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Effect of protective cultures on selected parameters of fresh cheese

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Abstract: Protective cultures are selected microorganisms that inhibit undesirable microbiota through metabolic activity, thereby improving the safety and shelf life of dairy products. This study evaluated the effect of protective cultures on the physicochemical, microbiological, and sensory properties of fresh cheese during 21 days of storage at 6–8 °C. Three variants were prepared: a control with the starter culture CHN-19 and two experimental samples containing FreshQ® Cheese 3 and FreshQ® 9 (*Lacticaseibacillus rhamnosus*). The application of protective cultures resulted in a slightly faster acidification but did not significantly affect dry matter or fat content. Microbiological analysis revealed higher counts of lactic acid bacteria and generally lower counts of psychrotrophic microorganisms in samples containing protective cultures on several storage days, while *Escherichia coli* and moulds were not detected. Sensory evaluation indicated that cheeses with protective cultures, particularly FreshQ® 9, maintained a pleasant flavour and appearance even after 21 days of storage, whereas the control sample became sensorially unacceptable after approximately two weeks. The application of protective cultures therefore enhances the sensory stability and microbial safety of fresh cheeses without adversely affecting their technological characteristics.

Keywords: dairy products; lactic acid bacteria; *Lacticaseibacillus rhamnosus*; microbial safety; sensory quality; physicochemical properties

Protective cultures consist of specifically selected microorganisms capable of inhibiting the growth of pathogens and spoilage microbiota. Their antimicrobial effect is primarily related to the production of organic acids such as lactic and acetic acid, which lower the pH and inhibit competing microorganisms (Kilcast and Subramaniam 2011). They may also generate hydrogen peroxide, which acts as an oxidative stress factor for sensitive microbes (Fischer and Titgemeyer 2023). Another important mechanism is the synthesis of low-molecular-weight metabolites and bacteriocins, which can disrupt membrane integrity or inhibit essential enzymatic pathways (Holzapfel et al. 1995).

In addition to metabolite formation, protective cultures contribute to food safety through competition for nutrients and ecological niches, preventing the establishment of undesirable microorganisms (Arqués et al. 2015). Certain species also employ quorum-sensing mechanisms, enabling them to detect environmental signals and adjust metabolic activity to outcompete spoilage flora (Bintsis and Papademas 2024).

The effectiveness of a protective culture depends on the initial contamination level, fermentation conditions, and storage environment. When the initial microbial load is high, the culture cannot fully restore safety, but it can significantly slow spoilage and extend shelf life (Kilcast and Subramaniam 2011).

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Lactic acid bacteria (LAB) are the most common protective cultures in dairy products due to their well-documented safety and antagonistic activity against undesirable microorganisms and pathogens. Their selection and use are based on the screening of phenotypic and genotypic characteristics to ensure the desired activity within a specific food matrix (Bintsis and Papademas 2024). Species such as *Lacticaseibacillus rhamnosus* and *Lacticaseibacillus paracasei* have been shown to prolong the shelf life of dairy products by suppressing the growth of yeasts and moulds, as well as heterofermentative lactic acid bacteria. These cultures can function as adjunct starters and typically exert minimal influence on sensory attributes. Some also provide additional protection against *Listeria monocytogenes*, offering a secondary barrier against contamination (Kilcast and Subramaniam 2011). The antifungal activity of *L. rhamnosus*, *Lactobacillus* spp., and *Leuconostoc* spp. has been demonstrated against *Penicillium commune*, *Mucor racemosus*, *Galactomyces geotrichum*, and *Yarrowia lipolytica* (Leyva Salas et al. 2018), as well as for *Lactiplantibacillus plantarum* (Ouiddir et al. 2019) and *Lactobacillus acidophilus* (Motalebi Moghanjougi et al. 2020). Moreover, some LAB strains are capable of binding aflatoxin B1, thereby reducing its bioavailability (Varsha and Nampoothiri 2016).

The growth rate and competitiveness of protective cultures depend on their adaptation to the substrate. Their effectiveness is influenced by internal and external factors such as redox potential, water activity, pH, and temperature. A sufficiently high inoculation level promotes dominance during fermentation. Besides metabolic activity, starter cultures occupy ecological niches, preventing the colonization of undesirable microorganisms. The simultaneous production of several antimicrobial metabolites is part of a complex system that allows protective cultures to remain active even in competitive environments (Holzapfel et al. 1995; Arqués et al. 2015).

