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Functional Burrata cheese enriched with *Lacticaseibacillus casei* ATCC 393: Insights into production, unique characteristics, and aromatic profile

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Abstract

The aim of this study was to improve the aromatic profile, functional attributes and extend shelf life by incorporating *Lacticaseibacillus casei* subsp. *casei* ATCC393 into Burrata cheese. Two-batches of cheese were produced; the probiotic-group (LC-BC), and the control-group (C-BC). The results revealed successful integration of this strain into Burrata cheese. The presence of *L. casei* was found to enhance the cheese environment, promoting its growth. This strain can be characterized by high productions of mainly ethyl-acetate and 2-heptanone compunds, and then acetoin, diacetyl, hexanoic-acid, and acetaldehyde compounds for Burrata cheese. The LC-BC, enriched with diacetyl, exhibited a creamy, slightly sweet, and buttery aroma, contributing to its distinct sensory profile. Consequently, the *L. casei* ATCC393 proved to be a valuable addition, enhancing the quality, sensory appeal, and shelf life of Burrata cheese. It was demonstrated that Burrata-Cheese is well-suited for probiotic applications, contributing valuable insights to the existing body of literature.

Keywords: *Lacticaseibacillus casei*; probiotic; Burrata cheese; aroma compounds; qPCR

Introduction

BurrataCheese is an 'pasta-filata' cheese made from cow's milk and cream. The pasta-filata cheeses include soft or semisoft varieties, typically consumed fresh or after a short ripening such as high- and low-moisture mozzarella (Minervini et al., 2017). Burrata cheese consists of two parts, an outer shell' and an inner filling parts. The outer shell is a mozzarella paste type which is called as 'fior di latte', and is made from cow's milk, while the inner filling is a mixture of fresh cream which is called as 'stracciatella', and mozzarella strips. Considered all features of this cheese from a different perspective, it may be thought as a substitute for the composition of mozzarella cheese, cream cheese and feta cheese. However, the awareness of this cheese and the studies (Trani et al., 2016) on it are quite limited. As, the milky liquid inner filling of Burrata cheese, whose outer sheet is enclosed with a fibrous texture, is a sheltered environment, it can be considered as a dairy medium where probiotic microorganisms can survive unabated throughout the shelf life of the product.

On the other hand, cheeses can be a very suitable carrier medium for probiotics, if they have a higher pH and adeqate buffering capacity, a solid and consistent matrix, and a relatively higher fat content in comparison with fermented milk products such as yogurt. A medium with these properties preserves the probiotics as they pass through the gastrointestinal tract (Cuffia et al., 2017). In the literature, there are some cheese studies containing probiotic microorganisms (Dantas et al., 2016). However, to our knowledge, studies on fresh pasta filata cheeses containing probiotic are very limited (Minervini et al., 2017), due to the high temperature kneading process applied in their production, but the inner filling of Burrata cheese is not subjected to this process.

The *L. casei* ATCC393 a strain belonging to the *Lacticaseibacillus casei* group (LCG), which includes *Lacticaseibacillus casei*, *Lacticaseibacillus paracasei* and *Lacticaseibacillus rhamnosus*. This group is of interest as probiotics (Kourkoutas et al., 2006). In addition, in recent years, many studies have revealed the importance of the gut microbiota in health promotion in the treatment or prevention of a number of diseases and disorders. LCG microorganisms are also known to have the potential to be used prophylactically or therapeutically in diseases associated with disruption of the gut microbiota. The effect of LCG microorganisms on diseases such as obesity, brain function, allergic diseases, cancer, diarrhea, oxidative stress has also been supported by scientific studies. Therefore, the use of such beneficial microorganisms in industry should be expanded (Wang et al., 2021). In this context, it was thought that this strain might be suitable for Burratta cheese.

Burrata cheese, categorized as a fresh cheese, has utilized *L. casei* ATCC393 to enhance its aroma-profile in this study. The aim of this study was to improve the aromatic profile, functional attributes and extend shelf life by incorporating *Lacticaseibacillus casei* subsp. *casei* ATCC393 into Burrata cheese.

Materials and methods

The cheese starter culture, consisting of *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris*, and *Streptococcus thermophilus*, was sourced from Danisco-Choozit-RA-LYO (50381, France). The probiotic strain, *L. casei* ATCC393, was acquired from the German Microorganisms and Cell Cultures Collection (DSMZ; Germany). Fermento, a liquid commercial rennet was employed as the coagulant enzyme. Chemicals, solutions, and were provided from Sigma-Aldrich Co. (St Louis, MO, USA) and SupelcoCo. (Bellefonte, USA).

Experimental design

Firstly, an 18-hour active culture of the probiotic strain was prepared, then the pellet containing live microorganisms was obtained after washing twice using a phosphate-buffered-saline (PBS) solution. Finally, 8 logs when added to cheese; it was resuspended with sterile-PBS equal to 0.5 McFarland value. Thus, a probiotic cheese culture was obtained. Meanwhile, a Burrata cheese containing the *L. casei* ATCC393 (LC-BC) and a control Burrata cheese (C-BC) was produced as three repetitions. The inner filling of Burrata cheese was prepared by adding cream fermented with *L. casei* to Mozzarella pieces. The outer part is formed by shaping Mozzarella cheese in hot water. The production methods was given in Figure 1. These were analyzed weekly for 21-days of storage.

Physicochemical/chemical analysis

Samples were collected under sterile conditions by homogenizing the inner filling and the Mozzarella outer part. Various parameters including titration acidity, pH measured using a pH-meter (WTW, Germany), moisture analyzed with a moisture-analyzer (RADWAGMA-50R, Poland), fat content (Gerber method), salt content (Mohr method), protein content (Kjeldahl method), total nitrogen, water-soluble nitrogen, maturation-index, water-activity were measured using a Novasina-LabSwift-aw device (Switzerland), and color (CIE L*a*b*) values determined using a 3NH YS3060 Konica color device (UV-Grating-Spectrophotometer, China) were assessed in the Burrata cheeses following established protocols (Standartları, 2007)).

Microbiological analysis

Lactobacillus spp. were quantified in De-Man-Rogosa and Sharpe (MRS; Merck) medium, and *Lactococcus* spp. and *Streptococcus* spp. in M17 medium, both at 37 °C for 48 hours (Condalab, Spain). Yeasts were detected on PDA medium with 0.14 % lactic acid at 25 °C for 5 days, coliforms on VRB-agar medium (Merck) for 24 h. at 37 °C. Total, psychrophilic, and proteolytic bacteria were counted in PCA medium after incubations at 4 °C (7-10 days), and 21 °C (72 h with 10 % sterile skim-milk and 10 % dry matter), respectively. Lipolytic bacteria were counted on Tributyrin agar (Merck) at 30 °C for 48 h. (Duran et al., 2022).

