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ORIGINAL PAPER

Molecular characterization of yeast flora and quality attributes of traditional gravyer cheese

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Abstract

This study aimed to characterize the yeast flora of traditional Kars Gravyer cheese produced in Türkiye using molecular methods, alongside evaluating its physicochemical and microbiological quality attributes. Thirty cheese samples were collected from retail points. DNA was isolated from 150 yeast colonies (5 per sample). Species identification was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the ITS1-5.8 S-ITS2 region and 26 S rDNA sequencing. Additionally, pH, dry matter, color parameters, and microbial counts were analyzed. Biotechnological analyses revealed that *Debaryomyces hansenii* was present in 20 cheese samples (66.7%), *Cryptococcus laurentii* in 8 cheese samples (26.7%), and both yeasts concurrently in two cheese samples (6.6%). In Gravyer cheeses, where the mean pH and dry matter values were determined as 5.96 ± 0.17 and 67.25 ± 2.12%, respectively, the count of lactic acid bacteria (7.45 ± 0.42 log CFU/g) was higher than the count of *Micrococcus/Staphylococcus* (5.65 ± 0.97 log CFU/g). The mean yeast-mould count was 4.48 ± 1.16 log CFU/g, with yeasts being more predominant than moulds in the samples, and no mould growth was observed in three samples. The *Enterobacteriaceae* count was found to be below 2.00 log CFU/g in 20 samples, whereas the average in 10 samples was 3.33 ± 1.13 log CFU/g. The inner and rind surfaces of the Gravyer cheese have different color values ($P < 0.01$), and the L* and b* values of the inner surface were higher than those of the rind surface ($P < 0.05$). As a result of the current study, *Debaryomyces hansenii* was identified as the predominant yeast species in the traditional Gravyer cheese.

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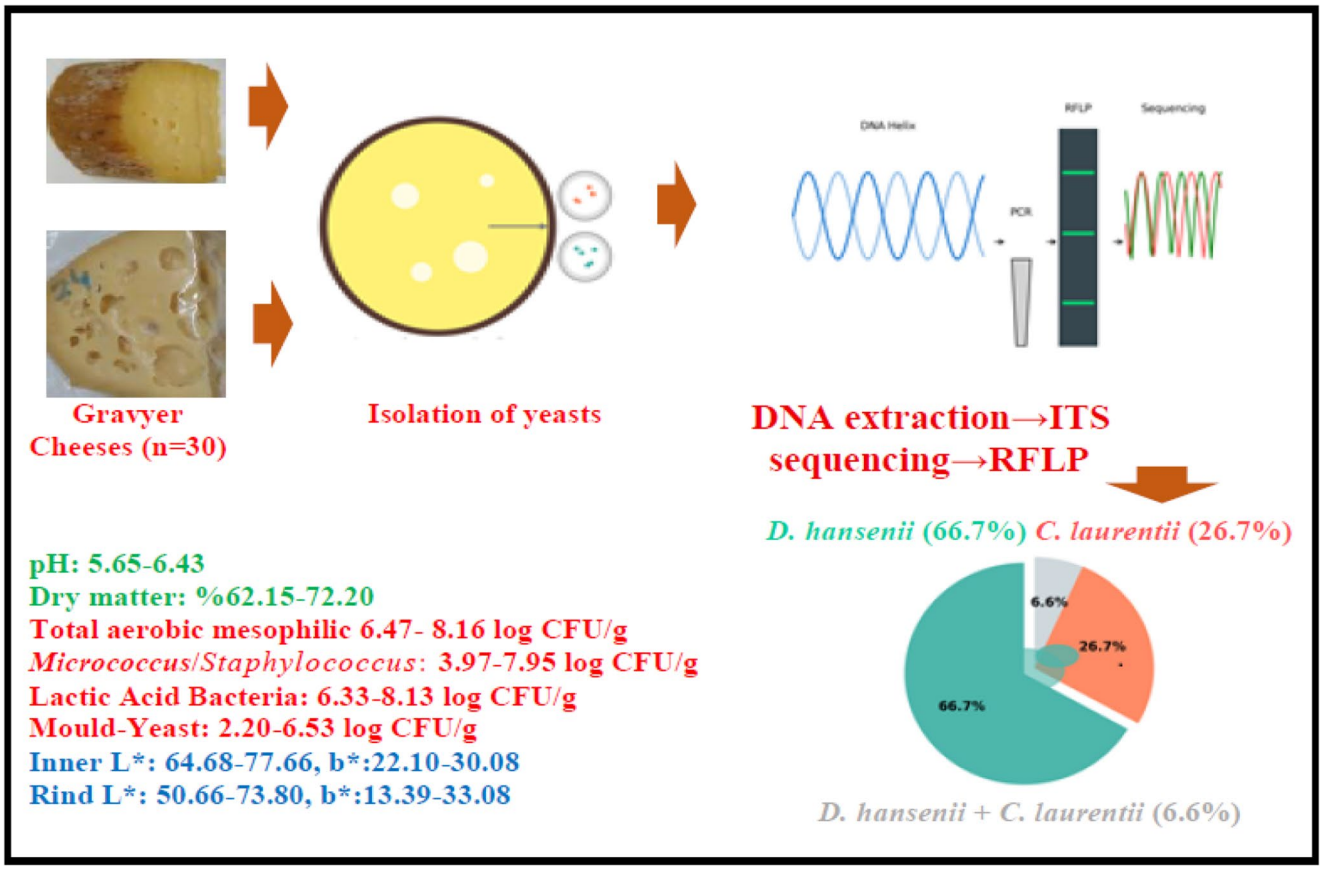
Graphical Abstract



Highlights

- Yeast flora in traditional Gravyer cheeses was detected for the first time in this study.
- Yeast species of isolates was determined using the PCR technique following sequencing of the ITS1-5.8S-ITS2 and 26 S rDNA regions.
- The dominant yeast flora in Kars Gravyer cheeses was detected as *Debaryomyces hansenii*.
- *Lactic acid bacteria and Micrococcus/Staphylococcus* was the predominant microbial population in Kars Gravyer cheeses.
- Among Kars Gravyer cheese samples showed significant differences in color, pore structure, and shape.

Keywords Gravyer cheese · *Debaryomyces hansenii* · *Cryptococcus laurentii* · Yeast flora · Microbial properties · Biotechnology · PCR-RFLP · Kars

Introduction

Traditional foods have gained strategic importance and increased popularity due to their perceived health benefits. Thus, consumers have started to recognize traditional foods more, and consumption has increased [1]. Named after the town of Gruyères in Switzerland, Gravyer cheese is a traditional dairy product and has been produced in Turkey's Eastern Anatolia region (Kars-Ardahan) since 1905. While small quantities are produced in modern facilities in the central Kars and Ardahan regions, Gravyer cheese is mostly produced using traditional methods in villages (Boğatepe, Dikme, Kümbetli, and Borluk). The main production center for Gravyer cheese is the village of Boğatepe, a settlement in Kars province [2]. Gravyer is one of the most widely made types of cheese in Kars, along with Kaşar, Tulum, and Civil. Gravyer is also one of about 18 commercially important cheese types around the world. It is grouped with Gruyère (France), Emmental (Switzerland), Fontina (Italy), and Samsø (Denmark), Gouda/Edam (Netherlands) [3]. Gravyer cheese, which uses cow's milk in its production, is generally produced from high-fat Zavot cows [4]. Due to its moisture

content (20–42%), Gravyer cheese is classified as a hard cheese and, in terms of its ripening method, as a porous cheese ripened with bacteria. It is also classified as a cheese produced through propionic acid fermentation due to the propionic acid bacteria that play a role in the formation of pores. Because the traditional production method involves dry salting, it is also classified separately from cheeses aged in brine [5, 6].

