were associated with cheese CBHM\textsubscript{2.25} (Table 2). However, Pande (2016) did not note any marked difference in the textural properties of Mozzarella cheese prepared using salt levels of 2.00 and 2.25% salt by weight of curd.

Table 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CCUM\textsubscript{1.75}</th>
<th>CBHM\textsubscript{1.75}</th>
<th>CCUM\textsubscript{2.25}</th>
<th>CBHM\textsubscript{2.25}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness (N)</td>
<td>21.80 ± 1.12\textsuperscript{a}</td>
<td>16.96 ± 0.42\textsuperscript{b}</td>
<td>20.93 ± 0.92\textsuperscript{a}</td>
<td>16.00 ± 0.70\textsuperscript{b}</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>0.258 ± 0.012\textsuperscript{a}</td>
<td>0.226 ± 0.008\textsuperscript{b}</td>
<td>0.252 ± 0.026\textsuperscript{a}</td>
<td>0.213 ± 0.008\textsuperscript{b}</td>
</tr>
<tr>
<td>Springiness (mm)</td>
<td>4.77 ± 0.08\textsuperscript{a}</td>
<td>4.27 ± 0.15\textsuperscript{b}</td>
<td>4.73 ± 0.20\textsuperscript{a}</td>
<td>4.21 ± 0.17\textsuperscript{b}</td>
</tr>
<tr>
<td>Gumminess (N)</td>
<td>562.07 ± 22.11\textsuperscript{a}</td>
<td>382.50 ± 16.10\textsuperscript{c}</td>
<td>526.56 ± 33.51\textsuperscript{b}</td>
<td>340.55 ± 25.78\textsuperscript{d}</td>
</tr>
<tr>
<td>Chewiness (N-mm)</td>
<td>26.78 ± 1.00\textsuperscript{a}</td>
<td>16.34 ± 1.13\textsuperscript{c}</td>
<td>24.89 ± 1.35\textsuperscript{b}</td>
<td>14.30 ± 0.60\textsuperscript{d}</td>
</tr>
<tr>
<td>Adhesiveness (N-mm)</td>
<td>0.312 ± 0.007\textsuperscript{c}</td>
<td>0.429 ± 0.005\textsuperscript{a}</td>
<td>0.296 ± 0.008\textsuperscript{d}</td>
<td>0.410 ± 0.008\textsuperscript{b}</td>
</tr>
</tbody>
</table>

The subscripts 1.75 and 2.25 represent the salt addition rate; CCUM and CBHM = Cheese made employing Protocol I and Protocol II respectively; Figures placed after ± indicate standard deviation, the values in each row that have different superscript letters are significantly (p < 0.05) different from each other; n = 5

Table 2

Cheese CBHM\textsubscript{1.75} had the maximum adhesiveness (i.e. 0.429 N-mm) values (Table 2). Cheese CCUM\textsubscript{1.75} had significantly (p < 0.05) higher gumminess and chewiness values than the other three cheeses. The maximum hardness associated with cheese CCUM\textsubscript{1.75} could be attributed to the lowest moisture and higher content of protein and ash in such cheese compared to the rest of the three cheeses (Table 2).

**Effect of salting rate on the baking characteristics of cheese**

The baking characteristics relevant to the end-use application of Pizza cheese as affected by the salting rate are shown in Table 3.
Table 3
Baking characteristics of Pizza cheeses as influenced by salt levels