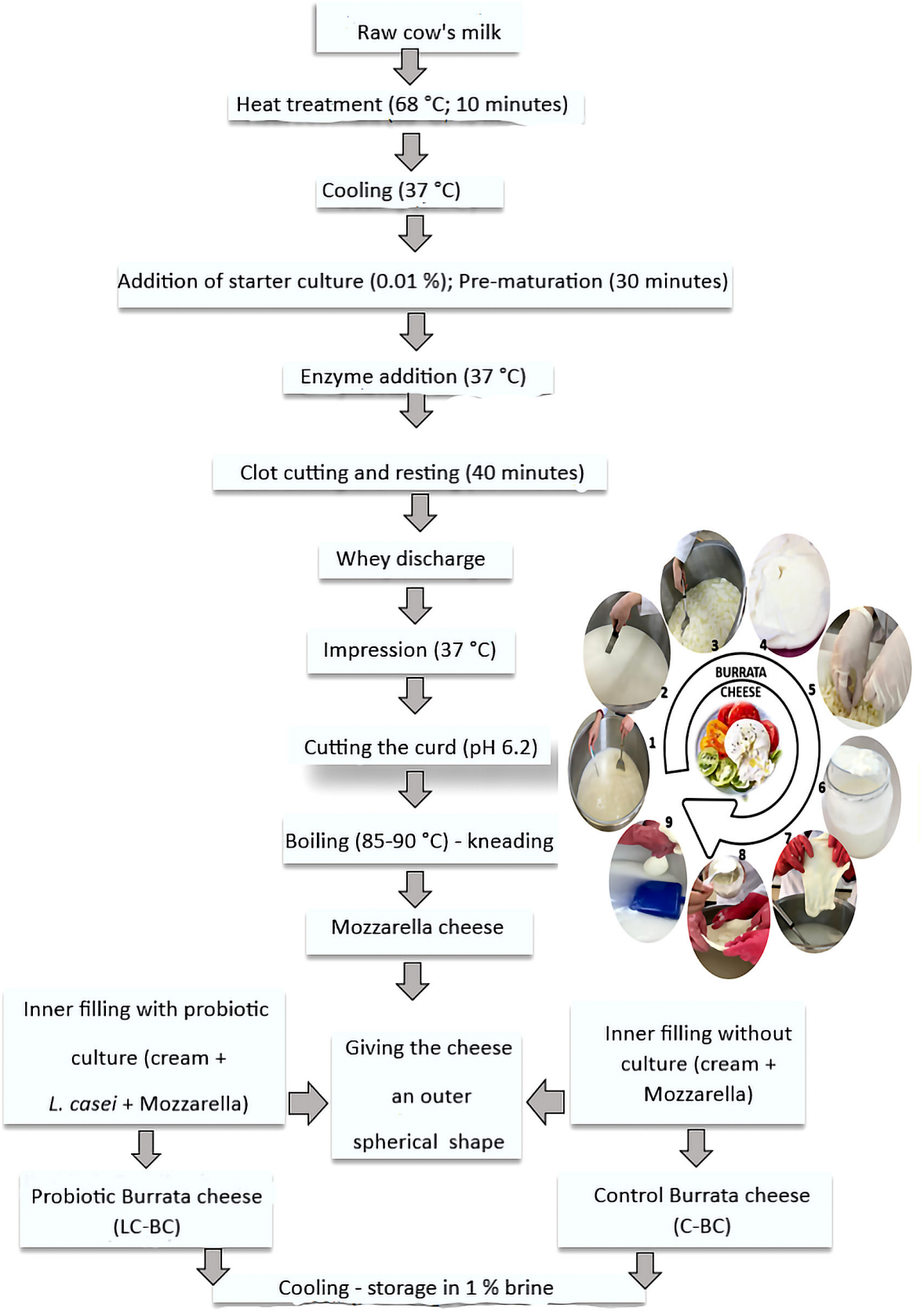

Figure 1. Functional Burrata cheese production flow diagram

DNA isolation- Real-time qPCR analysis

A QIAamp-DNA-Microbiome-Kit (Qiagen, Germany) was utilized for DNA-extraction from Burrata cheese samples. The DNA-isolation from the samples was conducted following the QIAamp-DNA-Microbiome-Kit protocol (Qiagen). Finally, the concentrations of all obtained isolates were determined using the NanoDrop-Device (Thermo, USA). Sample concentrations were standardized to 5 ng/µL.

DNA-isolates were analyzed using qRT-PCR. The CFX96-TOUCH-System by Bio-Rad (Hercules) was employed for the RT-qPCR study, utilizing the CFX (Win8-PDF-Reader, Microsoft-Office-Suite 2003/2007/2010/2013) and specific primers

(For *L. casei*: Lc1: 5'- GTGCTTGCACTGAGATTCGACTTA-3'; 61-84

Lc2: 5'- TGCGGTTCTTGGATCTATGCG-3'; 185–165). Taqman-probes (Oligomer) were incorporated to calculate Cq values and log values. Biorad-SSO advanced SYBR-green-master-mix was used for real-time PCR reactions. Reaction conditions for each sample were as follows: PCR reaction commenced at 98 °C for 3 minutes, followed by 40 cycles at 98 °C for 15 seconds, 60 °C for 30 seconds, and a gradual increase from 65 °C to 95 °C with a 0.5-degree increment.

Volatile aroma compounds analysis

Volatile compounds were assessed utilizing GC-MS (QP2010, Shimadzu, Japan) employing solid-phase micro-extraction (SPME) technology with some modifications, according to Karaçalı et al. (2018). A 2 g sample was placed into a vial, supplemented with 1 g NaCl, and 2.5 µL of internal standard (4-methyl-2-pentanol; 0.5 mL/L in dH_2O). A stabil-wax column (60 m length × 0.32mm i.d. × 0.25 µm thickness) and SPME fiber (2 cm–50/30 mm DVD/Carboxen/PDMS Stable Flex Supelco, USA) were employed. A program included holding at 40 °C for 1 min, ramping up at 7 °C/min to 100 °C (held for 5 min), increasing at 4 °C/min to 130 °C (held for 1 min), escalating at 2 °C/min to 180 °C (held for 1 min), and finally increasing at 15 °C/min to 250 °C (held for 4 min). Identification of compounds was achieved by comparing their retention indices and mass spectra with those in the libraries (Wiley6 and FFNSC). Linear-retention-indices (LRIs) were calculated using an alkane (C7-C30) series. Compound peak-areas were quantified relative to the internal-standard's peak-area, expressed in µg/kg.