Yeasts, which have important effects on the production of almost all traditional cheeses, contribute significantly to the flavor and typical appearance of the cheese, especially in cheeses that are ripened for a long time, such as Tilsit, Munster, Reblochon, and Gruyère. Raw milk and traditional cheeses have a potential for biotechnological utilization of advantageous yeast species [7]. Yeasts provide the necessary energy source for the yeast population by breaking down lactate, which they produce from lactose through the action of lactic acid bacteria during cheese production. Lactate breakdown causes deacidification of the cheese surface and an increase in pH, which encourages the growth of molds that contribute to ripening, as well as mostly aerobic and acid-sensitive bacteria. This mechanism demonstrates the effectiveness of using certain yeast strains as adjunct cultures in cheese varieties and explains the importance of yeast in cheese production [8].

Although Gravyer cheese is a traditional and economically important dairy product, the number of studies is quite limited [3, 4, 9–11], and the product has not been examined in detail in many aspects. In the studies, various physical, chemical, microbiological, and sensory properties of Gravyer cheese were determined [3, 10, 11] and the changes that occur during the ripening and storage processes by producing the cheese under controlled conditions were determined [9, 12]. It is known that yeasts have an effect on the quality characteristics of cheese during the production and ripening process and that each yeast exhibits a different effect. Therefore, determining the type of yeast that develops is important in characterizing the quality of cheeses and in developing starter or auxiliary cultures specific to the products. In studies, only the total colony counts of yeasts in Gravyer cheeses were determined. To date, no research has been conducted to determine the molecular characterization of traditional Gravyer cheeses at the yeast species level that are effective in the changes that occur during the production and ripening processes. Also, no previous study has identified yeast species in Kars Gravyer using ITS-RFLP sequencing [13, 14]. The PCR-RFLP technique was chosen for this study as it provides a cost-effective and reliable high-throughput screening method to differentiate and cluster a large number of isolates. While traditional culture-dependent methods are often insufficient for accurate discrimination of closely related species, PCR-RFLP allowed us to effectively group the isolates based on their genetic profiles without the prohibitive cost of sequencing every single isolate. Representative strains from these groups is then verified via sequencing to ensure accuracy. Furthermore, characterizing the native microbial biodiversity is essential for future technological research, indüstrial product standardization, and the potential cultivation of new cultures specific to this traditional product. The aim of this study is to describe the molecular characterization of yeast species in the microbial flora of traditional Kars Gravyer cheese using the ITS-RFLP technique for the first time.

Materials and methods

Material

As material in the study, thirty ($n=30$) Gravyer cheese samples were used (Fig. 1). The samples were sourced from Kars, Türkiye, a city where Gravyer cheese is produced intensively. The samples were randomly collected from thirty different dairy retail outlets that mainly sell Gravyer cheese. All cheeses selected were fully ripened commercial products (typically aged > 90 days) and ready for consumption. The samples were cut from whole Gravyer cheese in sizes representing the rind and interior at the point of sale (approximately 2 kg), then vacuum-packed, and transported to the laboratories under cold chain conditions (4–6 °C, approximately 48 h).

Methods

Biotechnological analyses

Yeast species identification was conducted on colonies grown on Potato Dextrose Agar (PDA, Neogen NCM0018A) adjusted to pH 3.5 with 10% tartaric acid. From each cheese sample, five colonies were randomly selected, for a total of 150 colonies. In selecting the individual colonies from each sample, the morphological and color characteristics of the colonies were considered. In this context, care was taken to ensure that all colonies with visually distinct characteristics were represented. This sampling strategy was chosen to obtain a representative snapshot of the dominant yeast populations while maintaining analytical feasibility. Relationships among the yeasts isolated from each colony were inferred using the Maximum Parsimony method [15, 16].

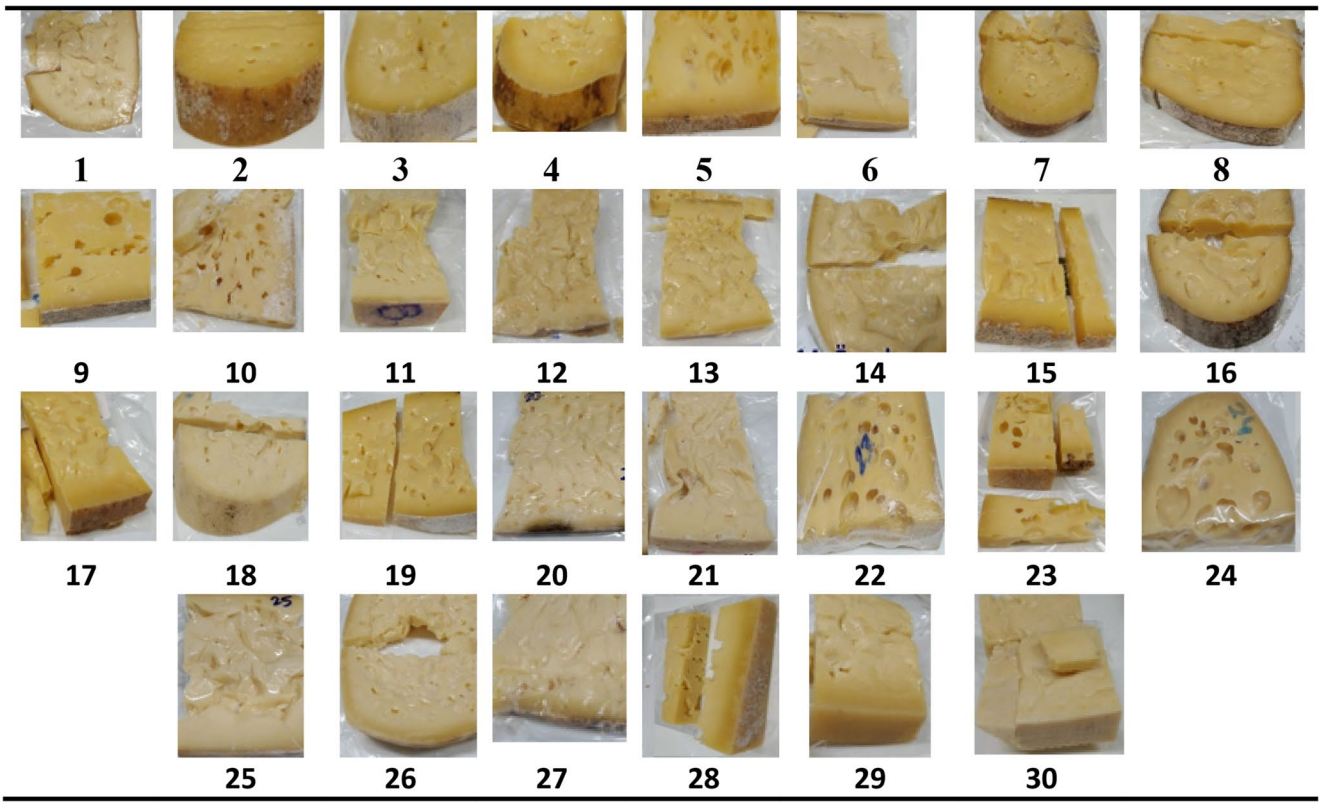


Fig. 1 Gravyer cheese samples analyzed in the study

DNA extraction

For yeast identification, the ITS1-5.8S-ITS2 and 26S rDNA markers were used. Genomic DNA was extracted with a commercial kit (Genomix, Talent, Trieste) following the manufacturer's instructions. Cells were collected from colonies on plates, frozen in liquid nitrogen, and homogenized prior to extraction [13, 14].

PCR amplification and RFLP (Restriction Fragment Length Polymorphism) analysis

The internal transcribed spacer (ITS) region of rDNA, which encompasses the ITS1-5.8S-ITS2 segment located between the 26 S and 18 S genes, was amplified using primers CNL12 F (5′-CTGAACGCCTCTAAGTCAG-3′) and CNS1 R (5′-GAGACAAGCATATGACTACTG-3′) in a thermal cycler [17]. PCR reactions (25 µL total volume) contained 100 ng template DNA, 2 mM $MgCl_2$, 1 mM dNTPs, 1 µL forward primer, 1 µL reverse primer, and 1 U Taq DNA polymerase. The thermal profile was: initial denaturation 94 °C for 85 s; 35 cycles of 95 °C for 35 s (denaturation), 58 °C for 55 s (annealing), and 72 °C for 2 min (extension); and a final extension at 72 °C for 10 min. PCR products were checked on 1% agarose gels containing 0.05% ethidium bromide[18]. To establish genetic profiles and avoid the analytical redundancy of sequencing every isolate, the PCR products of all 150 isolates were first subjected to RFLP analysis. Aliquots (10 µL) of PCR products were digested with the restriction enzymes HhaI, HapII, and MboI. These enzymes were selected because they provide high discriminatory power for identifying the most frequent yeast species in dairy products [14]. Based on the obtained restriction patterns, the isolates were clustered into distinct groups. Subsequently, to definitively confirm species identity, only representative strains from each RFLP group were subjected to Sanger sequencing using an ABI 3730xl Genetic Analyzer (Applied Biosystems, USA). The resulting raw sequences were edited in CodonCode, and phylogenetic trees and codon positions were determined using MEGA X [13, 14, 18].