<table>
<thead>
<tr>
<th>Baking characteristics</th>
<th>CCUM&lt;sub&gt;1.75&lt;/sub&gt;</th>
<th>CBHM&lt;sub&gt;1.75&lt;/sub&gt;</th>
<th>CCUM&lt;sub&gt;2.25&lt;/sub&gt;</th>
<th>CBHM&lt;sub&gt;2.25&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shredability</td>
<td>Excellent</td>
<td>Very Good</td>
<td>Excellent</td>
<td>Very Good</td>
</tr>
<tr>
<td>Melting time in oven at 230°C (s)</td>
<td>420.00 ± 10.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>484.00 ± 5.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>426.00 ± 2.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>488.60 ± 6.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Schreiber meltability (Arbitrary value)</td>
<td>4.21 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.49 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.09 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.33 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat leakage (cm²)</td>
<td>5.96 ± 0.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.73 ± 0.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.51 ± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.78 ± 0.24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stretch length (cm)</td>
<td>52.80 ± 1.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.30 ± 0.84&lt;sup&gt;c&lt;/sup&gt;</td>
<td>50.00 ± 2.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.20 ± 2.39&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The subscripts 1.75 and 2.25 represent the salt addition rate; CCUM and CBHM = Cheese made employing Protocol I and Protocol II respectively; Figures placed after ± indicate standard deviation, the values in each row that have different superscript letters are significantly (p < 0.05) different from each other; n = 5

Table 3

**Shredability**

To ensure proper distribution and melting of cheese on pizza pie and for other applications, the cheese needs to be sliced, diced, or shredded into separate entities of uniform size. Hence, the determinants of the overall functional properties of Pizza cheese involve shredability and resistance to clumping, post shredding (Jana and Tagalpallewar 2017). The cheese should be easy to shred into thin, long shreds.

Shredded cheeses are classified into various categories viz., ‘Excellent’, ‘Very Good’, ‘Good’ and ‘Fair’. ‘Excellent’ shredded cheeses shred easily and form thin, long shreds that do not mat. ‘Good’ shredded cheeses are slightly more difficult to shred and form thick, short shreds that may mat slightly. The cheeses that behaved in a manner that was construed to be midway between the previous two categories were labelled as ‘Very Good’ and those that behaved very poorly were categorized in the ‘Fair’ category. Pizza cheeses having the desired firmness yielded a greater proportion of longer shreds during the shredding process (Banville et al. 2014).

**Figure 1**

The shredability of the two CCUM cheeses was rated as ‘Excellent’, while the rating for the two CBHM cheeses was ‘Very Good’ (Table 3).

**Meltability (including melting period)**

‘Meltability’ is defined as the ability of cheese shreds to coalesce into a uniform, continuous layer at the heat of the baking oven. The cheese should melt easily (Jana and Tagalpallewar 2017). Cheese meltability is concerned with the disintegration of the protein matrix as a consequence of heating, with
consequent melting of entrapped fat and weakening of the protein-protein linkages culminating in the flowability of cheese (Lucey et al. 2003). Any delay in the melting of the cheese in the heat of the oven is unfavourable, as it may lead to browning and blistering of the cheese (Jana and Tagalpallewar 2017).

The melting periods of cheeses prepared by the same method (i.e. CCUM or CBHM) were statistically similar, irrespective of the level of salting. As expected, the two CBHM cheeses required a markedly higher melting period as compared to the two CCUM cheeses (Table 3).

**Fat leakage**

The tendency for liquid fat to separate from melted cheese and form oil pockets on the surface of baked cheese is known as ‘oiling-off’ or ‘free oil formation’. While a small amount of free fat is desirable in pizza cheese, excessive free fat can detract from its appearance, palatability and crispness (Rudan et al. 1999).

As noted for the meltability character, the fat leakage values of cheeses prepared using same method (i.e. CCUM, CBHM) were rated statistically alike. As anticipated, cheeses CBHM<sub>1.75</sub> and CBHM<sub>2.25</sub> had lower fat leakage values (i.e. 3.73 and 3.78 respectively); such values were significantly (p < 0.05) different from those associated with the two CCUM cheeses (Table 3).

Mozzarella cheese made using a higher (i.e. 3.0%) salt rate exhibited reduced fat leakage compared to cheeses containing a lower (0.4–2.5%) salting rate (Rowney et al. 1999; Pande, 2016).

**Stretchbility**

The ability of melted pizza cheese to form long, thin strands that do not break when stretched is known as ‘stretchability’. The United States Department of Agriculture has set a standard for mozzarella cheese in the United States, requiring a minimum stretch of 3.0 inches (7.62 cm) of continuous string (USDA 2013).