During milk fermentation, various metabolites such as acetic, lactic, propionic, phenyllactic, and hydroxyphenyllactic acids are produced. These compounds exhibit inhibitory activity against bacteria and fungi, primarily due to pH reduction and the diffusion of undissociated acid forms through cell membranes. This leads to intracellular acidification, protein denaturation, inhibition of transmembrane transport, and disruption of enzyme function and oxidative balance, ultimately inhibiting microbial growth (Nes and Johnsborg 2004; Bintsis and Papademas 2024). Other antimicrobial metabolites include hydrogen peroxide, fatty acids, diacetyl, acetoin, and bacteriocins. Diacetyl and acetoin can react with arginine residues in proteins, altering their structure, and both compounds are capable of interacting with DNA and contributing to its degradation (Cesselin et al. 2021; Bintsis and Papademas 2024).

Several previous studies have demonstrated the successful application of protective cultures in fresh cheese. Barukčić et al. (2021) reported that protective cultures improved microbial stability and maintained desirable sensory attributes during storage. Ibrahim and Awad (2018) showed that the use of *Lacticaseibacillus rhamnosus* effectively suppressed *Staphylococcus aureus* in fresh Domiati-type cheese while preserving sensory quality. Similar observations were described by Pappa et al. (2022), who found that protective cultures helped maintain flavour acceptability and delayed spoilage in fresh goat cheese. These findings provide strong evidence that protective cultures represent an effective biopreservation strategy for fresh dairy products.

Fresh cheeses are high-moisture, non-ripened dairy products with a relatively short shelf life, typically ranging from 7 to 14 days under refrigerated conditions, depending on hygienic quality, packaging, and storage temperature (Fox et al. 2017). Their rapid spoilage is mainly associated with the growth of psychrotrophic microorganisms and yeasts, which limits their commercial distribution and sensory acceptability.

The objective of this study was to evaluate the effect of protective cultures on the physicochemical, microbiological, and sensory properties of fresh cheeses during storage.

MATERIAL AND METHODS

Production of fresh cheeses. The production procedure followed the technological steps described in České cechovní normy (2022-07-13-0537). Three variants of fresh cheese were prepared: a control sample containing only a mesophilic starter culture (CHN-19; *Lactococcus lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis biovar diacetylactis*, *L. lactis* subsp. *lactis*, *Leuconostoc mesenteroides* subsp. *cremoris*), and two experimental variants containing protective cultures FreshQ® Cheese 3 (Q3) and FreshQ® 9 (Q9) (Chr. Hansen, Denmark). Both protective cultures include *Lacticaseibacillus rhamnosus*. The cultures were selected based on preliminary screening experiments.

Commercial standardised milk (Pilos, Czech Republic; 1.5% fat) was used for cheese production.

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The milk was poured into three thermostatic baths (VAC-STAR, Czech Republic), heated to 65 °C, and then cooled to 33 °C. At this temperature, the starter culture CHN-19 and the protective culture were added (0.1 g of each per 4 L of milk) and allowed to ferment for 40 min. calcium chloride (1.6 mL of 36% solution per 4 L of milk) and rennet (Fromase 220 TL BF, strength 1 : 5 333, dosage 1.6 mL per 4 L of milk) were subsequently added. Coagulation took 40 min at 33 °C. The curd was cut and filled into UV-sterilised cheese moulds. During the following 20 h, whey was drained off with occasional turning of the cheeses at 6–8 °C. The resulting cheeses weighed approximately 40 g each, corresponding to a yield of 18 pieces per 4 L of milk. Samples were stored at 6–8 °C for 21 days and subjected to further analyses.

Microbiological analysis during storage. Microbiological analyses were performed in duplicate for each variant on days 0, 7, 14, and 21 after production. The following microbial groups were determined:

Total viable counts (TVC): Plate Count Agar with skimmed milk (Biokar Diagnostics, France), 30 °C for 72 h.

Lactic acid bacteria (LAB): MRS Agar (Biokar Diagnostics, France), 30 °C for 72 h and 37 °C for 48 h under anaerobic conditions. The incubation at 30 °C for 72 h was used for mesophilic LAB such as *Lactococcus* and *Leuconostoc*, whereas the incubation at 37 °C for 48 h under anaerobic conditions was used for thermophilic or facultatively heterofermentative LAB.

***Escherichia coli* and coliform bacteria:** Chromogenic medium (*E. coli*/Coliform Agar, Harlequin® NCM1002, Neogen, UK), 37 °C for 24 h.

Psychrotrophic microorganisms: Determined using Plate Count Agar (Biokar Diagnostics, France). Samples were incubated at 7 °C for 10 days, and characteristic colonies were counted and expressed as CFU·g^{-1}.

Yeasts and moulds: Chloramphenicol Glucose Agar (Biokar Diagnostics, France), 25 °C for 72–120 h.

After incubation, characteristic colonies were counted, and results were expressed as colony-forming units per gram of cheese (CFU·g^{-1}).

Physicochemical analyses. The following parameters were evaluated on days 0, 7, 14, and 21 after production: pH, titratable acidity, dry matter, diacetyl intensity, and colour (CIELAB). Fat and total nitrogen content were determined at day 0. All analyses were carried out in triplicate, and mean values were reported.