Physicochemical analyses

Determination of pH value

For pH analysis, 10 g of cheese were combined with distilled water and homogenized using an Ultra-Turrax device prior to measurement. The pH meter was calibrated with buffer solutions at pH 4.0 and pH 7.0.

Determination of dry matter content

For dry matter determinations, 3–5 g of cheese were placed in aluminum drying dishes and held at 105 °C in an oven until constant weight. Dry matter content was calculated gravimetrically (% w/w).

Determination of instrumental color values

Instrumental color parameters (L*, a*, b*, C*, and h°) were measured using a reflectance colorimeter (Minolta CR-400). The optical probe was placed in direct contact with the cheese surface (rind) and cross surface. For each cheese, measurements were taken separately from the cross surface and the rind, with 10 technical replicates per region. Color was assessed according to the criteria of the International Commission on Illumination (CIE) based on the three-dimensional color space, yielding L* [lightness: 100 = white, 0 = black], a* [(+) = redness, (–) = greenness], and b* [(+) = yellowness, (–) = blueness]. Chroma (C*, saturation) was calculated as $\sqrt{(a*)^2 + (b*)^2}$, and hue angle (h°, dominant wavelength) as $h = \tan^{-1}\left(\frac{b*}{a*}\right)$.

Determination of microbiological counts

Microbiological analyses were performed on subsamples taken from the cut surface to represent both the center and the rind portions of the cheeses. Twenty-five grams from each sample were combined with 225 mL physiological saline (0.85% NaCl) and homogenized in a Stomacher for 3 min. Appropriate decimal dilutions were prepared and plated by the surface spread method. For total aerobic mesophilic counts (TAMB), Plate Count Agar (PCA, Merck 1.05463) was used, and plates were incubated at 30 °C under aerobic conditions for 48 h. For *Micrococcus*/*Staphylococcus* counts, Mannitol Salt Phenol-Red Agar (MSA, Merck 1.05404) was used with incubation at 30 °C under aerobic conditions for 48 h; counts were based on catalase-positive cocci. For lactic acid bacteria counts, de Man, Rogosa and Sharpe Agar (MRS, Merck 1.10660) was used with incubation at 30 °C under anaerobic conditions (Anaerocult A) for 48 h; counts were based on catalase-negative colonies. For yeast–mould counts, PDA (Neogen NCM0018A) adjusted to pH 3.5 with 10% tartaric acid was used; after plating appropriate dilutions, incubation was carried out at 25 °C for 5–7 days. At the end of incubation, colonies were selected and enumerated based on their morphology and pigmentation. For *Enterobacteriaceae* counts, Violet Red Bile Dextrose Agar (VRBD, Merck 1.10275) was used, with incubation at 30 °C under anaerobic conditions for 48 h. Anaerobic A was used for the anaerobic conditions; the overlay method was not used with VRBD agar. The colonies with a diameter of 1–2 mm were enumerated.

Statistical analyses

Data obtained for the investigated parameters were subjected to statistical analysis to determine the effect of each parameter. Statistical analyses were performed using statistical software (SPSS version 26.0) [19]. Prior to analysis, data normality was verified using the Shapiro-Wilk test ($p > 0.05$). Subsequently, One-way Analysis of Variance (ANOVA) was applied to determine significant differences between the cheese samples. Duncan's Multiple Range Test was used as a post-hoc test to compare the means at a significance level of $p < 0.05$. Differences between mean color values of the cut surface and the rind were evaluated using the paired t-test. Linear associations among continuous traits of Kars Gravyer cheeses were assessed with Pearson's correlation coefficient, while associations between yeast species and cheese traits were evaluated using Spearman's rho.

Results and discussion

The presence of different yeast species in the final stages of ripening and in ready-to-eat cheeses suggests that yeasts make a significant contribution to ripening and especially to the development of organoleptic properties. In this study, the predominant yeast species identified by isolation and identification analyses in Gravyer cheeses are presented in Table 1. In the yeast profile of the examined cheese samples, only *Debaryomyces hansenii* was detected in 66.7% (20 samples), and only *Cryptococcus laurentii* was detected in 26.7% (8 samples). The percentage of cheeses containing both yeast species together was 6.6% (2 samples). In one cheese sample, 60% of the yeast isolates (3 isolates, sample 1) were identified as *D. hansenii*, while in another sample, 80% (4 isolates, sample 16) were similarly identified. A total of 71.33% of the 150 yeast isolates from Gravyer cheeses were identified as *D. hansenii*, whereas 28.67% were identified as *C. laurentii* (Table 1). The prevalence of *D. hansenii* in cheese is likely due to its various physiological characteristics, such as its ability to grow at high salt concentrations, low temperature, water activity, and pH; to utilize lactate as its main carbon source; and to produce proteolytic and lipolytic enzymes [20].

The base compositions for all codon locations of the yeast sequences were as follows: for *Cryptococcus laurentii*, A + T = 55.208 and G + C = 44.791; for *Debaryomyces hansenii*, A + T = 64.151 and G + C = 35.84. In *C. laurentii*, the codon position with the highest A + T ratio

Table 1 Yeast species identified in traditional Kars Gravyer cheese samples

Samples number	Isolates number	Dominant yeast species	Samples number	Dominant yeast species	Samples number	Dominant yeast species
1	1	*D. hansenii*	11	*D. hansenii*	21	*C. laurentii*
	2	*D. hansenii*		*D. hansenii*		*C. laurentii*
	3	*D. hansenii*		*D. hansenii*		*C. laurentii*
	4	*C. laurentii*		*D. hansenii*		*C. laurentii*
	5	*C. laurentii*		*D. hansenii*		*C. laurentii*
2	1	*D. hansenii*	12	*D. hansenii*	22	*D. hansenii*
	2	*D. hansenii*		*D. hansenii*		*D. hansenii*
	3	*D. hansenii*		*D. hansenii*		*D. hansenii*
	4	*D. hansenii*		*D. hansenii*		*D. hansenii*
	5	*D. hansenii*		*D. hansenii*		*D. hansenii*
3	1	*D. hansenii*	13	*D. hansenii*	23	*C. laurentii*
	2	*D. hansenii*		*D. hansenii*		*C. laurentii*
	3	*D. hansenii*		*D. hansenii*		*C. laurentii*
	4	*D. hansenii*		*D. hansenii*		*C. laurentii*
	5	*D. hansenii*		*D. hansenii*		*C. laurentii*
4	1	*C. laurentii*	14	*D. hansenii*	24	*C. laurentii*
	2	*C. laurentii*		*D. hansenii*		*C. laurentii*
	3	*C. laurentii*		*D. hansenii*		*C. laurentii*
	4	*C. laurentii*		*D. hansenii*		*C. laurentii*
	5	*C. laurentii*		*D. hansenii*		*C. laurentii*
5	1	*D. hansenii*	15	*D. hansenii*	25	*C. laurentii*
	2	*D. hansenii*		*D. hansenii*		*C. laurentii*
	3	*D. hansenii*		*D. hansenii*		*C. laurentii*
	4	*D. hansenii*		*D. hansenii*		*C. laurentii*
	5	*D. hansenii*		*D. hansenii*		*C. laurentii*
6	1	*D. hansenii*	16	*D. hansenii*	26	*D. hansenii*
	2	*D. hansenii*		*D. hansenii*		*D. hansenii*
	3	*D. hansenii*		*D. hansenii*		*D. hansenii*
	4	*D. hansenii*		*D. hansenii*		*D. hansenii*
	5	*D. hansenii*		*C. laurentii*		*D. hansenii*
7	1	*C. laurentii*	17	*D. hansenii*	27	*D. hansenii*
	2	*C. laurentii*		*D. hansenii*		*D. hansenii*
	3	*C. laurentii*		*D. hansenii*		*D. hansenii*
	4	*C. laurentii*		*D. hansenii*		*D. hansenii*
	5	*C. laurentii*		*D. hansenii*		*D. hansenii*
8	1	*D. hansenii*	18	*D. hansenii*	28	*C. laurentii*
	2	*D. hansenii*		*D. hansenii*		*C. laurentii*
	3	*D. hansenii*		*D. hansenii*		*C. laurentii*
	4	*D. hansenii*		*D. hansenii*		*C. laurentii*
	5	*D. hansenii*		*D. hansenii*		*C. laurentii*
9	1	*D. hansenii*	19	*D. hansenii*	29	*D. hansenii*
	2	*D. hansenii*		*D. hansenii*		*D. hansenii*
	3	*D. hansenii*		*D. hansenii*		*D. hansenii*
	4	*D. hansenii*		*D. hansenii*		*D. hansenii*
	5	*D. hansenii*		*D. hansenii*		*D. hansenii*
10	1	*D. hansenii*	20	*D. hansenii*	30	*C. laurentii*
	2	*D. hansenii*		*D. hansenii*		*C. laurentii*
	3	*D. hansenii*		*D. hansenii*		*C. laurentii*
	4	*D. hansenii*		*D. hansenii*		*C. laurentii*
	5	*D. hansenii*		*D. hansenii*		*C. laurentii*