Sensory analysis

A scoring-test was employed to evaluate Burrata cheese. This test involved assessing various parameters in detail: appearance of the whole cheese (10 points), section structure after cutting (20 points), filling (10 points), smell (25 points), and taste (35 points). During early orientation sessions, 10 panellists were trained with Burrata cheese manufactured without any treatment The evaluation was conducted by a panel of the semi-trained and experienced panelisties who were familiar with this cheese properties. The LC-BC and C-BC samples were presented in plates bearing a random three-digit number, and a glass of water were given between each sample.

Statistical analysis

This study was arranged in three replications and two parallels after preliminary trials. Data were compared by one-way-Anova analysis with a Tukey-multiple range test, used to determine significance among differences ($p<0.05$). Besides, t-Student's test was performed to compare the sample containing the probiotic strain and the control sample.

Results and discussion

Physicochemical properties of Burrata cheese containing probiotic L. casei strain

For cheese production, the exploration of metabolic traits is crucial in identifying strains with aroma potential, a key factor in modifying or enhancing cheese flavor. Prior to their integration into large-scale fermentation processes, a thorough screening of potential adjunct strains is imperative. Dairy companies are actively expanding their culture collections with strains isolated from diverse sources to influence aroma formation in fermented dairy products (Smid and Kleerebezem, 2014). Consequently, strain selection should consider technological properties relevant to cheese production, such as acidifying activities.

A statistically significant difference in titration acidity, expressed as a percentage of lactic acid, was observed between the C-BC and LC-BC groups during the storage days ($p<0.05$) (Table 1). The titration acidity of the C-BC sample was significantly lower than that of the LC-BC sample on the 1st day ($p<0.05$), but it was found to be significantly higher on subsequent storage days ($p<0.05$). This difference is believed to stem from the fermentation of the inner filling cream with *L. casei* in the LC-BC sample. Throughout the 21-day storage period, the increase in titration acidity in terms of lactic acid was less pronounced in the LC-BC sample compared to the C-BC sample. This is attributed to the inhibitory effect of *L. casei* on the decrease in acidity. Along with lactic acid production, the development of *L. casei* results in the production of acetic acid, propylene glycol, acetoin, diacetyl, bacteriocins, and exopolysaccharides, imparting different aroma profiles to the cheese. The influence of these metabolites is thought to slow down the development of acidity, which is one of the reasons why *L. casei* is recommended in cheese production.

The pH range for the C-BC sample was 6.20-4.68 pH, while the pH range for the LC-BC sample was 5.80-5.22. On the 1st day, the pH values of the C-BC sample were significantly higher than those of the LC-BC sample ($p<0.05$), attributed to the fermentation of the cream with the *L. casei* in the

Table 1. *Physicochemical properties of Burrata cheese containing a probiotic L. casei strain*

Samples**	Storage days	Titration acidity (% LA)	pH value	Water activity (aw)	Salt (NaCl)	Moisture (%)	Fat (%)	Total nitrogen (%)
C-BC	1	0.28±0.02^{Ab}	6.20±0.06^{Aa}	0.894±0.02^{Aa}	0.76±0.12^{Cb}	60.62±0.33^{Bb}	24.75±0.18^{Aa}	1.08±0.21^{Bb}
	7	0.59±0.02^{Ba}	5.22±0.13^{Bb}	0.893±0.01^{ABa}	0.81±0.12^{Bb}	62.15±0.35^{Ab}	24.25±0.24^{Ba}	1.19±0.06^{Aa}
	14	0.62±0.03^{Ba}	4.92±0.09^{Cb}	0.894±0.02^{Aa}	0.83±0.05^{Ba}	62.24±0.29^{Ab}	24.75±0.31^{Aa}	1.20±0.08^{Ab}
	21	0.64±0.02^{Ba}	4.68±0.08^{Db}	0.891±0.03^{Bb}	0.88±0.06^{Ab}	61.41±0.28^{ABb}	24.50±0.46^{Ba}	1.15±0.08^{ABb}
LC-BC	1	0.37±0.02^{Aa}	5.80±0.06^{Ab}	0.893±0.03^{Aa}	0.84±0.08^{Ba}	61.21±0.36^{Ba}	24.25±0.37^{Aa}	1.37±0.28^{Aa}
	7	0.39±0.02^{Ab}	5.62±0.09^{Ba}	0.893±0.02^{Aa}	0.88±0.12^{Aa}	63.09±0.19^{Aa}	24.00±0.41^{Aa}	1.16±0.06^{Ba}
	14	0.53±0.02^{Bb}	5.42±0.08^{Ca}	0.891±0.03^{Ab}	0.83±0.05^{Ba}	63.53±0.21^{Aa}	24.65±0.25^{Aa}	1.41±0.13^{Aa}
	21	0.57±0.03^{Bb}	5.22±0.09^{Da}	0.894±0.02^{Aa}	0.91±0.08^{Aa}	63.96±0.26^{Aa}	24.95±0.30^{Aa}	1.20±0.17^{Ba}

Samples	Storage days	Water soluble nitrogen (%)	Protein (%)	Maturation index	Color values		
					L*	a*	b*
C-BC	1	0.12±0.06^{Aa}	6.88±0.21^{Bb}	11.14±0.38^{Aa}	92.30±0.19^{Aa}	-1.62±0.11^{Aa}	9.47±0.15^{Aa}
	7	0.12±0.02^{Aa}	7.60±0.06^{Aa}	10.44±0.08^{Ba}	92.75±0.25^{Aa}	-1.46±0.05^{Ab}	9.11±0.19^{Aa}
	14	0.11±0.03^{Ba}	7.69±0.21^{Ab}	9.02±0.35^{Ca}	95.31±0.27^{Ba}	-0.72±0.09^{Bb}	8.94±0.36^{Aa}
	21	0.11±0.0^{4Bb}	7.33±0.08^{ABb}	9.90±0.23^{BCa}	95.96±0.15^{Ba}	-0.85±0.11^{Bb}	6.62±0.19^{Ba}
LC-BC	1	0.11±0.01^{Bb}	8.76±0.70^{Ba}	8.06±0.67^{Bb}	92.63±0.21^{Aa}	-1.37±0.10^{Ab}	8.59±0.16^{Ab}
	7	0.10±0.01^{Cb}	7.42±0.15^{Ca}	8.89±0.25^{Bb}	92.39±0.09^{Aa}	-1.33±0.23^{Aa}	8.83±0.21^{Ab}
	14	0.11±0.05^{Ba}	9.03±0.33^{Aa}	7.54±0.25^{Cb}	95.84±0.16^{Ba}	-0.86±0.09^{Ba}	7.39±0.20^{Bb}
	21	0.13±0.06^{Aa}	7.69±0.42^{Ca}	10.61±0.09^{Aa}	95.59±0.22^{Ba}	-0.98±0.11^{Ba}	6.75±0.10^{Cb}

