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RESEARCH ARTICLE
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Functionality of Pizza Cheese as Affected Using *Saccharomyces boulardii* Adjunct Culture During Refrigerated Storage

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ABSTRACT

Background: Mozzarella/Pizza cheese remains the preferred choice for topping pizza. There is currently no information available on the production of Pizza cheese employing *Saccharomyces boulardii* as an adjunct culture. The research was conducted to study the influence of *S. boulardii* adjunct culture on the functional properties of Pizza cheese during refrigerated storage.

Methods: Experimental Pizza cheese was manufactured using a blend of homogenized and unhomogenized milks (1:1; cheese designated as CBHM). Control cheese was prepared from unhomogenized milk involving stretching of curd, post whey draining.

Result: The baking properties *viz*., meltability as well as melting time were markedly affected by T, P and the interaction T×P. The fat leakage and stretch length of cheeses were markedly affected by T and P only. The melt and fat leakage values exhibited marked increase with the advancement of the storage period. It is recommended to consume such cheese before 21 days since CBHM cheese elicits the desirable baking characteristics up to 21 days.

Key words: Cheddaring, Homogenized milk, Pizza cheese, Refrigerated storage, *Saccharomyces boulardii*.

INTRODUCTION

Mozzarella cheese (particularly the Pizza cheese type, also known as low-moisture part-skim Mozzarella) is the preferred choice with regard to topping on pizza. Historically, the production of Mozzarella cheese utilized the traditional technique of using a "starter culture". Various researchers have prepared Mozzarella/Pizza cheese employing the Starter Culture (SC) technique utilizing adjunct cultures such as *Lactobacillus acidophilus* and *L. rhamnosus*. (Ortakci *et al*., 2012; Cuffia *et al.,* 2017; Akarca and Yildirim, 2022). There is no information available on the manufacture of Pizza 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.

Saccharomyces boulardii as a probiotic agent

Diarrhoea and other gastro-intestinal (GI) diseases induced by the consumption of antimicrobial drugs were ameliorated when using *S. boulardii*. The probiotic potential of *S. boulardii* can be understood since such microbe can survive the transit through the GI system and inhibits various pathogens, both in *vitro* and in *vivo*. Such yeast was resistant to antibiotics (tetracycline, erythromycin and vancomycin) (Czerucka *et al*., 2007).

Therapeutic virtues of *Saccharomyces boulardii*

S. boulardii has been used as a probiotic in vegetable juices, ice cream and in fermented 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 host. Improvement in the gut flora, immunological modulation, avoiding enteric infections, diarrhoea and inflammatory bowel disease are some of the advantages gained when consuming food product containing such probiotic yeast. Water-soluble vitamins such as thiamine, riboflavin, biotin, pyridoxine and folic acid as well as antibacterial substances such as bacteriocins (such as leucocin C) were produced by *S. boulardii* (Staniszewski and Kordowska-Wiater, 2021).

Effectiveness on humans through ingestion of *S. boulardii*

The effectiveness on prospective persons through ingestion of *S. boulardii* can be gauged by the below delineated reports.

- *S. boulardii* endures the passage to its target organ (*i.e.* colon), despite much of the oral dose getting impaired (count in stool was 100-1000 times lower than intake of oral dose), surviving oral doses were effective, usually at levels ≥10^8/g.
- *S. boulardii* thrives longer in individuals with altered gut microbiota in contrast to those who have not been exposed to antibiotics.

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- *S. boulardii* is best adapted at body temperature (*i.e*. 37°C).
- *S. boulardii* endures bile and gastric acid in lyophilized form. *S. boulardii* is resistant to proteolysis.
- After 3 days of oral consumption, *S. boulardii* count reached steady-state concentrations and was eliminated within 3-5 days of its discontinuation.
- Presence of some types of fibers (*i.e*. psyllium) raised the levels of *S. boulardii* by 22.0 per cent; pectin failed to show such effect (Kelesidis, 2012).

Viability of *S. boulardii* under harsh conditions

Dairy food products serve as an ideal medium for the delivery of probiotic microbes into the human GI tract. Probiotic microorganisms need to survive at body temperature (*i.e*. 37°C), be resistant to bile and stomach acids and tolerate the competitive environment in the intestinal lumen. *S. boulardii* grows well at 37°C (ideal temperature for most strains varies between 22 and 30°C). *S. boulardii* can reach high concentration in the colon, if adequate amount is consumed (Ansari *et al*., 2021).

Potential health benefits exerted by *S. boulardii* 28 strain

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 (IBS) with constipation and prevented gastrointestinal infections.