was region 2, while in *D. hansenii*, it was region 3. The markers chosen based on codon locations were evidently suitable (Fig. 2). The Unweighted Pair Group Method Algorithm (UPGMA) tree illustrated sequence differences [13, 14] among colonies to assess intra- and interspecies percentage differences (Fig. 3). The findings indicate that colonies of *D. hansenii* and *C. laurentii* were molecularly distinct from one another. The species boundary for both species was established at 1.76%. Species differentiation was quantified at 0.02%, with differences noted at minimal levels. The *D. hansenii* species is recognized as the predominant flora in Danbo, Asiago, Tilsit, Gruyère, Raclette, Brazilian Minas, Fontina, Munster, Divle Obruk, Gubbeen, Limburger, Epoisses, Havarti, Saint-Nectaire and Pecorino Romano cheeses [7]. Our present research findings indicate the similarity of Kars Traditional Gravyer cheese with these varieties of traditional cheeses. A study on Gubbeen cheese found the predominant yeast species as *D. hansenii* at 624 isolates, *Candida catenulata* at 135 isolates, and *Candida lusitaniae* at 62 isolates [20]. Banjara et al. [21] identified *D. hansenii* as the predominant fungus in a survey of 44 distinct cheeses, finding its presence in 63% of the samples analyzed. A study on the classic French cheese Tomme d'Orchies identified *D. hansenii* as a commonly occurring microorganism in this cheese [22]. Freitas et al. [23] conducted a study on the lipolytic and proteolytic activities of bacteria and yeasts isolated from Picante cheese, revealing that *C. laurentii* exhibited significant lipolytic activity. But, Banerjee et al. [24] reported that *C. laurentii* is a non-neoformans *Cryptococcus* and a rare human pathogen capable of inducing fungemia. However, *C. laurentii* is also frequently isolated from the natural flora of traditional dairy products and is generally considered an environmental contaminant rather than a direct hygiene failure in this context, though its presence warrants monitoring.

Table 2 presents the Spearman's rho correlation study that elucidates the links between yeast species and physicochemical as well as microbiological characteristics. A statistically significant correlation ($p<0.05$) was identified between the inner surface b* and C* values and the samples where the two yeast species coexisted. This suggests a potential association where the coexistence of both species might relate to lower b and C values (r = -0.371), rather than a definitive causal impact on quality, possibly indicating competitive interactions between them. A negative association was seen between samples containing the *D. hansenii* species and the inner surface L* and h values, alongside a positive correlation with the inner surface a* value; however, these effects lacked statistical significance ($p>0.05$) (Table 2).