Active acidity (pH): measured using a WTW pH 95 meter with a combined puncture electrode (SenTix, Germany).

Titratable acidity: determined by the Soxhlet–Henkel method using 0.25 mol·L^{-1} NaOH and phenolphthalein as indicator.

Fat content: analysed according to the van Gulik method (IDF Standard 221:2008), based on dissolution of non-fat solids in sulfuric acid followed by centrifugation in a butyrometer.

Total nitrogen: determined by the Kjeldahl method according to AOAC Official Method 991.20 (AOAC 2005). The nitrogen content (protein and non-protein) was expressed as grams per 100 g of sample. Samples were digested with sulfuric acid and a copper catalyst, distilled after addition of NaOH, and the released ammonia was captured in boric acid and titrated to calculate crude protein content.

Dry matter: measured by drying the sample at 102 ± 2 °C to constant weight according to IDF Standard 21:1987.

Diacetyl intensity: assessed visually based on colour reaction in an alkaline environment (40% NaOH, 30 min), where diacetyl forms a pink complex with creatine according to the spectrophotometric procedure described by Engström et al. (2021). Intensity was rated from + (very weak, slightly aromatic) to +++++ (very strong, highly aromatic).

Colour (CIELAB): measured using a CM-3500d spectrophotometer (Konica Minolta, Japan) with CW-100s software under standard illuminant D65, geometry d/10, and an 8 mm aperture. Each sample was measured three times and averaged. The total colour difference was calculated as:

$$\Delta E^*_{ab} = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2} \quad (1)$$

where: ΔL^* – the difference in lightness; Δa^* – the difference on the green–red axis; Δb^* –the difference on the blue–yellow axis between samples.

Sensory analysis. Sensory evaluation was performed according to ISO 6658 (2005) using a 10-member trained panel. Samples were assessed in individual booths under standardised lighting and temperature conditions. Evaluations were carried out on a 100-mm unstructured line scale, where the left end corresponded to the lowest and the right end to the highest intensity or pleasantness.

The following attributes were evaluated:

External appearance (0 = unpleasant, 100 = very pleasant)

Texture (crumbliness) (0 = non-compact, crumbly; 100 = compact)

Colour (0 = grey; 100 = white, milky)

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Aroma (0 = unpleasant; 100 = pleasant)
Hardness in the mouth (0 = very hard; 100 = very soft)
Flavour (0 = unpleasant, impure; 100 = very pleasant)
Sour flavour (0 = absent; 100 = very intense)
Creamy flavour (0 = absent; 100 = very intense)
Salty flavour (0 = absent; 100 = very intense)
Foreign/untypical flavour (0 = absent; 100 = very intense)
Overall impression (0 = unacceptable; 100 = fully acceptable)

Panellists were instructed to rinse their mouths with water between samples. Results were recorded digitally, converted to numerical values (0–100), and statistically analysed.

A ranking test was also performed, in which panellists ordered the samples from most to least preferred.

Statistical analysis. All data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using Microsoft Excel 365 with the Real Statistics Resource Pack (Release 9.9). Normality of data distribution was assessed using the Shapiro–Wilk test. For comparison of two independent groups (control vs. Q3 and control vs. Q9), the non-parametric Mann–Whitney U test was applied. Differences among more than two groups or across storage days were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. For repeated measurements within the same variant over storage time, paired t-tests were employed. Statistical significance was accepted at $P < 0.05$. Graphical visualization of sensory data was performed using radar plots.

RESULTS AND DISCUSSION

Physicochemical analysis of fresh cheeses. The results of the physicochemical analysis of fresh cheeses are summarised in Table 1. Initial chemical parameters determined on day 0 included fat and total nitrogen content. The obtained values corresponded well with those reported by Alexa et al. (2018), who found 22% dry matter, 15.5% protein, and 8% fat in control samples of fresh cheese. Similar results were described by Barukčić et al. (2021), who compared several fresh cheese variants, including samples containing protective cultures. Their protein contents ranged between 13.7% and 14.8%, with the highest values observed in cheeses with protective cultures. In the present experiment, no statistically significant differences were found among variants ($P > 0.05$), indicating that the applied cultures did not alter the gross composition of the cheese matrix.