The stretch lengths of all the cheeses markedly differed from each other. The maximum stretch length (i.e. 52.80 cm) was associated with cheese CCUM<sub>1.75</sub>; the cheeses exhibiting still lower stretch, in descending order, were as follows: CCUM<sub>2.25</sub> > CBHM<sub>1.75</sub> > CBHM<sub>2.25</sub> (Table 3).

The impaired stretch character noted for the two CBHM cheeses (vs. CCUM cheeses) could be ascribed to the negative influence on such properties as a result of ‘mixed milk’ containing homogenized milk. Homogenization treatment meted to cheese milk is reported to impair the stretchability of Pizza/Mozzarella cheeses (Rowney et al. 2003; Patel 2022).

**Effect of salting rate on the sensory scores of cheeses judged as pizza topping**

To ascertain the sensory acceptability of the four Pizza cheeses viz., CCUM<sub>1.75</sub>, CCUM<sub>2.25</sub>, CBHM<sub>1.75</sub> and CBHM<sub>2.25</sub>, the cheeses topped on pizza pie were subjected to sensory evaluation. The sensory scores of all four cheeses evaluated as pizza topping are represented in Fig. 2.
The rate of salting of cheese curd affects the final salt content of Pizza cheese. The salt content of cheese has been observed to have an impact on baking characteristics including shredding, melting, fat leakage, stretching and the sensory perception of chewiness when it is used in pizza pies (Rowney et al. 1999; Jana and Tagalpallewar 2017). Hence, the Pizza cheeses made using two manufacturing protocols (i.e. to yield cheeses CCUM and CBHM), salted at two levels (viz. 1.75 and 2.25%) at the salting stage, were subjected to sensory evaluation as a topping on pizza pie.

The appearance scores of the Pizza cheeses remained unaffected by the two salting rates applied during their manufacture. All the sensory scores of the two CCUM cheeses were statistically similar. The flavour score of cheese CBHM$_{2.25}$ (i.e. 7.30) was markedly higher than that of counterpart cheese CBHM$_{1.75}$. The two CBHM cheeses had statistically similar scores for attributes such as stringiness, chewiness and total sensory aspects (Table 4).

The highest flavour and total scores were associated with cheeses CCUM$_{1.75}$ (i.e. 8.15 out of 10.0) and CCUM$_{2.25}$ (i.e. 40.37 out of 50.0) respectively, while the lowest scores for such attributes were associated with cheese CBHM$_{1.75}$ (i.e. 7.30 and 38.32 respectively).

The total sensory score (i.e. 41.63 out of 50.0) as well as scores for flavour (i.e. 8.40 out of 10.0) and stringiness (i.e. 8.58 out of 10.0), were superior for Mozzarella cheese prepared utilizing 2.5% salt in contrast to cheeses made using lower (i.e. 2.0, 3.0%) levels. The Mozzarella cheeses made using dry salting at a level of 2.0 or 2.5% had sensory scores (judged as pizza topping) for appearance, melting and chewiness that were statistically similar (Pande 2016).

**Microbial count of cheeses as influenced by the salting rate**

The main objective of studying the two levels of salt in producing Pizza cheese was to ascertain the survivability of the adjunct *S. boulardii* culture (purported to be a probiotic) in addition to the thermophilic
LAB used in its manufacture. The microbial counts of Pizza cheeses as influenced by the levels of salt addition are shown in Table 4.

Table 4

The LAB as well as the *S. boulardii* count of the cheeses (viz., CCUM and CBHM) were significantly (*p* < 0.05) influenced by the salt addition rate and the two protocols of cheese manufacturing. The highest and lowest counts of both LAB were associated with cheeses CBHM$_{1.75}$ (i.e. 9.02 log$_{10}$ cfu/g) and CCUM$_{2.25}$ (i.e. 8.16 log$_{10}$ cfu/g) respectively. In a similar fashion, the highest and least count of *S. boulardii* were associated with cheeses CBHM$_{1.75}$ (i.e. 6.02 log$_{10}$ cfu/g respectively) and CCUM$_{2.25}$ (i.e. 4.69 log$_{10}$ cfu/g) respectively (Table 4). This clearly indicated that the use of a higher salt level (i.e. 2.25%) was somewhat inhibitory to the growth of both *S. boulardii* and LAB.