***LC-BC: Sample with probiotic L. casei; C-BC: control sample **a,b: The difference between two samples on each storage day is shown with different lower case letters and analyzed by Student's t-test (p<0.05). A,B,C: The difference between each sample on different storage days is shown with different upper case letters and analyzed by Tukey's multiple range test (p<0.05)*

LC-BC sample. Throughout the 21-day shelf life, the pH values decreased significantly in both samples (p<0.05). However, the pH reduction in the LC-BC group was found to be slower than that in the C-BC group, believed to be due to the acid-slowing properties of the *L. casei* used in the fermentation of the cream in the LC-BC sample. Due to its heterofermentative nature, genrally *L. casei* strains have an innate tolerance to acid stress, making it particularly valuable in the industry, especially for probiotic bacteria. In previous studies, the pH values of Burrata cheeses have shown variations in the range of 6.21 to 6.51 (Rea et al., 2016). Upon reviewing the literature, Minervini et al. (2017) conducted a comprehensive study on microbiological, chemical, and sensory analyses of Burrata cheese throughout a 16-day shelf life, which is the only study available in the literature. In present study, the observed reduction in pH values over the 21-day period in Burrata cheeses aligns with the findings of Minervini et al. (2017).

Throughout the storage period, no significant changes were observed in water activity, salt, moisture, fat, total nitrogen, water-soluble nitrogen, maturation index, and protein values among Burrata cheeses (Table 1).

In present study, the water activity values of Burrata cheese samples varied between 0.891 and 0.894 throughout their shelf life. Water activity values reported in previous studies on Burrata cheese range from 0.963 to 0.998 (Rea et al., 2016; Minervini et al., 2017). The relatively lower water activity values in the produced Burrata cheeses are believed to be attributed to the comparatively lower cream content used in the inner filling during production. Similar to Minervini et al. (2017), who examined water activity changes during storage, present study did not observe a significant difference in water activity values between groups throughout the shelf life (p>0.05).

In present study, the salt content of the Burrata cheeses varied between 0.76 % and 0.91 %. Previous research indicates that salt levels in the Burrata cheeses can range from 0.12 % to 0.99 % depending on the raw materials and production methods employed (Rea et al., 2016; Costantino et al., 2020). It was found that the salt content in the Burrata cheeses closely resembled the values reported by Rea et al. (2016), who produced traditional Burrata cheeses. The higher salt content observed in present study is attributed to the 21-day storage of the Burrata cheeses in a 1 % saline solution.

The moisture content of the Burrata cheese in present study ranged from 60.62 % to 63.96 %, while the fat content varied between 24.25 % and 24.95 %. These values align with findings from existing literature (Rea et al., 2016; Costantino et al., 2020).

The protein content of the Burrata cheeses produced in present research was observed to be lower than values reported in the literature. This discrepancy is thought to arise from the lower protein content in cow's milk used in production compared to the traditional use of buffalo milk in Burrata cheese production (Costantino et al., 2020). Additionally, Burrata cheese is a filled cheese containing cream and Mozzarella pieces. The protein difference between the samples is thought to be due to a higher proportion of cream taken during sampling.

Color values of the Burrata cheese samples in terms of CIE L*, a*, and b* are provided in Table 1. The use of probiotics in Burrata cheese production did not cause a significant change in

the lightness (L*) value. In present study, the Burrata cheeses exhibited a higher L* value compared to other studies (Akarca and Yıldırım, 2022), which could be attributed to differences in fat content in the milk composition or storage conditions. Notably, present study revealed negative a* values, a decrease in b* values, and lower b* values compared to other studies (Akarca and Yıldırım, 2022).

Microbial content of Burrata cheese containing a probiotic L. casei strain

Throughout the shelf life, the count of *Lactobacillus* spp. in the LC-BC group was significantly higher than in the C-BC group ($p<0.05$; Table 2A). After 21 days of shelf life, an increase in the number of *Lactobacillus* spp. was observed in both groups compared to the first day ($p>0.05$), aligning with literature findings on short-shelf-life and freshly consumed cheeses (Akarca and Yıldırım, 2022). Statistically significant differences were not observed in the investigated microbial parameters, including mesophilic bacteria, coliforms, yeast-mold, *Streptococcus* spp., *Lactococcus* spp., psychrophilic bacteria, proteolytic bacteria, and lipolytic bacteria counts between both groups of Burrata cheese.

Literature studies on the change in the count of total mesophilic bacteria during storage yield diverse results (Rea et al., 2016; Minervini et al., 2017). In present study, a non-significant increase in the count of total aerobic mesophilic bacteria was observed over the shelf life in both groups of Burrata cheese. This increase might stem from the cheese's high moisture content or the cream used in the inner filling, creating a favorable environment for microbial development.

Hygiene control analyses in C-BC and LC-BC groups revealed no development of the coliform group and yeast-mold throughout the 21-day shelf life. When compared to similar studies (Rea et al., 2016), this absence could be attributed to the lower pH and water activity values of the

Table 2. *Microbiological content of Burrata cheeses (A) and amount of L. casei strain in Burrata cheeses (B) (log CFU/g)*

A. Microbiological contents					
Samples*	**Storage days**	***Lactobacillus* spp.**	**Total mesophyll bacteria**	**Coliform group**	**Yeast-mould**
C-BC	1	7.26 ± 0.10^{Bb}	7.73 ± 0.03^{Bb}	n.d.	n.d.
	7	7.87 ± 0.09^{ABb}	8.17 ± 0.10^{Ab}	n.d.	n.d.
	14	8.01 ± 0.09^{Ab}	8.19 ± 0.08^{Ab}	n.d.	n.d.
	21	8.12 ± 0.02^{Ab}	8.38 ± 0.11^{Aa}	n.d.	n.d.
LC-BC	1	8.62 ± 0.09^{Aa}	8.05 ± 0.12^{Aa}	n.d.	n.d.
	7	8.59 ± 0.07^{Ba}	8.32 ± 0.10^{Aa}	n.d.	n.d.
	14	8.61 ± 0.06^{Aa}	8.34 ± 0.08^{Aa}	n.d.	n.d.
	21	8.65 ± 0.09^{Aa}	8.38 ± 0.07^{Aa}	n.d.	n.d.
Samples**	**Storage days**	***Streptococcus* spp. and *Lactococcus*spp.**	**Total psychrophilicbacteria**	**Proteolytic bacteria**	**Lipolytic bacteria**
C-BC	1	8.35 ± 0.07^{Aa}	6.68 ± 0.13^{Aa}	8.30 ± 0.26^{Bb}	n.d.
	7	8.34 ± 0.09^{Aa}	6.91 ± 0.05^{Aa}	8.53 ± 0.33^{Aa}	n.d.
	14	8.35 ± 0.07^{Aa}	7.23 ± 0.09^{Ba}	8.51 ± 0.31^{Aa}	n.d.
	21	8.38 ± 0.11^{Aa}	7.34 ± 0.10^{Ba}	8.35 ± 0.03^{Bb}	n.d.
LC-BC	1	8.43 ± 0.06^{Aa}	6.95 ± 0.10^{Aa}	8.51 ± 0.32^{Ba}	n.d.
	7	8.42 ± 0.07^{Aa}	7.20 ± 0.11^{Bb}	8.53 ± 0.24^{Ba}	n.d.
	14	8.41 ± 0.04^{Aa}	7.28 ± 0.08^{Ba}	8.65 ± 0.28^{Aa}	n.d.
	21	8.40 ± 0.08^{Aa}	7.33 ± 0.07^{Ba}	8.53 ± 0.17^{Ba}	n.d.