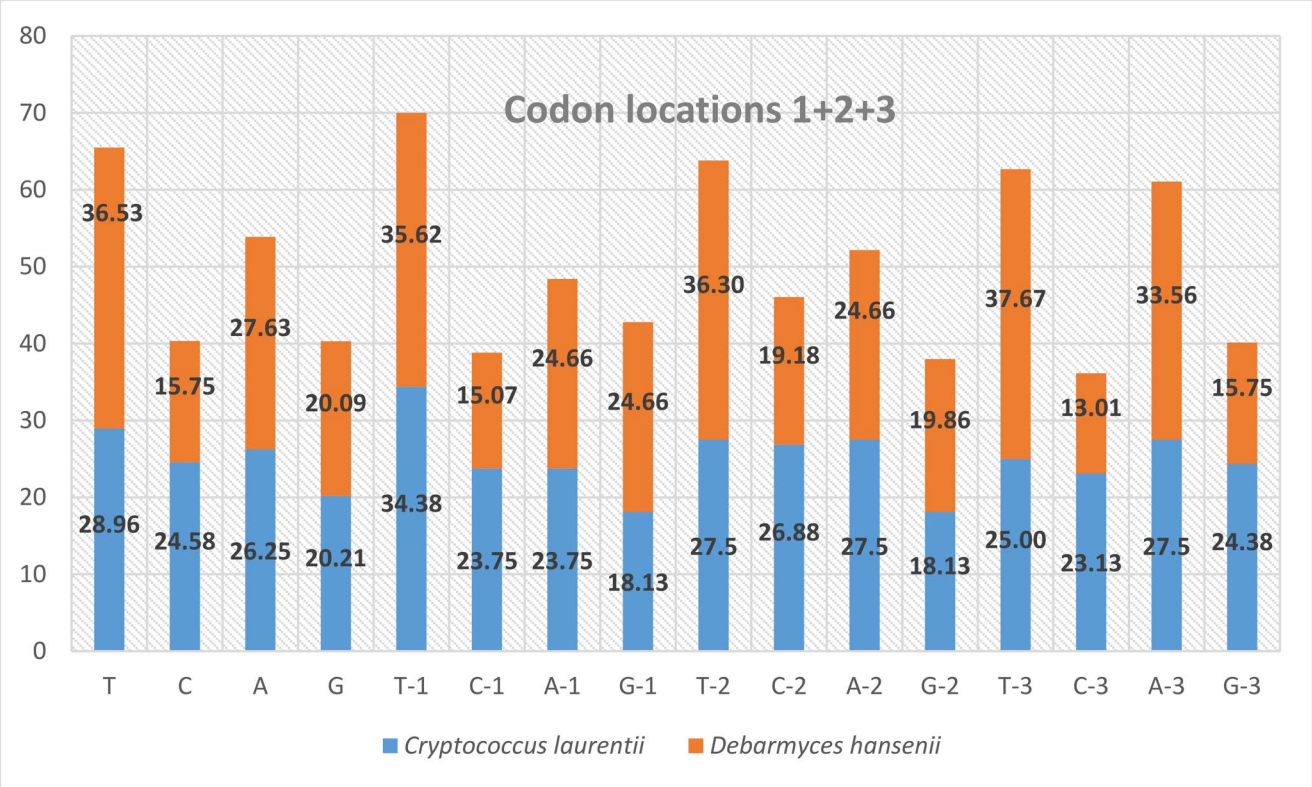


Fig. 2 Codon locations of the identified yeast species

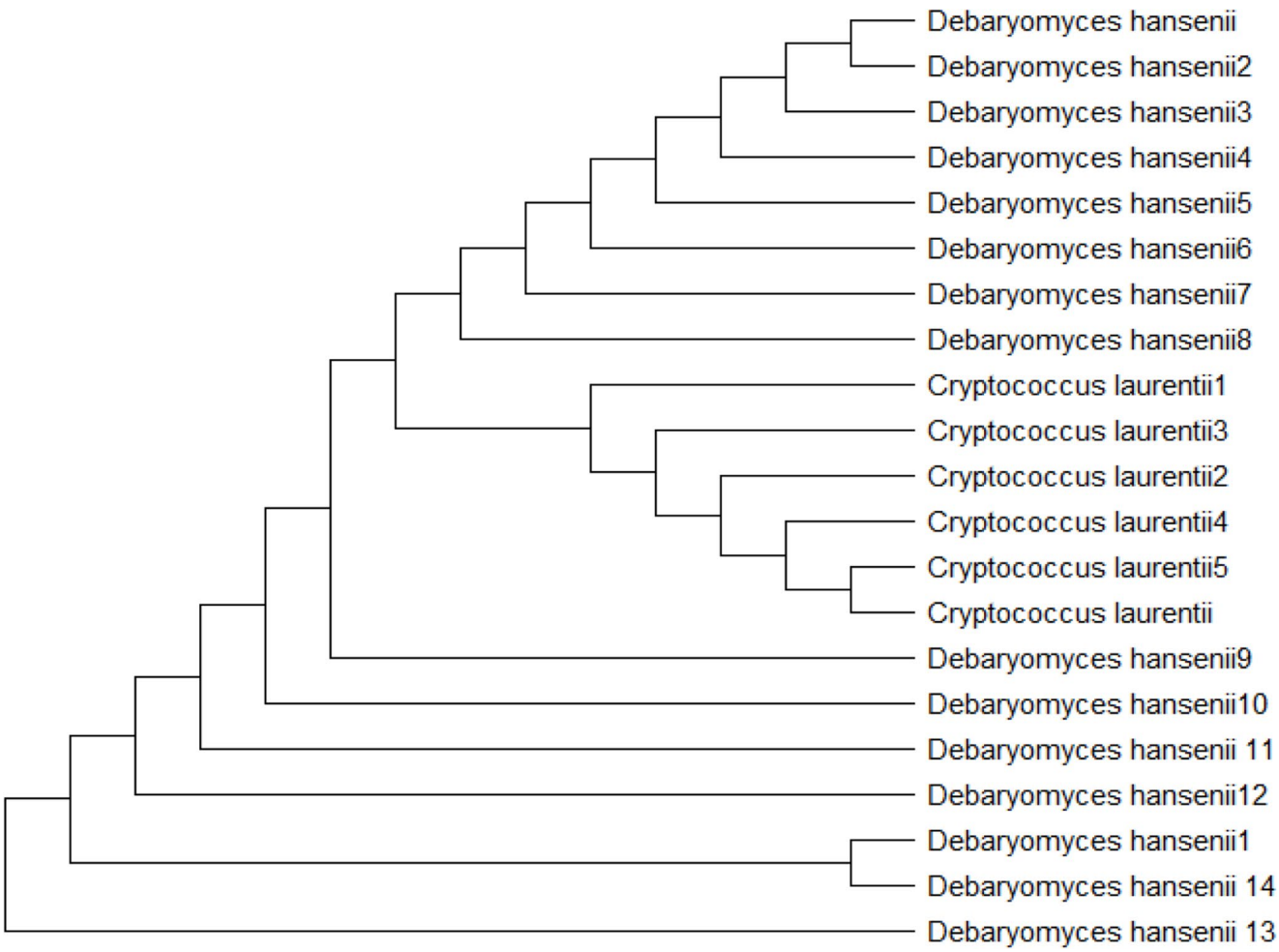


Fig. 3 Variations in the identified species inside the UPGMA tree

D. hansenii is a heterogeneous yeast exhibiting high genetic and phenotypic diversity. The high abundance of *D. hansenii* in cheeses is due to its ability to grow at low a_w values, high salt concentrations, and low temperatures [20, 25]. Furthermore, although the samples were vacuum-packaged, creating an anaerobic environment, *D. hansenii* is a facultative anaerobe capable of surviving under such conditions, which supports its isolation as the dominant species. *D. hansenii* also has mould growth inhibitory effects in dairy products [26, 27]. Ferreira and Viljoen [25] determined that using *D. hansenii* as a secondary flora, in addition to lactic acid bacteria, in Cheddar cheese production is beneficial because its interaction with lactic acid bacteria is positive and contributes to flavor development during cheese ripening. Overgrowth of *D. hansenii* can alter the typical appearance and sensory characteristics of cheeses without reducing their overall quality [7].

There is limited research on the occurrence of *Cryptococcus laurentii* in cheeses and its impact on quality. A study on the surface fungal microflora of Reblochon de Savoie cheese from France identified *Candida famata* (*D. hansenii*) as the predominant yeast species, with *C. laurentii* also observed in limited quantities [28]. Freitas et al. [29], in their study of Picante cheese, a hard cheese manufactured in Portugal from a blend of sheep and goat milks, found no presence of *C. laurentii* on day 0 of ripening; however, they identified that 11.1% of the yeast isolates on day 9 of ripening were of this species. In the same study, this yeast was detected on days 25 and 83 of ripening, and on day 110, it was determined that it constituted 12.5% of the yeast isolates. Biagiotti et al. [30] identified the *C. laurentii* species in the cave where Fossa cheese was created in Italy prior to ripening but did not locate this species in the cheese post-ripening.

The mean values and statistically significant differences among samples of pH ($P<0.01$), dry matter ($P<0.01$), and microbiological characteristics ($P<0.01$) determined in the analyzed cheese samples are presented in Table 3. The pH levels of Gravyer cheeses were found to range from 5.65 to 6.43, with a mean of 5.96 ± 0.17. The pH values acquired

Table 2 Spearman's rho correlations

Yeast	DM	pH	TAMB	MY	M/S	LAB	Inner Area				Rind Area				*D. hansenii*	*C. laurentii*
							L*	a*	b*	C*	h°	L*	a*	b*	C*	h°		
D. hansenii	0.005	0.014	0.137	-0.063	0.299	-0.076	-0.148	0.281	-0.073	-0.090	-0.206	0.009	-0.062	0.083	0.089	0.059	1.000	-1.000**
C. laurenti	-0.005	-0.014	-0.137	0.063	-0.299	0.076	0.148	-0.281	0.073	0.090	0.206	-0.009	0.062	-0.083	-0.089	-0.059	-1.000**	1.000
D. hansenii+*C. laurentii*	0.015	-0.340	-0.216	0.201	-0.293	-0.100	-0.077	-0.100	-0.371*	-0.371*	0.355	-0.185	0.232	-0.201	-0.216	-0.247	-0.224	0.224

** $P<0.01$; * $P<0.05$

DM: Dry matter, *TAMB*: Total aerobic mesophilic bacteria, *M/S*: *Micrococcus/Staphylococcus*, *MY*: Mould-yeast, *LAB*: Lactic acid bacteria

in the study correspond closely to those established in previous research on this topic [4, 11, 31]. The differences in pH values ($P<0.01$) determined in the samples examined within the scope of the research indicate that there are significant differences in the production and ripening processes of cheeses offered to the market.

The Pearson correlation coefficients (r) for the parameters analyzed in the study are presented in Table 4. A statistically significant correlation was observed between pH and the counts of LAB and TAMB ($p<0.05$). LAB convert lactose into lactic acid, hence lowering the pH in cheese; consequently, an increase in LAB counts is anticipated to result in a fall in pH. Nevertheless, our current investigation established that pH did not diminish in correlation with the rise in LAB counts. In samples exhibiting elevated LAB counts, pH levels were typically seen to be high as well. The pH values of samples exhibiting elevated LAB were predominantly within the 5.80–6.00 range, with only two samples registering pH values below 5.8. Notwithstanding the fermentation of lactose by LAB and the resultant accumulation of lactic acid during cheese production, the elevated pH values observed are attributed to the degradation of lactic acid by yeasts and moulds during the ripening phase, the production of ammonia, the deamination of amino acids, and the catabolism of fatty acids into methyl ketones [32]. Conversely, interactions between yeasts and between yeasts and bacteria influence the development of the cheese surface ecology; in the presence of yeasts and moulds, lactic acid is digested, resulting in the production of NH_3. Consequently, the surface pH of cheeses increases from 4.8 to 5.2 to 6–8.2, facilitating the proliferation of acid-sensitive bacteria and salt-tolerant [33]. In the current study, a positive correlation was observed between TAMB and pH ($r=0.397$, Table 4). Conversely, Souza et al. [34] identified a negative correlation between TAMB counts and pH in their investigation of Serrano cheese, concluding that the total flora comprised acid-resistant bacteria.