The microbial count displayed in Table 4 also revealed that cheeses prepared from ‘mixed milk’ involving 50.0% homogenized milk (i.e. CBHM) had a significantly (*p* < 0.05) higher count of both LAB and of *S. boulardii* than the counterpart cheeses made using another method (i.e. CCUM). Such responses regarding LAB and *S. boulardii* count of cheeses held true at each level of salting used in the preparation of Pizza cheese. However, a comparison of the microbial count of cheeses could not be made in light of the availability of the literature.

The *S. boulardii* and LAB counts of Pizza cheese tended to be lower when using higher levels (i.e. 2.25 vs. 1.75% w/w of curd) of salt. It is recommended to use salt @ 1.75% when employing *S. boulardii* culture as an adjunct starter for Pizza cheese making.

**CONCLUSIONS**

To produce health-promoting Pizza cheese utilizing *S. boulardii* an adjunct starter, it is recommended that cheese makers to adopt a specific standardized cheesemaking protocol. Such a cheesemaking protocol involves (a) the use of a ‘milk blend’ comprising of unhomogenized and homogenized (1.96 and 0.98 MPa pressure) milk as the starting material, (b) inoculating cheese milk with *S. boulardii* adjunct culture @ 3.5 g/100 kg milk along with yoghurt culture (*S. thermophilus* and *L. bulgaricus*) @ 6.0 g per 100 kg milk, (c) salting @ 1.75% by weight of cheese curd and (d) adopting plasticizing conditions of 80°C with contact period of 2–3 min. with cheese curd. Cheese obtained employing such a standardized cheesemaking protocol was preferred over cheese CCUM containing a lower level of salt (1.75% w/w of curd) as used in preparing cheese CBHM owing to the higher count of both *S. boulardii* and LAB. Moreover, the composition (i.e. moisture, FDM) of cheese CBHM conformed to the FSSR regulation and had acceptable sensory and baking properties for their end use application on pizza pie. The lower level of salt used during Pizza cheese making enabled a higher *S. boulardii* and LAB count in the product in contrast to a higher level of salt.

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

CCUM – Cheese from unhomogenized milk following cheese making involving cheddaring stage

CRD – Completely randomized design

FDM – Fat-on-Dry Matter

IMCU – International Milk Clotting Units

LAB – Lactic Acid Bacteria

SC – Starter Culture

TA – Titratable Acidity

Declarations

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AUTHORS CONTRIBUTION

Ankit Bihola designed, planned and conducted the experiments, examined the data and wrote the manuscript; Atanu H. Jana conceived the research, aided in refining the design and edited the manuscript; Satish C. Parmar contributed to the physicochemical analyses of cheeses and validation of the data; and Priyanka Suvera and Adil Shaikh helped in the conduct of experiments, review of literature and statistical analysis of the data.

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CONFLICT OF INTEREST

The authors have no conflicts of interest/no competing interests.

DATA AVAILABILITY

The authors will make all the data available based on demand.


**Figures**
Figure 1

Shredded form of Pizza cheeses prepared using two salt levels viz., (A) CCUM_{1.75} (B) CBHM_{1.75} (C) CCUM_{2.25} (D) CBHM_{2.25}

Figure 2

Sensory scores of Pizza cheeses as influenced by salt levels

1.75 and 2.25 represent the salt addition rate; CCUM and CBHM = Cheese made employing Protocol I and Protocol II respectively; n = 5
Figure 3

Stretch behaviour of Pizza cheeses as influenced by the level of salt viz., (A) CCUM$_{1.75}$ (B) CBHM$_{1.75}$ (C) CCUM$_{2.25}$ (D) CBHM$_{2.25}$