B. Amount of *L. casei* ATCC 393 in Burrata cheeses during storage period		
Samples for qPCR	**qPCR count (log CFU/mL)*** **	
The *L. casei* ATCC 393 in the growth medium (MRS broth)	10.382±0.095	
The Filling with *L.casei* ATCC 393	10.901±0.080	
Burrata cheeses on the first day and storage days	**LC-BC sample**	**C-BC sample**
The 1. day of BC-cheeses	10.362 ± 0.009^{C}	n.d.
The 7. day of BC-cheeses	11.137 ± 0.005^{B}	n.d.
The 14. day of BC-cheeses	12.403 ± 0.012^{A}	n.d.
The 21. day of BC-cheeses	12.398 ± 0.107^{A}	n.d.

LC-BC: sample with probiotic L. casei; C-BC: control sample n.d. not-detected. **a,b: The difference between two samples on each storage day is shown with different lower case letters and analyzed by Student's t-test ($p<0.05$). A,B,C: The difference between each sample on different storage days is shown with different upper case letters and analyzed by Tukey's multiple range test ($p<0.05$).*mean value±SD calculated from three different qPCR runs*

Burrata cheeses produced in present study compared to other studies on pasta filata cheeses.

In the Burrata cheese containing the *L. casei* stain produced in present research, a decrease in the count of aroma group bacteria, as observed in the study by Minervini et al. (2017) during the 21-day storage period, was noted. The total number of psychrophilic bacteria reached a significantly higher level at the end of the 21-day shelf life compared to the first day in both C-BC and LC-BC groups ($p<0.05$). However, in line with similar studies (Minervini et al., 2017), it can be interpreted that the *L. casei* used in the LC-BC group slows down the psychrophilic bacterial development.

In both statistically significant Burrata cheese samples, the count of proteolytic bacteria remained stable and did not change over the 21-day shelf life. Arora et al. (1990) found that *L. casei*, with its low protease and strong peptidase activity, reduces bitterness in cheese and provides positive effects texturally. While the literature has examined the count of proteolytic bacteria in Burrata cheeses, the changes observed in present study align with similar studies on pasta filata cheeses (Mozzarella) (Akarca and Yıldırım, 2022).

No development of lipolytic bacteria was observed in the Burrata cheeses produced in present study over the 21-day storage period. In literature studies, there is no specific investigation into the development of lipolytic bacteria in cheese. In a study evaluating the enzymatic activities of *L. casei* strains (Arora et al., 1990), it was determined that *L. casei* strains have relatively weaker esterase and lipase activities compared to other *Lactobacillus* species.

Change in amount of L. casei ATCC393 in Burrata cheeses during storage process

The *L. casei* content of the Burrata cheese samples (1st, 7th, 14th, and 21st days) was determined using the qPCR test (Table 2B). No study in the literature has investigated the attachment of probiotic bacteria to the Burrata cheese using qPCR, making this research the first to apply the qPCR test to quantify probiotic bacterial counts in probiotic Burrata cheese. Previous studies in the literature have highlighted the effectiveness and speed of qPCR as a tool for determining probiotic bacterial counts (Dreier et al., 2021). Especially, diacetyl is a natural compound that contributes to a buttery or creamy flavor in certain fermented products, including some types of cheese and yogurt. The production of diacetyl is influenced by various factors such as the specific strain of *Lactobacillus casei*, fermentation conditions, and the composition of the substrate. Detecting the presence of bacteria through PCR in this study serves as confirmation of the source responsible for diacetyl production.

The analysis revealed the attachment of *L. casei* strain to the Burrata cheese. Throughout the storage period, no significant change was observed in the *L. casei* strain content of the Burrata cheeses ($p>0.05$). Additionally, analyses conducted during the initial 14 days indicated that the Burrata cheese provided a favorable environment for the development of the *L. casei* strain, promoting its growth.

Volatile compound profile related to aroma of Burrata cheese containing a probiotic L. casei strain

The LC-BC and the C-BC samples produced in the study were examined in terms of the effect of probiotic microorganism on volatile compounds production on the first day and during the 21-day storage period. According to the results, a total of 13 volatile aroma compounds and carbon-dioxide were determined above the detectable limits, 6 compounds from the ketone group, 2 compounds from the alcohol group, 3 compounds from the acid group, and compound from each of the aldehyde and ester groups (Table 3; Figure 2A).

When the analysis results of the fresh samples produced on the first day were examined, the highest number of ketone group compounds were detected in both samples, followed by the acid group. The compound group with the highest total amount was the acid and alcohol groups, respectively, in the culture prepared with the probiotic strain, while the ketone group followed by the alcohol group in the cheese samples produced ($p<0.05$). In addition, the total major aroma compounds in the LC-BC sample containing probiotic *L. casei* strain from cheese samples were higher than those in the control sample (Figure 2).