S. boulardii showed antidiarrheal activity by restoring the normal balance of microorganisms in the intestines. Such organism inhibited bacterial toxins from binding to 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. 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).

The investigation was carried out to study the functional properties of Pizza cheese during refrigerated storage prepared using *S. boulardii* as an adjunct starter through technological interventions to obtain the desired viable counts. This research generated helpful data for cheesemakers who intend to produce Pizza cheese containing lactic acid bacteria (LAB) with *S. boulardii*.

MATERIALS AND METHODS

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

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

Pizza cheesemaking

Pizza cheese was made using 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] employing the SC method of Patel (2022) using *S. boulardii* as an adjunct culture (cheese designated as CBHM).

Control Pizza cheeses with and without inclusion of *S. boulardii* adjunct culture were prepared from standardized (3.2% milk fat), pasteurized (72°C/no hold) mixed milk as per the process of Patel *et al.* (1986) employing the SC method (moulding water temperature was 95±3°C) to compare the functional properties of experimental cheese with control cheeses. Pizza cheeses were vacuum-packed in polyethylene bags (~80.0 μm thick) and stored at 7±1°C until they were organoleptically acceptable (for up to 21 days). The description and conditions of Pizza cheesemaking are provided in Appendix 1.

The *S. boulardii* (unique 28) adjunct culture was incorporated into cheese milk during Pizza cheesemaking [control (C2) and experimental] and by 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.

Appendix 1: Descriptions and conditions of pizza cheesemaking.

Cheeses	Designated code	Level of *S. boulardii* (g/ 100 kg milk)	Plasticizing conditions
Cheese from unhomogenized milk	CUM (C1)	-	92°C, 4-5 min.
Cheese from unhomogenized milk with *S. boulardii* culture	CUMY(C2)	3.50	92°C, 4-5 min.
Cheese from 'milk blend' (homogenized: unhomogenized milks; 1:1, w/w) using *S. boulardii* culture (Experimental)	CBHM	3.50	80°C, 2-3 min.

Note: Salting with NaCl @ 1.75 per cent w/w of cheese curd in all cases.

Baking characteristics of cheese

The shredability of cheese was assessed subjectively by manual shredding through a 2.0 mm pore diameter stainless steel shredder. Meltability and fat leakage were determined by the procedure described by Rajani *et al.* (2021). Fat leakage was determined using the procedure described by Breene (Rajani *et al*., 2021). The stretch length of pizza cheese was tested using the 'Fork Test' (USDA, 2013) with a 4-pronged fork.

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 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 (Patel, 2022). Both of these factors led to cheese curds getting plasticized at a much lower temperature.

Based on the plasticizing conditions adopted in preparing control cheeses and 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 for 4.5 min. and 79.0°C for 2.5 min. respectively.

Changes in the functional properties of pizza cheeses during refrigerated storage

Fresh mozzarella cheese is not suitable for pizza because it melts into a tough, rubbery and grainy consistency with 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.

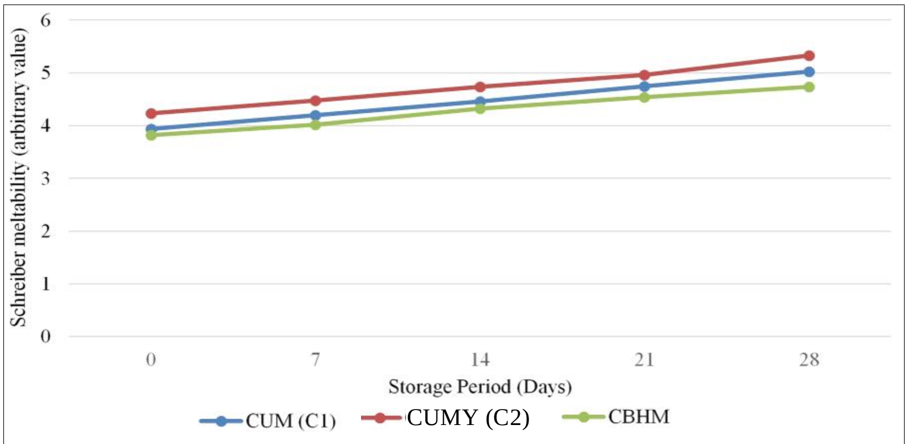
Changes in the baking qualities of cheese

Two specific baking qualities of Pizza cheeses *viz*., meltability and fat leakage were assessed throughout refrigerated storage (7±1°C) for up to 28 days. During actual baking trials, the time needed for the cheese shreds to melt in the heat of the oven and stretch length (*i.e*. Fork stretch test) were assessed. Hence, these two parameters were assessed only until the 21st day, coinciding with the actual pizza baking trials. The Pizza cheeses did not retain the ease of shredding, after 3 weeks of refrigerated storage.