A decreasing trend in active acidity (pH) was observed during storage (Table 1), consistent with the continuous production of lactic acid by lactic acid bacteria. The initial pH ranged between 4.71 and 4.80, dropping to 4.58–4.61 by day 21. These values agree with the findings of Arvanitoyannis et al. (2011), who reported a reduction from pH 4.66 to 4.47 during storage. Statistical evaluation (one-way ANOVA, $P < 0.05$) confirmed faster acidification in cheeses containing

Table 1. Basic physicochemical parameters of fresh cheeses (mean ± SD) during storage at 6–8 °C for 21 days

Day	Variant	pH	°SH	Lipids (%)	Proteins (%)	Dry matter (%)	Diacetyl
	control	4.72 ± 0.14	99.12 ± 0.2	7.00 ± 0	17.08 ± 0.28	27.54 ± 0.61	+++
0	+ Q3	4.80 ± 0.01	100.98 ± 0.1	7.00 ± 0	15.06 ± 1.26	28.03 ± 0.37	++++
	+ Q9	4.71 ± 0.01	100.68 ± 0.64	7.00 ± 0	15.70 ± 0.11	29.21 ± 0.11	++++
	control	4.67 ± 0.09	109.07 ± 1.04	ns	ns	29.09 ± 0.22	+++
7	+ Q3	4.67 ± 0.01	105.99 ± 0.88	ns	ns	27.70 ± 0.10	++++
	+ Q9	4.66 ± 0.00	107.17 ± 0.00	ns	ns	28.76 ± 0.19	++++
	control	4.63 ± 0.01	113.05 ± 0.15	ns	ns	30.40 ± 0.32	++++
14	+ Q3	4.62 ± 0.01	111.75 ± 0.25	ns	ns	29.79 ± 0.06	+++++
	+ Q9	4.61 ± 0.00	110.35 ± 0.45	ns	ns	29.11 ± 0.11	+++++
	control	4.61 ± 0.00	114.75 ± 0.05	ns	ns	30.19 ± 0.05	++++
21	+ Q3	4.58 ± 0.00	113.50 ± 0.00	ns	ns	30.02 ± 0.14	+++++
	+ Q9	4.58 ± 0.00	109.80 ± 0.30	ns	ns	29.18 ± 0.40	+++++

Diacetyl intensity scale: + (very weak, slightly aromatic) to +++++ (very strong, highly aromatic)
Q3 – FreshQ® Cheese 3; Q9 – FreshQ® 9; ns – not specified; °SH – Soxhlet-Henkel degree (titratable acidity)

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FreshQ® 3 and FreshQ® 9 compared with the control. The titratable acidity increased correspondingly, reflecting the accumulation of organic acids, as also reported by Pavunc et al. (2012).

The dry-matter content increased gradually during storage (Table 1), which is typical for high-moisture fresh cheeses as whey continues to be released. The initial dry-matter values (27.5–29.2%) were comparable to those of commercial products (approximately 30%; Fox et al. 2017). Moisture loss may also be promoted by residual rennet activity stimulating syneresis. According to Riahi et al. (2007), water loss during ripening or storage is commonly associated with dehydration processes influenced by pH and salt content. In the present study, ANOVA confirmed significant differences ($P < 0.05$) on days 7 and 14. Post-hoc tests revealed that cheeses with FreshQ® 3 showed significantly higher dry-matter values on day 7, while FreshQ® 9 differed from the control on day 14.

The diacetyl reaction produced positive results in all samples (Table 1). On day 0, the control variant exhibited moderate pink coloration (moderately aromatic), whereas cheeses containing protective cultures displayed a stronger colour (highly aromatic). This trend persisted throughout storage, with both treated variants showing a marked increase in diacetyl intensity by days 14 and 21. These findings correspond with the known ability of *L. rhamnosus* to produce diacetyl as a bioprotective metabolite (Jyoti et al. 2003), suggesting that FreshQ® 9 contributed to enhanced flavour formation through its metabolic activity.

Microbiological analysis. The results of the microbiological analysis of fresh cheeses are summarised in Table 2. Throughout storage, the highest microbial counts were recorded for total viable microorganisms, ranging from 7 to 10 log CFU·g^{-1}. These high values mainly reflect the presence of lactic acid bacteria (LAB) from the starter and protective cultures, whose activity is both expected and desirable. While total viable counts and LAB counts showed similar trends across all variants ($P > 0.05$), the counts of psychrotrophic microorganisms were consistently lower in the cheeses containing protective cultures. Both FreshQ® 3 and FreshQ® 9 exhibited significantly reduced psychrotrophic populations compared with the control on several storage days ($P < 0.05$), indicating a clear suppressive effect of the protective cultures on cold-tolerant spoilage microflora.

Considering that the typical shelf life of fresh cheeses rarely exceeds 14 days, the reduced counts of psychrotrophic microorganisms observed in samples containing protective cultures on several storage days represent a technologically relevant improvement in microbial stability.