On the first day, when the compounds were analyzed one by one, it was observed that the amount of all compounds was higher in the LC-BC sample ($p<0.05$). While acetoin, diacetyl and carbon dioxide were especially prominent in the LC-BC sample, acetoin, carbon dioxide acid and butanoic acid were prominent in the C-BC sample. Since the threshold value of acetoine (800 mg/L) is higher than the other compounds, its effectiveness is less. Therefore, diacetyl is the compound that determines the difference in the LC-BC sample and butanoic acid in the C-BC sample. Diacetyl provides a sweet, buttery and creamy flavor in fermented dairy products, especially fresh cheeses (Reyes-Díaz et al., 2020). In the case of LC-BC, diacetyl makes this creamy, slightly sweet and buttery flavor more pronounced. The diacetyl and acetoin compounds were thought to be formed as a result of the metabolic pathways of the added probiotic strain. *L. casei* strains convert lactase to pyruvic acid, which is converted to α-acetolactate. Diacetyl is formed by oxidative decarboxylation of this compound. Acetoin is formed by the conversion of α-acetolactate by the enzyme acetate decarboxylase or diacetyl by the enzyme reductase (Yebra et al., 2007). Furthermore, Díaz-Muñiz et al. (2006) showed that *L. casei* strain is responsible for acetoin production in ripened cheese. In another study (Peralta et al., 2014), the increase in the amount of diacetyl in hard type cheese using *L. paracasei* I90 strain was associated with this strain.

Of the other compounds, carbon dioxide, hezanoic acid, ethanol, ethyl acetate, acetone and acetaldehyde compounds were determined to be higher in the LC-BC sample, respectively, than in the control sample. Carbon dioxide and ethanol are acumulate in the cheese due to the facultative-heterofermentative feature of *L. casei* (Inês and Falco, 2018). Lactic acid bacteria with this feature can also produce organic acids other than lactic acid and/or

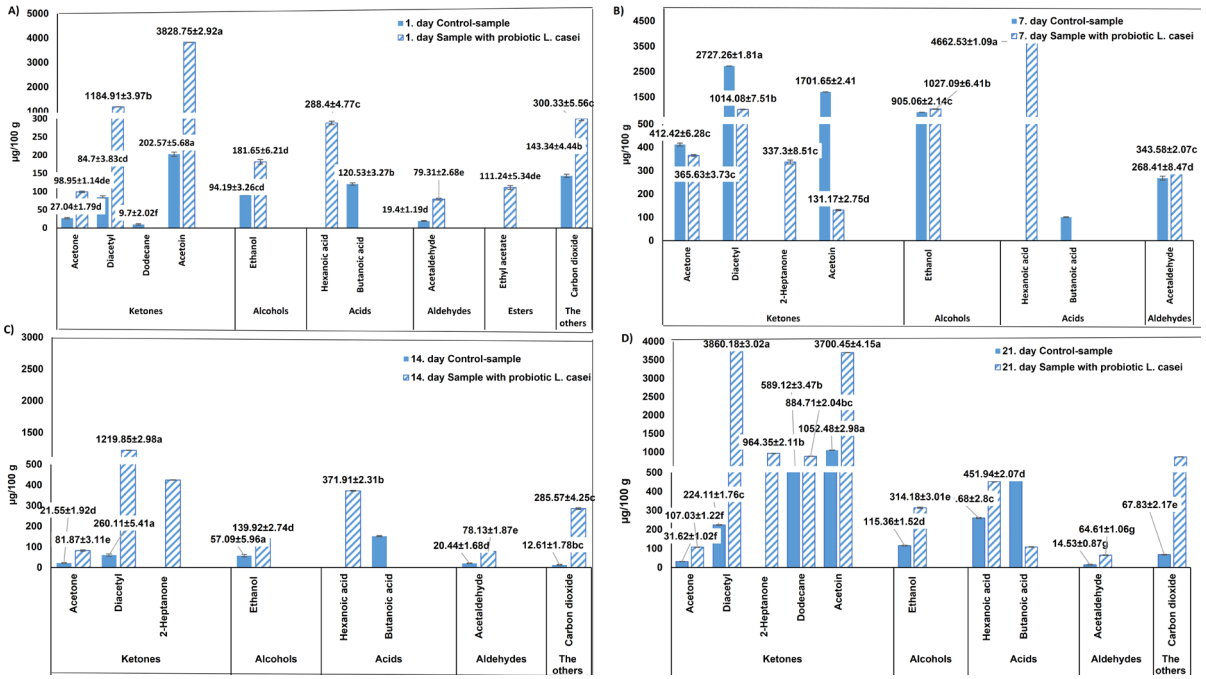

Figure 2. *Major aroma compounds of Burrata cheese containing a probiotic L. casei strain. (A) 1st day (B) 7th day (C) 14th day and (D) 21st ay of storage period*

compounds such as ethanol and carbon dioxide (Licandro-Seraut et al., 2014). Other compounds, ethyl acetate, acetone, and acetaldehyde, have also been associated with the presence of *L. casei* strain (Wang et al., 2021). Acetone, as a product of the citrate metabolism of the *L. casei* strain, is produced by decarboxylase from acetoacetate synthesized from acetyl-coA (Díaz-Muñiz et al., 2006). Acetone; it is associated with a sweet, called minty flavor. Ethyl acetate is produced biologically in mitochondria by the enzyme ethanol and acetyl-coA ethanol acetyltransferase. In addition, lipases are known to catalyze the esterification of alcohols with acids to obtain esters in the organic phase (Kourkoutas et al., 2006). This indicates that a strain with lipase activity contributes to extracellular ethyl ester formation (Zhang et al., 2020). In a study, the effect of *L. casei* group strains on the change of aroma compounds was investigated in a model system, and in this study, *L. casei* strains were shown to be responsible for ethyl acetate, acetone, acetaldehyde compounds (Stefanovic et al., 2017).

As for acids, only hexanoic acid was detected in the LC-BC sample, and only butanoic acid was detected in the C-BC sample. In addition, hexanoic acid has a cheesy, slightly sour, odor, while butanoic acid has a rancid, pungent, sour odor. In addition, butanoic acid is known to have an undesirable flavor in cheese when it has a high value (Marangoz and Bostan, 2020). This particularly highlights the example of LC-BC with the addition of *L. casei* ATCC393. It was thought that this situation gave the LC-BC sample a softer, more floral, fresh taste compared to the control. However, with a small amount of butanoic acid, these results were also supported by sensory analysis results (Figure 3). In a study, the production process and 14-day storage period of a fermented milk sample using probiotic *L. casei* Zhang in its fermentation were examined in terms of fingerprints of volatile metabolites. Similarly, in this study, hexanoic acid was found to be higher in samples using probiotic-strains (Sun et al., 2021). Hexanoic acid arises through lipid degradation or carbohydrate metabolism (Goswami et al., 2018). For this reason, it can be caused by both the strain and by the effect of the enzyme released by the strain. Besides the effect of hexanoic acid on the aroma of the product, it is likewise a precursor of another aroma compound, methyl-ketones (aroma precursor) since it is ß-keto-acid (Wang et al., 2021). On the other hand, in the metabolic pathway of butanoic acid, aceto-acetyl-CoA is formed from acetyl-CoA, from which butyl-CoA is formed. As a final step, butanoic acid is formed from butryl-CoA. As it can be understood from here, the probiotic strain used in the reactions of acetaldehyde and ethanol formation from acetyl-CoA, and/or acetoin formation from aceto-acetyl-CoA following acetoacetate reaction was thought to be effective. In addition, it can be predicted that the conversion of this strain to butyl-CoA is limited.