Meltability

There was a progressive increase in the Schreiber meltability of all the cheeses throughout the storage period. The changes in the meltability of cheeses were significantly ($p<0.05$) affected by T, P and the interaction T × P. All three cheeses differed markedly from each other with regard to their meltability; the maximum (5.33) and minimum (3.82) Schreiber meltability (as an arbitrary value) were associated with cheeses C2 and CBHM respectively (Fig 1). Improvement in the meltability of Pizza cheese during storage is a positive feature, especially for their end use application. In this regard, control cheese C2 fared superiorly during storage.

The meltability (determined by the Disc method) of fresh Mozzarella cheeses made from homogenized (2.45 MPa



CUM (C1)- Cheese from unhomogenized milk; CUMY (C2)- Cheese from unhomogenized milk with *S. boulardii* (yeast) culture; CBHM- Cheese from 'milk blend' comprising homogenized and unhomogenized milks (1:1, w/w); n=4.

Fig 1: Changes in the Schreiber meltability of pizza cheeses during storage.

pressure) and unhomogenized milks was 6.82 and 20.24 cm^2 respectively. The meltability of such cheeses increased upon storage; the values at 4 weeks of storage were 26.83 and 38.25 cm^2 respectively (Abd El-Gawad *et al.*, 2012). The proteolytic changes taking place during the ageing of cheese brought about by rennet, indigenous milk enzymes and starter enzymes were responsible for the noted improvement in the meltability of cheeses (Jana and Tagalpallewar, 2017).

Melting time in the oven

There was a progressive reduction in the melting time of all the cheeses during their refrigerated storage period up to 21 days. Such a reduction in the melting time of cheeses was found to be markedly different as noted at each 7 days interval of storage, until the 21st day. The changes in the melting period of cheeses were significantly ($p<0.05$) affected by T, P and the interaction T × P. Cheese C2 required a minimum time (*i.e*. 298.33 s) to melt in the heat of the oven; cheese CBHM took maximum time to melt (*i.e*. 360.0 s) as was expected (Table 1).

The decrease in the melting period for the cheeses during refrigerated storage is consistent with the pattern of increasing Schreiber meltability values shown in Table 1.

Fat leakage

A progressive increase in the fat leakage of all the Pizza cheeses was evident during the refrigerated storage period. The changes in the fat leakage of cheeses were significantly ($p<0.05$) affected by T and P; interaction T × P remained unaffected. Cheese C2 had significantly ($p<0.05$) higher (*i.e.* 6.06 cm^2) fat leakage than the other two cheeses. As anticipated, cheese CBHM was associated with the least (*i.e.* 4.17 cm^2) fat leakage; the presence of homogenized milk in the 'milk blend' used for cheesemaking led to such observed phenomena. The increase in the fat leakage of cheese was significant ($p<0.05$) at each 7 days interval of storage, until the 28th day (Table 2).

Abd El-Gawad *et al.* (2012) reported an increase in the fat leakage of Mozzarella cheeses, irrespective of the homogenization (2.45 MPa pressure) treatment meted to the cheese milk (standardized to 3.0% fat), during the storage period at 4°C. The fat leakage (cm^2) of homogenized milk cheese was 58.26 and 90.45 at storage periods of 0 and 4 weeks respectively; the fat leakage of untreated milk cheeses at the pertinent storage period was 64.87 and 104.16 cm^2 respectively.

Table 1: Changes in the melting time in oven of Pizza cheeses during storage.

Cheeses	Melting time (s) in oven at storage period (days)			Mean (Treatment)
	7	14	21	
CUM (C1)	440.00^b	311.25^e	184.25^h	311.83^q
CUMY (C2)	433.75^c	296.50^f	164.75^i	298.33^r
CBHM	481.25^a	360.75^d	238.00^g	360.00^p
Mean (Period)	451.67^a	322.83^b	195.67^c	-
Source of variation	Treatment (T)		Period (P)	T × P
SEm±	0.98		0.98	1.70
CD (0.05)	2.85		2.85	4.94
CV (%)		1.05		

CUM (C1)- Cheese from unhomogenized milk; CUMY (C2)- Cheese from unhomogenized milk with *S. boulardii* (yeast) culture; CBHM-Cheese from 'milk blend' comprising homogenized and unhomogenized milks (1:1, w/w); the values indicated row and column wise having differing superscripted alphabets differs significantly ($p<0.05$) from each other; n=4.