The dry matter content in Gravyer cheese samples varied from 62.15% to 72.2%, with a mean of 67.25 ± 2.12%. Sample 22 had the lowest dry matter value ($P<0.05$), while sample 5 demonstrated the highest value. There is no difference ($P>0.05$) in terms of dry matter between samples 3, 6, 8, 9, 10, 19, 28 and 30 (Table 3). The TS 2174 Gravye cheese standard stipulates that the moisture content must not exceed 38% [35]. All cheeses examined in this study adhere to the values outlined in the standard. Research on Kars Gravyer cheese has indicated that its dry matter content varies between 63.2 and 80.2% [3, 4, 10, 11]. In the manufacture of Gravyer cheese, the dry matter content rises [9], influencing several aspects of the product. In this context, Table 4 illustrates a substantial correlation between the product's dry matter content and total aerobic mesophilic bacteria

Table 3 Mean values of pH, dry matter, and microbiological characteristics of traditional Gravyer cheeses (The values given for each sample are the average of 3 analysis)

Samples Number	pH	Dry matter (%)	Total aerobic mesophilic bacteria (TAMB) (log CFU/g)	Micrococcus/ Staphylococcus (M/S) (log CFU/g)	Lactic Acid Bacteria (LAB) (log CFU/g)	Mould-Yeast (MY) (log CFU/g)	Enterobac-teriaceae (log CFU/g)
1	5.81 ± 0.02^{jk}	70.31 ± 0.08^{c}	6.78 ± 0.07^{l}	5.09 ± 0.18^{o}	6.66 ± 0.30^{k}	4.99 ± 0.06^{e}	3.21 ± 0.12^{cd}
2	$5.87 \pm 0.04^{g-k}$	68.49 ± 0.26^{e}	7.22 ± 0.10^{ijk}	7.22 ± 0.97^{c}	6.95 ± 0.17^{jk}	3.64 ± 0.13^{h}	2.77 ± 0.21^{de}
3	5.65 ± 0.02^{l}	66.35 ± 0.37^{l}	7.29 ± 0.15^{ij}	5.82 ± 0.12^{ijk}	6.95 ± 0.17^{jk}	5.32 ± 0.19^{d}	<2.00
4	$5.85 \pm 0.01^{h-k}$	68.99 ± 0.97^{de}	6.74 ± 0.14^{l}	5.52 ± 0.08^{lm}	6.70 ± 0.13^{k}	5.94 ± 0.07^{b}	2.95 ± 0.16^{cd}
5	5.97 ± 0.33^{k}	72.20 ± 0.02^{a}	7.04 ± 0.06^{g}	4.84 ± 0.10^{op}	$7.19 \pm 0.17^{g-j}$	5.48 ± 0.11^{cd}	<2.00
6	5.78 ± 0.01^{k}	66.59 ± 0.06^{l}	7.71 ± 0.08^{de}	4.86 ± 0.12^{op}	7.62 ± 0.22^{cde}	4.91 ± 0.27^{e}	2.10 ± 0.17^{f}
7	$5.87 \pm 0.01^{g-k}$	71.13 ± 0.43^{b}	6.82 ± 0.06^{l}	5.54 ± 0.21^{lm}	7.00 ± 0.18^{j}	3.40 ± 0.09^{hij}	<2.00
8	$5.90 \pm 0.05^{g-k}$	66.49 ± 0.02^{l}	7.71 ± 0.06^{de}	6.99 ± 0.31^{cd}	$7.50 \pm 0.33^{d-g}$	6.33 ± 0.31^{a}	<2.00
9	$5.87 \pm 0.00^{g-k}$	67.22 ± 0.10^{k}	7.93 ± 0.05^{bc}	7.70 ± 0.17^{b}	$7.37 \pm 0.20^{e-i}$	6.50 ± 0.27^{a}	<2.00
10	$6.04 \pm 0.01^{c-f}$	66.54 ± 0.43^{l}	7.09 ± 0.08^{jk}	6.23 ± 0.06^{gh}	7.11 ± 0.04^{ij}	6.26 ± 0.16^{a}	<2.00
11	$5.96 \pm 0.02^{e-h}$	67.59 ± 0.06^{ijk}	7.29 ± 0.10^{ij}	6.09 ± 0.18^{ghi}	$7.25 \pm 0.16^{f-j}$	5.39 ± 0.16^{d}	<2.00
12	5.83 ± 0.01^{ijk}	67.74 ± 0.08^{ij}	6.47 ± 0.02^{m}	4.78 ± 0.15^{pq}	6.33 ± 0.12^{l}	4.03 ± 0.02^{f}	<2.00
13	$5.93 \pm 0.01^{f-j}$	67.28 ± 0.04^{k}	7.70 ± 0.09^{de}	5.06 ± 0.10^{o}	$7.40 \pm 0.24^{e-i}$	2.20 ± 0.17^{k}	<2.00
14	$5.90 \pm 0.03^{g-k}$	68.85 ± 0.12^{ef}	7.36 ± 0.18^{hi}	3.97 ± 0.06^{s}	7.53 ± 0.18^{cde}	3.10 ± 0.09^{j}	2.10 ± 0.17^{f}
15	6.19 ± 0.01^{b}	67.77 ± 0.04^{ij}	7.32 ± 0.03^{hi}	6.03 ± 0.11^{ghi}	7.17 ± 0.02^{hij}	3.24 ± 0.22^{ij}	<2.00
16	5.81 ± 0.01^{jk}	65.17 ± 0.10^{n}	7.38 ± 0.14^{hi}	4.14 ± 0.24^{s}	$7.69 \pm 0.01^{b-e}$	5.74 ± 0.16^{bc}	<2.00
17	6.17 ± 0.02^{b}	68.39 ± 0.04^{gh}	7.22 ± 0.08^{ijk}	5.64 ± 0.04^{klm}	$7.22 \pm 0.11^{f-j}$	3.51 ± 0.08^{hi}	<2.00
18	5.80 ± 0.03^{jk}	69.31 ± 0.58^{d}	7.58 ± 0.03^{efg}	5.67 ± 0.06^{kl}	7.63 ± 0.30^{cde}	3.65 ± 0.08^{gh}	<2.00
19	5.83 ± 0.06^{ijk}	66.62 ± 0.03^{l}	7.95 ± 0.03^{b}	7.95 ± 0.03^{a}	7.81 ± 0.27^{abc}	4.99 ± 0.11^{e}	<2.00
20	6.10 ± 0.02^{bcd}	64.46 ± 0.05^{o}	7.92 ± 0.01^{bc}	5.05 ± 0.22^{op}	7.90 ± 0.04^{abc}	3.97 ± 0.12^{fg}	<2.00
21	$5.96 \pm 0.03^{e-j}$	63.42 ± 0.09^{p}	7.72 ± 0.05^{de}	5.12 ± 0.20^{no}	7.86 ± 0.10^{abc}	3.68 ± 0.37^{gh}	<2.00
22	$6.07 \pm 0.01^{b-e}$	62.15 ± 0.10^{q}	7.95 ± 0.01^{b}	5.96 ± 0.20^{hij}	$7.68 \pm 0.16^{b-e}$	3.70 ± 0.14^{gh}	2.30 ± 0.30^{ef}
23	$5.98 \pm 0.02^{d-h}$	68.01 ± 0.04^{hi}	7.49 ± 0.15^{fgh}	6.55 ± 0.09^{ef}	$7.41 \pm 0.03^{e-i}$	6.53 ± 0.09^{a}	<2.00
24	$5.90 \pm 0.03^{g-k}$	67.48 ± 0.03^{jk}	7.59 ± 0.07^{ef}	4.90 ± 0.05^{op}	$7.46 \pm 0.16^{e-h}$	4.96 ± 0.04^{e}	<2.00
25	$5.97 \pm 0.02^{e-h}$	65.52 ± 0.06^{mn}	7.22 ± 0.20^{ijk}	6.45 ± 0.30^{fg}	$7.64 \pm 0.02^{b-e}$	3.98 ± 0.07^{fg}	<2.00
26	$5.88 \pm 0.01^{g-k}$	69.22 ± 0.06^{de}	7.40 ± 0.08^{ghi}	5.72 ± 0.12^{jkl}	7.69 ± 0.07^{bcd}	4.23 ± 0.03^{f}	<2.00
27	6.21 ± 0.12^{bc}	65.70 ± 0.06^{m}	7.80 ± 0.24^{bcd}	6.78 ± 0.16^{de}	7.89 ± 0.09^{abc}	3.31 ± 0.10^{ij}	5.31 ± 0.52^{a}
28	6.14 ± 0.04^{bc}	66.64 ± 0.04^{l}	7.87 ± 0.11^{bcd}	4.57 ± 0.15^{q}	7.87 ± 0.10^{abc}	3.23 ± 0.40^{ij}	4.69 ± 0.05^{b}
29	6.43 ± 0.03^{a}	65.49 ± 0.02^{mn}	8.16 ± 0.08^{a}	5.46 ± 0.06^{lm}	8.13 ± 0.12^{a}	4.09 ± 0.13^{f}	4.51 ± 0.44^{b}
30	6.00 ± 0.01^{dg}	66.25 ± 0.06^{l}	7.74 ± 0.17^{cde}	5.37 ± 0.10^{mn}	7.99 ± 0.06^{ab}	4.21 ± 0.14^{f}	3.39 ± 0.36^{c}
Minimum	5.65 ± 0.02	62.15 ± 0.10	6.47 ± 0.02	3.97 ± 0.06	6.33 ± 0.12	2.20 ± 0.17	2.10 ± 0.17
Maximum	6.43 ± 0.03	72.20 ± 0.02	8.16 ± 0.08	7.95 ± 0.03	8.13 ± 0.12	6.53 ± 0.09	5.31 ± 0.52
Mean ± SD	5.96 ± 0.17	67.25 ± 2.12	7.45 ± 0.42	5.65 ± 0.97	7.42 ± 0.45	4.48 ± 1.16	3.33 ± 1.13
P	**	**	**	**	**	**	**