Since the shelf life of the Burrata cheese variety is already known as 14 days, which is determined in the literature, the produced cheeses were examined in a 21-day/3-week storage period. In this process (Figure 2B, C and D), the amount of acetone, one of the ketone compounds, increased in both cheese samples at the end of the first week of storage, and decreased in the next two weeks, reaching values close to the value of the cheeses on the first day. However, the value

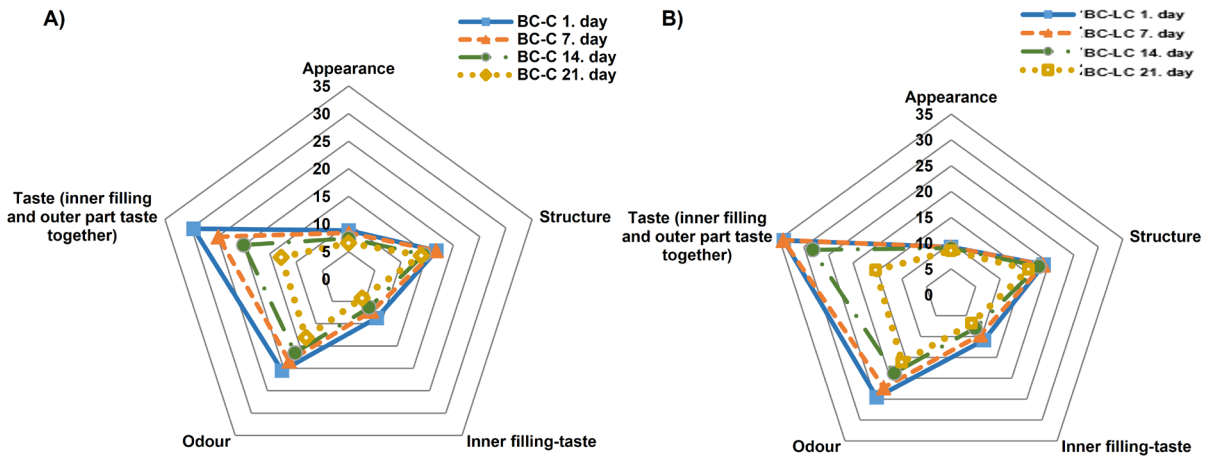

Figure 3. *Changes in sensory values of Burrata cheese containing a probiotic L. casei strain. (A) Control sample and (B) Burrata cheese sample containing a probiotic L. casei strain.*

of the LC-BC sample remained higher than the control sample. Diacetyl, on the other hand, increased in the control sample at the end of the first week and approached the LC-BC sample. The LC-BC sample remained approximately the same. During the ongoing process, diacetyl had a more stable value in the control sample, while it increased in the LC-BC sample, especially in the last week. On the other hand, 2-heptanone giving a fruity flavor similar to a green banana in fresh cream increased only in the LC-BC sample during the 3-week period, while it was not detected in the control sample. As for acetoin, an increase in the control sample and a decrease in the LC-BC sample were observed in the first week. At the end of the second week, this compound could not be detected in both samples, and at the end of the last week, it increased again, even more increase was observed in the LC-BC sample compared to the control sample and its value approached the value of the first day. Dodecane, an undesirable compound in cheese, was detected only after three weeks and was slightly higher in the LC-BC sample. When ketone compounds were evaluated together, it was thought that *L. casei* strain preserved the aromatic acceptability of the product, since acetone, diacetyl, acetoin, which contributed positively to the aroma properties of the product on the first day, were determined at higher values in the LC-BC sample. On the other hand, acetaldehyde, the only aldehyde compound determined in the analysis, also supported this situation. Since acetaldehyde is an intermediate product, it increased in the first week and decreased for both samples during the ongoing process, close to the values of the first day. However, the higher value of the LC-BC sample relative to the control sample was maintained.

When hexanoic and butanoic acids were examined during the storage period, it was observed that hexanoic acid increased during the first week, then decreased in the second week and remained approximately stable in the third week. The butanoic acid increased in the control sample in the second week and increased in the third week, while it increased only in the third week in the LC-BC sample. However, the value of the control sample is much higher than that of the LC-BC sample. Accordingly, it was thought that the added strain had a negative effect on the formation of butanoic acid, which gives an undesirable rancid flavor in cheese samples.

As a result; in terms of aroma, it was observed that the product was not very suitable for consumption for 21-days due to the increases in mainly dodecane and butanoic acid in the produced cheese samples, but in general, the aromatic properties of the Burrata cheese with added *L. casei* ATCC393 strain deteriorated more slowly. The *L. casei* strain can be characterized by a high productions of mainly ethyl acetate and 2-heptanone compunds, and then acetoin, diacetyl, hexanoic acid, acetaldehyde and CO_2 compounds for use in Burrata cheese production.

Sensory evaluation of Burrata cheese containing a probiotic L. casei strain

The sensory evaluation data of Burrata cheese with panelists are presented in Figure 3. Besides, The sensory evaluation form is given in Table S1 (Suplement-1). Throughout the shelf life, the sensory parameter scores of the LC-BC sample were consistently higher than those of the C-BC group, and this difference reached statistical significance only on the 7th day ($p<0.05$). The reduction in scores in the C-BC samples was attributed to perceived sourness and bland taste, especially originating from a bitter taste perception for both groups on the 21st day.

Consistent with studies in the literature, a slight melting was observed in the Burrata cheeses produced towards the end of the 21-day shelf life. Factors such as hydration of the protein matrix by absorbed water during storage (Kindstedt et al., 2010), proteolysis, salt concentration in the brine, and decreasing acidity are believed to contribute to melting in cheese towards the end of its shelf life.