Importantly, *Escherichia coli* was not detected in any of the samples, indicating that hygienic conditions were maintained throughout production and storage. Coliform bacteria were present only in very low numbers in samples Q3 and Q9 immediately after manufacture but were not detected in subsequent analyses. This confirms proper hygiene practices and the possible inhibitory effect of protective cultures. Similar results

Table 2. Mean microbial counts (log CFU·g^{-1}, $n = 2$) in fresh cheese samples during storage at 6–8 °C for 21 days

Day	Variant	TCM	Lactic acid bacteria		*E. coli* and coliform bacteria		Psychr. m.	Micromycetes		
			LAB 30 °C	LAB 37 °C anaer.	*E. coli*	other colif. bac.		total	yeasts	moulds
0	control	8.67	5.70	2.70	nd	nd	8.27	nd	nd	nd
	+ Q3	8.74	7.83	7.99	nd	0.70	8.06	nd	nd	nd
	+ Q9	8.59	7.80	7.84	nd	0.88	6.06	nd	nd	nd
7	control	9.02	5.96	3.07	nd	nd	6.81	2.53	2.53	nd
	+ Q3	9.12	8.19	8.18	nd	nd	6.20	0.40	nd	0.40
	+ Q9	8.89	8.16	8.28	nd	nd	7.81	nd	nd	nd
14	control	8.98	6.02	3.70	nd	nd	8.08	2.40	2.40	nd
	+ Q3	9.07	8.40	8.43	nd	nd	8.32	nd	nd	nd
	+ Q9	8.82	8.30	8.43	nd	nd	7.79	0.40	0.40	nd
21	control	7.44	5.24	3.40	nd	nd	6.56	nd	nd	nd
	+ Q3	8.28	8.20	8.14	nd	nd	7.28	nd	nd	nd
	+ Q9	8.32	8.17	8.19	nd	nd	7.12	2.26	2.26	nd

TCM – Total count of microorganisms; Psychr. m. – Psychrotrophic microorganisms; nd – not detected; LAB – lactic acid bacteria; Q3 – FreshQ® Cheese 3; Q9 – FreshQ® 9

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were reported by İncili et al. (2020), who demonstrated the ability of *Pediococcus acidilactici* to inhibit *E. coli* O157:H7 during refrigerated storage.

Yeasts and moulds were found only sporadically and in very low counts in all cheese variants (Table 2). The growth of micromycetes is expected to be reduced in samples containing protective cultures, which are known to exhibit antifungal activity. *Lacticaseibacillus rhamnosus* and other LAB species have previously been shown to inhibit *Penicillium commune*, *Mucor racemosus*, *Galactomyces geotrichum*, and *Yarrowia lipolytica* (Leyva Salas et al. 2018). In the present study, however, mould growth in the control variant was also minimal, and thus no statistically significant differences among variants were observed ($P > 0.05$).

Overall, the microbiological results demonstrate that the application of protective cultures did not negatively affect the natural microbial balance of the fresh cheese but contributed to slightly better microbial stability during storage.

Although samples containing protective cultures showed lower counts of psychrotrophic microorganisms on several storage days, this effect was not observed uniformly throughout the entire storage period. Such variability can be attributed to the natural heterogeneity of fresh cheese microflora, low initial contamination levels, and the competitive interactions between starter, protective, and background microbiota. In high-moisture fresh cheeses, minor fluctuations in psychrotrophic counts are common and do not necessarily contradict the overall trend of improved microbial stability.

Colour evaluation. The colour parameters of fresh cheeses are presented in Table 3. The lightness values (L^*) ranged between 84 and 90, showing no statistically significant differences among the variants ($P > 0.05$). Such high L^* values indicate a light, creamy-white appearance typical for fresh cheese. This stability of brightness throughout storage suggests that neither of the applied protective cultures affected the optical characteristics of the cheese surface.

The chromatic coordinate a^* remained negative and close to zero, reflecting a slight shift toward the green region of the CIELAB colour space. No significant differences were observed among samples during storage. Similarly, the b^* coordinate remained positive, corresponding to yellow tones, with values ranging between 7.8 and 9.4. The slight increase in b^* at the end of storage indicates a minor tendency toward more yellow coloration, possibly due to moisture loss and concentration of solids. Comparable trends were described by Juan et al. (2008) in ewe's milk cheeses, where b^* values increased from 8.99 to 9.88 during prolonged storage.

For comparison, Yasin and Shalaby (2013) reported colour values of cottage-type cheeses as $L^* \approx 88.4$, $a^* \approx -9.17$, and $b^* \approx 14.73$, which corresponds well with the range observed in this study. Overall, the results demonstrate that the addition of protective cultures did not significantly affect the visual colour characteristics of fresh cheeses during refrigerated storage.