±; Standard Deviation, **; $P < 0.01$

a-l: Means of the same column having the same letters are not significantly different at $P > 0.05$ (Duncan's test) between the means of samples for pH values and *LAB* counts

a-r: Means of the same column having the same letters are not significantly different at $P > 0.05$ (Duncan's test) between the means of samples for dry matter values

a-m: Means of the same column having the same letters are not significantly different at $P > 0.05$ (Duncan's test) between the means of samples for *TAMB* counts

a-s: Means of the same column having the same letters are not significantly different at $P > 0.05$ (Duncan's test) between the means of samples for *M*/*S* counts

a-f: Means of the same column having the same letters are not significantly different at $P > 0.05$ (Duncan's test) between the means of samples for *Enterobacteriaceae* counts

($r = -0.615$, $p < 0.01$), LAB ($r = -0.550$, $p < 0.01$), the cross-section L* value ($r = -0.368$, $p < 0.05$), and the outer surface L* value ($r = -0.391$, $p < 0.05$). Microbial growth during production is suppressed based on moisture concentration and hence water activity. During this procedure, the loss of moisture in the product results in a reduction of its gloss. As the dry matter content increases, the L* value, indicating the product's brightness, lowers. Our current analysis revealed

Table 4 Linear correlations (Pearson correlation coefficients) among dry matter content (DM), pH, total aerobic mesophilic bacteria (TAMB), yeast-mould, *Micrococcus/Staphylococcus* (M/S), lactic acid bacteria (LAB), and inner and rind area attributes L*, a*, b*, C*, and h°

		DM	pH	TAMB	MY	M/S	LAB	Inner Area					Rind Area			
								L*	a*	b*	C*	h°	L*	a*	b*	C*
pH		-0.292														
TAMB		-0.615**	0.397*													
MY		0.076	-0.327	-0.121												
M/S		-0.049	0.254	0.201	0.311											
LAB		-0.550**	0.451*	0.872**	-0.210	0.073										
Inner Area	L*	-0.368*	0.321	0.453*	-0.286	0.151	0.549**									
	a*	-0.216	0.023	-0.081	-0.266	-0.042	0.051	0.364*								
	b*	0.014	0.342	0.292	-0.123	0.073	0.179	-0.009	-0.323							
	C*	0.020	0.339	0.290	-0.115	0.074	0.175	-0.018	-0.344	1.000**						
	h°	0.209	-0.213	-0.105	0.339	0.003	-0.162	-0.349	-0.795**	-0.312	-0.291					
Rind Area	L*	-0.391*	-0.157	0.255	0.116	0.148	0.225	0.220	0.109	-0.318	-0.319	0.089				
	a*	0.312	-0.055	-0.412*	0.077	0.195	-0.355	-0.259	0.003	0.027	0.027	-0.024	-0.358			
	b*	0.260	-0.005	-0.255	-0.225	0.040	-0.157	0.192	0.318	0.179	0.170	-0.433*	-0.448*	0.609**		
	C*	0.265	0.003	-0.258	-0.219	0.039	-0.159	0.183	0.308	0.185	0.177	-0.426*	-0.460*	0.619**	0.999**	
	h°	-0.280	0.090	0.392*	-0.052	-0.201	0.338	0.232	-0.068	0.025	0.026	0.061	0.317	-0.980**	-0.570**	-0.575**

** $P<0.01$; * $P<0.05$

DM: Dry matter, *TAMB*: Total aerobic mesophilic bacteria, M/S: *Micrococcus*/*Staphylococcus*, *LAB*: Lactic Acid Bacteria, *MY*: Mould-Yeast

a statistically significant inverse correlation between the dry matter ratio and the L* value ($p<0.05$). The L* values for both the inner surface ($r=-0.368$) and the outer surface ($r=-0.391$) are associated with the dry matter ratio (Table 4).

Microbiological counts

Significant differences were found in TAMB counts in Gravyer cheese samples ($P<0.01$), counts ranged from a minimum of 6.47 log CFU/g to a maximum of 8.16 log CFU/g, with an average of 7.45±0.42 log CFU/g (Table 3). These counts are similar to other research results [9, 11]. In our study, the TAMB count was often seen to range from 7 to 8 log CFU/g. Only four samples (1, 4, 7, and 12) exhibited a count below 7 log CFU/g. In one sample (29), it exceeded 8 log CFU/g. These counts are considered standard values for matured cheeses. The variation in TAMB count correlates with the LAB count ($r=0.872$; $p<0.01$), inner surface L* value ($r=0.453$; $p<0.05$), outer surface a* ($r=-0.412$; $p<0.05$), and h values ($r=0.392$; $p<0.05$) (Table 4). This parameter, which indicates the total microbial load of the cheese, is influenced by factors such as the microbial load of the milk used as raw material, the heat treatment applied to the milk, the conditions of cheese production, ripening/storage conditions, and the application of starter cultures.