Table 3. Volatile compounds related to aroma of Burrata cheese containing a probiotic L. casei strain (µg/100 g sample)

Compounds	The Burrata Cheeses (LC-BC and C-BC samples) and Storage Days (1-21)								Culture for BP-LC (First day)
	1. day		7. day		14. day		21. day		
	C-BC	LC-BC	C-BC	LC-BC	C-BC	LC-BC	C-BC	LC-BC	
Ketones									***Ketones***
3-Methyl-2-butanone	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	155.1±3.47^{g}
Acetone	27.04±1.79^{dB}	98.95±1.14^{deA}	412.42±6.28^{cA}	365.63±3.73^{cB}	21.55±1.92^{dB}	81.87±3.11^{eA}	31.62±1.02^{fB}	107.03±1.22^{fA}	348.04±5.65^{f}
Diacetyl	84.7±3.83^{cdB}	1184.91±3.97^{bA}	272.26±1.81^{dB}	1014.08±7.51^{bA}	260.11±5.41^{aB}	1219.85±2.98^{aA}	224.11±1.76^{cB}	3860.18±3.02^{aA}	n.d
2-Heptanone	n.d	n.d	n.d	337.3±8.51^{c}	n.d	424±1.01^{b}	n.d	964.35±2.11^{b}	n.d
Dodecane	9.7±2.02^{e}	n.d	n.d	n.d	n.d	n.d	589.12±3.47^{bB}	884.71±2.04^{bcA}	n.d
Acetoin	202.57±5.68^{aB}	3828.75±2.92^{aA}	1701.65±2.41^{aA}	131.17±2.75^{dB}	n.d	n.d	1052.48±2.98^{aB}	3700.45±4.15^{aA}	n.d
Alcohol									***Alcohol***
Ethanol	94.19±3.26^{cdB}	181.65±6.21^{dA}	905.06±2.14^{bB}	1027.09±6.41^{bA}	57.09±5.96^{cB}	139.92±2.74^{dA}	115.36±1.52^{dB}	314.18±3.01^{eA}	1981.12±6.83^{d}
Dodecanol	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	15255.63±4.92^{a}
Acids									***Acids***
Acetic acid	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	7946.06±2.32^{c}
Hexanoic acid	n.d	288.4±4.77^{c}	n.d	4662.53±1.09^{a}	n.d	371.91±2.31^{b}	260.68±2.80^{cB}	451.94±2.07^{dA}	10640.34±5.52^{b}
Butanoic acid	120.53±3.27^{b}	n.d	101.31±1.12^{e}	n.d	152±2.04^{b}	n.d	532.17±1.74^{bA}	108.24±1.53^{fB}	2201.04±3.27^{d}
Aldehydes									***Aldehydes***
Acetaldehyde	19.4±1.19^{dB}	79.31±2.68^{eA}	268.41±8.47^{dB}	343.58±2.07^{cA}	20.44±1.68^{dB}	78.13±1.87^{eA}	14.53±0.87^{gB}	64.61±1.06^{gA}	208.83±5.36^{fg}
Esters									***Esters***
Ethyl acetate	n.d	111.24±5.34^{de}	n.d	n.d	n.d	n.d	n.d	n.d	398.15±1.98^{f}
Others									***Others***
CO_2	143.34±4.44^{bB}	300.33±5.56^{cA}	n.d	n.d	12.61±1.78^{bcB}	285.57±4.25^{cA}	67.83±2.17^{eB}	867.42±1.08^{cA}	1299.75±3.06^{e}

**LC-BC: sample with probiotic L. casei; C-BC: control sample n.d. not-detected. **^{a,b},...: Differences between the components constituting the aroma profile of each sample on each storage day are shown with different lower case letters and analyzed with Tukey's multiple range test (p<0.05). A,B: Differences between two samples for each component on each storage day are shown with different upper case letters and analyzed with Student's t-test (p<0.05).*

In the LC-BC samples, the perceived sweetness, thought to be of bacterial origin, positively influenced the overall rating. Sweetness and creamy taste perceptions showed similarity to aroma compounds. Moreover, especially in terms of taste parameter, the value of the LC-BC sample on the 14th day of the storage process is very close to the value of the control sample on the first day (Figure 3). Accordingly, it has been determined that adding the *L. casei* strain in the production of the Burrata cheese has a positive effect on the sensory preferability in general, especially on smell and taste. The effect of using a probiotic strain was consistent with similar studies in the literature (Minervini et al., 2017; Akarca and Yıldırım, 2022).

Conclusions

BurrataCheese, characterized by its creamy texture and unique visual presentation, is a fresh pasta filata cheese originating from Italy and gaining popularity across Europe and the United States. The incorporation of the *L. casei* strain into Burrata cheese not only enhances its aroma-profile but also distinguishes it within the category of fresh pasta filata cheeses. This addition has positively impacted both the sensory appeal and the shelf life of Burrata cheese. The fresh nature and filled structure of Burrata cheese create an ideal environment for the development of probiotic bacteria. The *L. casei* strain can be characterized by a high productions of mainly ethyl acetate and 2-heptanone compunds, and then acetoin, diacetyl, hexanoic acid, acetaldehyde and CO_2 compounds for use in Burrata cheese production. This study observed an increase in certain compounds, such as dodecane and butanoic acid, during storage, which may negatively impact flavor. Future research could explore strategies to mitigate these undesirable compounds through different fermentation conditions or probiotic culture combinations. Additionally, while qPCR confirmed the presence of *L. casei* in the cheese, further studies could investigate its long-term viability and interaction with other bacteria, as well as its potential effects on product quality and human health. Analytical findings suggest that Burrata cheese serves as an excellent vehicle for expanding the array of probiotic products. Notably, there is a scarcity of literature on Burrata cheese, and this study makes a valuable contribution to the existing body of knowledge. This research marks the first exploration of probiotic additions to Burrata cheese in the literature.

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Funkcionalni Burrata sir obogaćen *Lacticaseibacillus casei* ATCC 393: Uvid u proizvodnju, jedinstvene karakteristike i aromatski profil

Sažetak

Cilj ovog istraživanja bio je poboljšati aromatski profil, funkcionalna svojstva i produžiti rok trajanja sira Burrata pomoću *Lacticaseibacillus casei* subsp. *casei* ATCC393. Proizvedene su dvije serije sira; probiotička (LC-BC) i kontrolna (C-BC) skupina. Rezultati su pokazali uspješnu integraciju ovog soja u sir Burrata. Utvrđeno je da je sir dobar medij za rast *L. casei* te da on proizvodi visoke koncentracije različitih spojeva uglavnom etil-acetata i 2-heptanona, a zatim acetoina, diacetila, heksanske kiseline i acetaldehida. Sir LC-BC, obogaćen diacetilom, pokazao je kremastu, blago slatku i maslastu aromu, što je pridonijelo njegovom jasnom senzorskom profilu. *L. casei* ATCC393 se pokazao kao vrijedan dodatak, poboljšavajući kvalitetu, senzorske karakteristike i rok trajanja Burrata sira. Pokazalo se da je Burrata sir vrlo prikladan za primjenu probiotika.

Ključne riječi: *Lacticaseibacillus casei*; probiotik; Burrata sir; aromatski spojevi; qPCR

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