Sensory analysis. The radar chart in 1 illustrates the sensory evaluation of all parameters on day 0. It is evident that the individual cheese variants differed only

Table 3. CIELAB colour parameters (mean ± SD, $n = 3$) of fresh cheeses during storage at 6–8 °C for 21 days

Day	Variant	L^*	a^*	b^*
0	control	85.53 ± 0.16	−0.79 ± 0.04	7.58 ± 0.15
	+ Q3	84.38 ± 0.79	−0.83 ± 0.05	8.48 ± 0.32
	+ Q9	87.49 ± 0.24	−0.95 ± 0.03	8.27 ± 0.26
7	control	86.18 ± 0.16	−0.93 ± 0.01	8.41 ± 0.20
	+ Q3	86.97 ± 0.35	−1.04 ± 0.02	8.13 ± 0.21
	+ Q9	86.76 ± 1.25	−0.95 ± 0.02	7.98 ± 0.32
14	control	90.03 ± 0.27	−1.11 ± 0.01	8.02 ± 0.08
	+ Q3	90.11 ± 0.66	−1.11 ± 0.04	8.86 ± 0.41
	+ Q9	89.62 ± 1.12	−1.13 ± 0.04	8.44 ± 0.11
21	control	85.39 ± 0.26	−1.04 ± 0.06	9.42 ± 0.25
	+ Q3	86.19 ± 0.39	−1.07 ± 0.03	8.51 ± 0.06
	+ Q9	86.26 ± 0.73	−1.12 ± 0.08	9.31 ± 0.48

L^* – lightness (100 = white, 0 = black); a^* – positive values indicate red, negative values indicate green; b^* – positive values indicate yellow, negative values indicate blue; Q3 – FreshQ® Cheese 3; Q9 – FreshQ® 9

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minimally. The descriptors of appearance, consistency, and colour were rated between 80 and 90 points on the 0–100 scale. The attribute *firmness in mouth* reached an average score of approximately 62 points across all samples, indicating a rather soft texture (0 = very hard, 100 = very soft).

In the *flavour* descriptor, the highest mean score was obtained for the Q9 variant (80 points), which also achieved the highest score in *acid taste*. The control and Q3 variants reached approximately 73 points in both attributes. Due to high variability among panellists, these differences were not statistically significant ($P > 0.05$).

The *off-flavour* descriptor was not recorded by any assessor, and *salty taste* was also absent, corresponding to the fact that no salt was added during production. The *overall impression* of all three variants was evaluated very positively, ranging from 75 to 82 points. Although statistical analysis did not confirm significant differences among variants, the ranking test indicated the following preference order: control sample > Q9 > Q3.

According to Engström et al. (2021), protective cultures differ from starter cultures in that they are often non-fermentative and have minimal impact on sensory attributes, whereas starter cultures develop during fermentation and influence flavour, aroma, and texture. Adjunct or multifunctional cultures may act as intermediates between these two groups (Vítová et al. 2011).

Figure 2 presents the mean values of sensory descriptors for all cheese variants on day 7 of storage, when similar results to those obtained immediately after production were expected. The attributes of external appearance, texture, colour, and aroma remained at comparable levels, ranging between 75 and 85 points. No statistically significant differences ($P > 0.05$) were observed for these parameters. The *hardness in the mouth* descriptor also remained stable at approximately 62 points.

However, the *flavour* descriptor was rated considerably lower compared with day 0, reaching only 52–57 points. For each sample, a paired *t*-test ($P < 0.05$) confirmed a statistically significant decrease in flavour pleasantness between the initial evaluation and day 7 of storage.

The intensity of *sour flavour* did not change in the control or Q3 variant, whereas in Q9 it markedly decreased from 81 to 57 points. This difference was statistically significant both compared with the control and with the initial (day 0) value of Q9. Another significant difference ($P < 0.05$) was detected in the *foreign/untypical flavour* descriptor, which some panellists described as slightly bitter or pungent.

The *overall impression* scores decreased to an average of 60 points across variants. A statistically significant reduction was confirmed only for Q3 and Q9 ($P < 0.05$). The ranking test indicated that the best-rated sample was Q9, followed by Q3, while the control sample received the lowest scores. Compared with the initial evaluation, where the variants showed almost no differences,

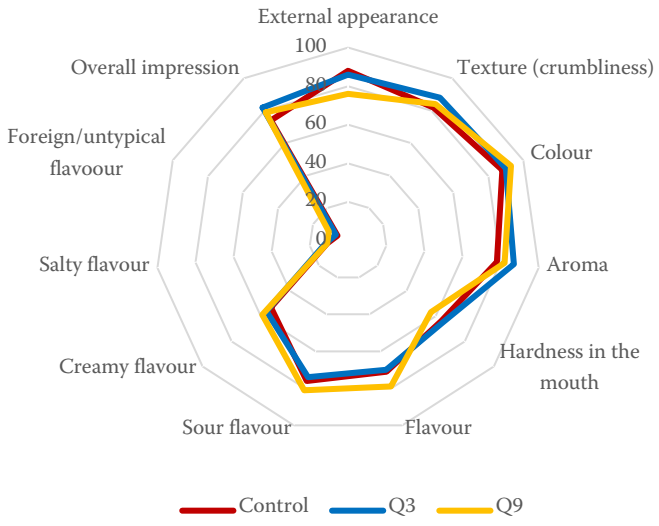

Figure 1. Radar chart showing mean sensory scores of fresh cheese variants immediately after production (day 0 of storage)