Table 2: Changes in the fat leakage of pizza cheeses during storage.

Cheeses	Fat leakage (cm^2) during storage (days)					Mean
	0	7	14	21	28	(Treatment)
CUM (C1)	5.51	5.67	5.86	6.04	6.37	5.89^q
CUMY (C2)	5.72	5.88	6.05	6.24	6.43	6.06^p
CBHM	3.79	3.94	4.18	4.34	4.59	4.17^r
Mean (Period)	5.00^e	5.16^d	5.36^c	5.54^b	5.80^a	-
Source of Variation		Treatment (T)		Period (P)		T × P
SEm±		0.016		0.020		0.035
CD (0.05)		0.044		0.057		NS
CV (%)			1.30			

CUM (C1)- Cheese from unhomogenized milk; CUMY (C2)- Cheese from unhomogenized milk with *S. boulardii* (yeast) culture; CBHM-Cheese from 'milk blend' comprising homogenized and unhomogenized milks (1:1, w/w); the values indicated row and column wise having differing superscripted alphabets differs significantly ($p<0.05$) from each other; n=4.

Table 3: Changes in the stretch length of Pizza cheeses during storage.

Cheeses	Stretch length (cm) during storage (days)			Mean (Treatment)
	7	14	21	
CUM (C1)	50.50	41.50	32.50	41.50^{p}
CUMY (C2)	50.75	39.50	30.25	40.17^{q}
CBHM	43.75	34.00	25.75	34.50^{r}
Mean (Period)	48.33^{a}	38.33^{b}	29.50^{c}	-
Source of variation	Treatment (T)		Period (P)	T × P
SEm±	0.329		0.329	0.569
CD (0.05)	0.953		0.953	NS
CV (%)			2.94	

CUM (C1)- Cheese from unhomogenized milk; CUMY (C2)- Cheese from unhomogenized milk with *S. boulardii* (yeast) culture; CBHM-Cheese from 'milk blend' comprising homogenized and unhomogenized milks (1:1, w/w); the values indicated row and column wise having differing superscripted alphabets differs significantly (p<0.05) from each other; n=4.

The fat leakage of Pizza cheese made using thermophilic SC from mixed milk (cow and buffalo milk) increased from an initial value of 5.61 to 6.49 cm^2 over a span of 28 days; the storage temperature was 7°C (Rajani, 2021).

Stretchability

Pizza baking trials were conducted only until the 21st day of storage, owing to impairments in the shredding property beyond this period. Hence, the 'fork stretch test' was performed only thrice during the storage period.

The stretch length of Pizza cheeses was significantly (p<0.05) affected by T and P; interaction T × P did not have any marked effect. Cheese C1 exhibited the maximum stretch length (*i.e.* 41.50 cm); such stretch length was significantly (p<0.05) superior when compared to the pertinent character of the remaining two cheeses. Cheeses C2 and CBHM exhibited stretch lengths of 40.17 and 34.50 cm respectively; such a difference in the stretch length was also found to be significant (p<0.05). The decrease in the stretch length of cheeses during refrigerated storage was significantly (p<0.05) different, as noted at each 7 days interval until the 21st day (Table 3). However, all cheeses in the present investigation stretched to a level well above the minimum (*i.e.* 7.62 cm) specified by USDA (2013), irrespective of the storage period.

The stretch length of Mozzarella cheese (measured by dipping 5.0 g cheese in hot water at 85°C for 1 min.) reported at a specific storage interval 4°C is as follows: 0 day: 33.33-41.00 cm; 3rd day: 22.66-36.00 cm; 7th day: 16.33-30.33 cm (Baskaran, 2015). The stretch length of Pizza cheese made using thermophilic SC declined from an initial length of 40.00 cm as noted on the 7th day to 37.50 cm on the 21st day of storage (7°C) (Rajani, 2021).

CONCLUSION

To produce health-promoting Pizza cheese with desirable functional properties using *S. boulardii* as an adjunct starter, it is recommended that cheese makers adopt a specific standardized protocol for CBHM cheese. Cheese CBHM was preferred over control cheeses made employing the SC method containing *S. boulardii* adjunct culture at the same level as in preparing cheese CBHM owing to acceptable baking characteristics for its end-use application on pizza pie in addition to the higher count of both *S. boulardii* and Lactic Acid Bacteria (LAB). Since refrigerated storage beyond 21 days led to a deterioration in the functional properties of cheese, only 3 weeks of refrigerated storage is advocated to provide health benefits from consumption of such cheese along with acceptable functional characteristics.

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Conflict of interest

The authors have no conflicts of interest.

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