Q3 – FreshQ® Cheese 3; Q9 – FreshQ® 9

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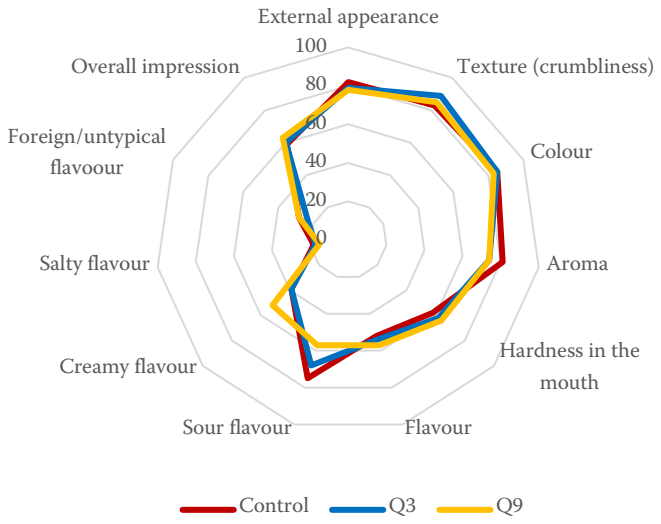

Figure 2. Radar chart showing mean sensory scores of fresh cheese variants after 7 days of storage

Q3 – FreshQ® Cheese 3; Q9 – FreshQ® 9

more pronounced variations were recorded after 7 days of storage. It is likely that the higher sensory scores of Q9 were associated with its lower perceived acidity.

Barukčić et al. (2021) also focused on the sensory aspects of fresh cheeses and noted that it is often difficult to identify a single best sample in terms of organoleptic properties. However, they tended to favour the sample containing a protective culture, which maintained high sensory scores throughout the storage period.

Similarly, Pappa et al. (2022) compared the sensory properties of fresh cheeses made with combinations of mesophilic and thermophilic cultures and with mesophilic culture alone. During 60 days of storage, no statistically significant differences were observed between the samples.

Ibrahim and Awad (2018) also applied sensory analysis in their evaluation of fresh cheese samples. In cheeses produced solely with lactic acid bacteria strains, panellists did not detect any negative sensory attributes. However, cheeses made with the protective strain *Lacticaseibacillus rhamnosus* achieved the highest overall sensory scores, primarily due to their lower perceived acidity.

Figure 3 presents the mean values of sensory descriptors for all variants on day 14 of storage. No statistically significant differences ($P > 0.05$) were confirmed for most descriptors. A significant decrease in the *flavour* score was observed in the control sample, whereas the Q9 variant showed an increase in flavour pleasantness compared with previous evaluations.

The ranking test produced similar results to those obtained after 7 days of storage: the Q9 variant was rated highest, followed by Q3, while the control sample received the lowest evaluation. Panellists frequently described the control and Q3 variants as having a slightly bitter and overly acidic flavour.

The fourth sensory evaluation (day 21 of storage) was influenced by the visible growth of microbial colonies. All panellists were informed of the sample condition, and tasting was performed only on microbiologically safe samples. Figure 4 shows distinct differences compared with the previous evaluation. The control variant was clearly assessed as unacceptable for consumption. Visible mould and yeast growth were accompanied by an intense unpleasant odour. For this sample, only *texture* and *colour* could be evaluated; both descriptors did not differ from the other variants, reaching scores of 93–94 points.

The Q3 and Q9 variants performed considerably better. The *external appearance* of both samples was rated similarly (~83 points), *texture (crumbliness)* reached 93–94 points, and *colour* (94 points) remained at comparable levels to those observed on day 14. *Aroma* was evaluated similarly for both variants, with Q3 reaching 83 points and Q9 89 points. The descriptor *hardness in mouth* indicated a slightly softer texture, with a decrease of 6–9 points in both variants; however, this difference was not statistically significant ($P > 0.05$).

The *flavour* descriptor was rated lower, showing a noticeable and statistically significant decline ($P < 0.05$). The *sour flavour* intensity increased in both samples, and statistical analysis confirmed significance at $P < 0.05$. The *creamy flavour* intensity dropped

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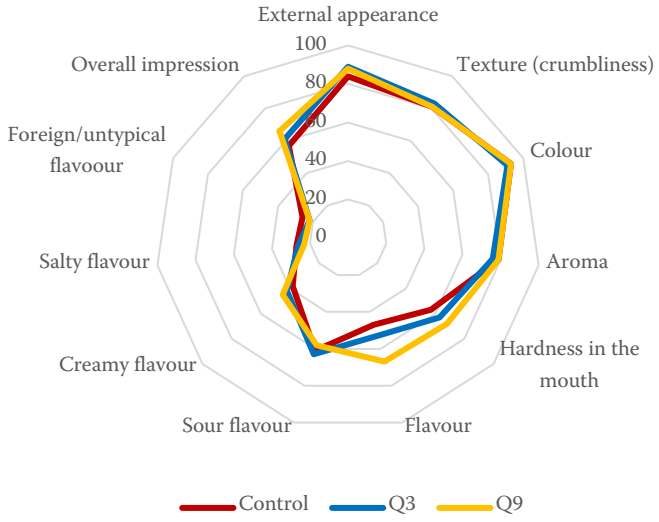

Figure 3. Radar chart showing mean sensory scores of fresh cheese variants after 14 days of storage

Q3 – FreshQ® Cheese 3; Q9 – FreshQ® 9

to nearly the lowest end of the scale, reaching only 3 points in both variants – a statistically significant decrease compared with the previous evaluation.

Panellists also reported higher *foreign/untypical flavour* intensity, with the Q9 variant scoring worse (70 points) than Q3 (55 points). This represented a several-fold increase compared with day 14, and the difference between variants was statistically significant ($P < 0.05$).

In terms of *overall impression*, the Q9 variant was rated more favourably than Q3, maintaining better sensory quality after 21 days of storage.

The ranking test produced results similar to those obtained in the previous analysis. The Q9 variant achieved the highest overall ranking, followed by Q3, while the control variant was rated the lowest.

The control sample was verbally described by the panellists as unacceptable and inedible, with visible growth of moulds and yeasts and a strong yeast-like odour. The Q3 variant was considered still acceptable for consumption, though slightly bitter with a noticeable aftertaste. Some reserve samples of this variant also showed visible dark microbial colonies.

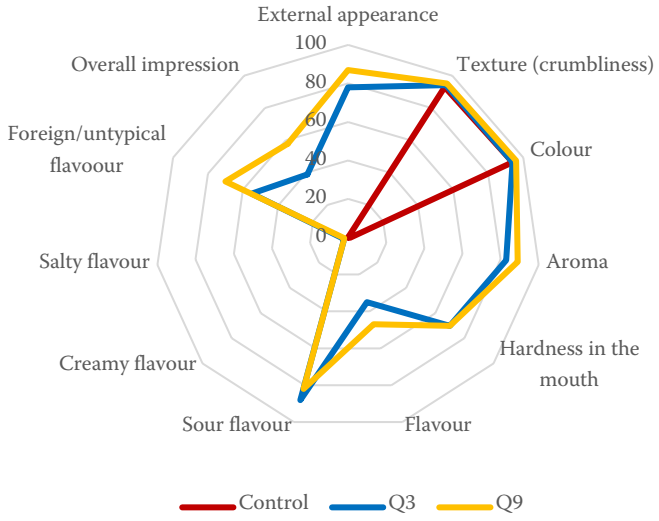

Figure 4. Radar chart showing mean sensory scores of fresh cheese variants after 21 days of storage

Q3 – FreshQ® Cheese 3; Q9 – FreshQ® 9

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In contrast, the Q9 variant was evaluated as free from off-flavours, distinctly acidic, and overall acceptable. Unlike the other variants, it did not exhibit a bitter taste, and no microbial contamination was detected.

CONCLUSION

The results of this study demonstrated that the use of protective cultures in fresh cheese production had no significant impact on basic physicochemical or colour parameters. Nevertheless, the application of *Lacticaseibacillus rhamnosus* (FreshQ® 9) and the multi-strain culture FreshQ® Cheese 3 contributed to improved microbiological and sensory stability during refrigerated storage. From a practical perspective, the application of protective cultures, particularly FreshQ® 9, enabled fresh cheeses to retain acceptable microbiological and sensory quality beyond the commonly reported 7–14-day shelf life, extending their usability up to 21 days under refrigerated conditions.

While no *Escherichia coli* or moulds were detected in any of the samples, cheeses containing protective cultures showed lower counts of psychrotrophic microorganisms on several storage days compared with the control, indicating improved microbial stability during refrigeration. Although this effect was not uniform across all sampling points, it is technologically relevant given the short shelf life and high moisture content of fresh cheeses. Throughout the 21-day storage period, the Q9 variant maintained a more pleasant aroma and flavour and was rated as the most acceptable by the sensory panel.

These findings confirm that protective cultures can enhance the safety and sensory shelf life of fresh cheeses without adversely affecting their technological properties. The use of such cultures therefore represents an effective bioprotective strategy that can reduce the need for chemical preservatives and support the production of clean-label dairy products.

Future research should focus on optimising the selection and concentration of protective cultures for different cheese types, exploring their interactions with native microbiota, and evaluating their performance under various storage conditions to further improve sensory stability and microbial safety.

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