Significant differences were found in *Micrococcus*/*Staphylococcus* (M/S) counts in Gravyer cheese samples ($P<0.01$), and counts varied from 3.97 log CFU/g to 7.95 log CFU/g, with a mean of 5.65±0.97 log CFU/g (Table 3). The obtained mean M/S counts align with previously reported findings [3, 11]. In the current study, samples 2, 9, and 19 surpassed 7 log CFU/g, despite the overall mean ($P<0.05$). Significant variation was noted: 13 samples ranged from 5 to 6 log CFU/g, six samples were between 4 and 5 log CFU/g, and only one sample, sample 14, was below 4 log CFU/g ($P<0.05$, Table 3). Many studies have reported elevated levels of micrococci in various cheese types, where they represent a predominant microflora. In Calabres, a cheese of Spanish origin, 82% of *Micrococcaceae* isolates were identified as micrococci, while 18% were classified as staphylococci [36]. Bacteria exhibit greater proliferation on the rinds of ripened cheeses compared to the interior and are inhibited at pH levels below 5.5. In our samples, pH values exceeding 5.5 likely promoted the growth of *Micrococcaceae*. In the absence of added starter culture in fermented and ripened dairy products, *Micrococcaceae* arise from the natural microflora of raw milk, typically comprising no more than approximately 1% of the bacterial population. *Micrococci* are characterized as non-motile cocci, Gram-positive, aerobic, and catalase-positive. All species are capable of growth in 5% NaCl, with certain species able to proliferate at concentrations of 10–15% NaCl. Many species exhibit yellow, orange, or red colonies and are primarily located on the rinds of smear-ripened cheeses; however, accurate species identification frequently remains ambiguous. Surface microflora of Comté cheese is predominantly characterized by their presence [37]. Surface-ripened cheeses are characterized by a complex microbiota on their surface that generates a distinctive red–orange smear. Gravyer is produced using thermophilic starter cultures and is typically salted through brining. As a result, salt-tolerant yeasts such as *Candida*, *Debaryomyces*, *Pichia*, *Saccharomyces*, *Kluyveromyces*, *Rhodotorula*, and *Hansenula*, along with *Geotrichum candidum*, proliferate on the rind and convert lactate into CO_2 and H_2O. These alterations promote the proliferation of bacteria from the genera *Micrococcus*, *Staphylococcus*, and several *Corynebacterium* species. French Gruyère, or Gruyère de Comté, features an orange rind and contains *Corynebacterium*, *micrococci*, and yeasts [37]. Burri [38] conducted a study on 12 red-smear cheeses, including Appenzeller, Tilsiter, Gruyère, and Limburger, identifying Brevibacterium and *Arthrobacter/Micrococcus* as the predominant genera in the surface microflora, in addition to *staphylococci*, *corynebacteria*, *enterococci*, and *lactococci*. *Micrococcus luteus* has been identified in firm cheeses including Livarot, Taleggio, and Scimudin, as well as in the semi-hard Gubbeen. Additionally, *Micrococcus* spp. have been found in soft Havarti and semi-hard Danbo [32]. *Staphylococcus equorum* is the most commonly found staphylococcal species in cheeses. Other species such as *Staphylococcus xylosus*, *S. vitulinus*, *S. saprophyticus*, *S. sciuri*, *S. lentus*, and *S. succinus* may also be present in cheese environments [8]. In a study of four Spanish cheeses (San Simón da Costa, Cebreiro, Tetilla, and Arzúa-Ulloa), 26 isolates were classified as *Staphylococcaceae*, 17 as *Micrococcaceae*, and 50 as yeasts [39]. In a study of 25 traditional French raw-milk cheeses, 26% of the isolates were identified as strains of *S. equorum* and *S. xylosus* [40]. Place et al. [41] identified *Staphylococcus equorum subsp. linens* as a novel subspecies derived from Swiss raw-milk cheeses. In a study conducted on Vorarlberger Bergkäse (VB), a hard cheese type produced traditionally from raw cow's milk in Austria, it was shown that *Staphylococcus equorum* and *Candida* species were predominant in the fresh product, *Psychrobacter* and *Debaryomyces* species were predominant in the early stages of ripening (14–30 days), and *Corynebacterium*, *Brevibacterium*, *S. equorum*, and *Scopulariopsis* species were predominant in the cheese rind in the late stages of ripening (90–160 days) [42].

The LAB are characterized as non-motile, non-spore-forming, facultative anaerobes, Gram-positive, and catalase-negative. LAB are crucial in cheese production, with certain species involved in fermentation and others influencing

cheese ripening. Starter *lactococci* are the predominant microflora in the cheese production process. During the production process, their counts can reach up to 10^9 CFU/mL, with levels decreasing as the cheese matures due to environmental factors including high salt concentration, low humidity, pH, and temperature [43]. Although our current study found that LABs constitute the dominant microflora in traditional Kars Gravyer cheeses, the differences between the samples are significant ($P<0.01$). The counts ranged from a minimum of 6.33 log CFU/g to a maximum of 8.13 log CFU/g, with an average of 7.42 ± 0.45 log CFU/g (Table 3). In general, LAB constitute the dominant flora in many types of cheese, including Gravyer cheeses. Topuk and Sezer [11] reported an average count of LAB at 6.67 ± 1.29 log CFU/g based on their analysis. Çetinkaya and Öz [9] reported LAB counts of 7.69 log CFU/g in samples from Kars Gravyer cheeses on day 0 and 6.28 log CFU/g on day 90 during the ripening period. The highest count of LAB was reported on the 25th day of ripening, measuring 8.54 log CFU/g. Kamber et al. [3] reported an average LAB count of 5.1×10^5 CFU/g in nine Gravyer cheeses. A study on Swiss Gruyere cheese, which has similar characteristics to traditional Kars Gravyer cheese, revealed that 90% of the LAB isolated from the cheeses were *Lactobacillus casei* and *L. rhamnosus* [44]. A positive correlation exists between the counts of LAB in Gravyer cheeses and the L* value of inner surface ($p<0.01$, Table 4).

Significant differences ($P<0.01$) were found among the samples examined in terms of yeast-mould count. The count ranged from 2.20 log CFU/g to 6.53 log CFU/g, with an average of 4.48 ± 1.16 log CFU/g. The yeast-mold count was between 3 and 4 log CFU/g in 12 (40%) of the Gravyer samples. In 8 samples (26.7%), it was between 4 and 5 log CFU/g. Only 5 samples (16.7%) had a yeast-mould count between 5 and 6 log CFU/g. These results indicate that the yeast-mould count in Gravyer cheeses is predominantly between 3 and 5 log CFU/g (Table 3). Topuk and Sezer [11] and Kamber et al. [3] reported an average yeast-mould count of 2.10 ± 1.39 log CFU/g and 3.9×10^4 CFU/g in their research on Kars Gravyer cheeses. Çetinkaya and Öz [9] found a significant change in the yeast-mould count during the ripening process of Kars Gravyer cheese. Researchers determined that Kars Gravyer cheeses exhibited an average yeast-mould count of 1.34 ± 2.41 log CFU/g throughout the 90-day ripening period, with a highest count of 5.80 log CFU/g observed on the 25th day of ripening. In a study on the surface microflora of Gravyer cheese, it was stated that yeast developed on the surface, and *D. hansenii* was at a level of 7.9×10^2 CFU/g [45]. A study on Gruyere and Beaufort cheeses revealed that the genera *Debaryomyces*, *Candida*, *Rhodotorula*, and *Kluyveromyces* proliferated on the cheese surface, with populations reaching up to 10^9 CFU/g during the initial three weeks of ripening, subsequently declining to around 10^5 CFU/g for the end of the ripening period [46].

The *Enterobacteriaceae* family comprises bacteria including coliforms, fecal coliforms, *Escherichia coli*, the *E. coli* O157:H7 serotype, *Yersinia enterocolitica*, *Salmonella*, and *Shigella*. Significant differences ($P<0.01$) were found among the samples examined in terms of *Enterobacteriaceae*. The *Enterobacteriaceae* were undetectable (<2.00 log CFU/g) in 66.7% (20 samples) of Gravyer cheese samples. In the 10 samples, the average count was 3.33 ± 1.13 log CFU/g. The values determined in the three samples (6, 14, 22) are quite close to 2.00 log CFU/g (Table 3). Topuk and Sezer [11] reported the absence of *E. coli* in Gravyer cheeses available in Kars, with an average *Enterobacteriaceae* count of 1.39 ± 0.98 log CFU/g. According to the TS 2174 Gravyer standard [35], Gravyer cheese must not contain *E. coli*. Additionally, the presence of coliform bacteria should not exceed 10^2. Samples 27 exhibited the highest values ($P<0.05$), as 5.31 log CFU/g indicating a potential food hygiene hazard. Çetinkaya and Öz [9] reported the absence of coliform bacteria in samples collected at various intervals during the ripening period of Kars Gravyer cheese. The source of *Enterobacteriaceae* group microorganisms in cheeses is attributed to raw milk and contamination from personnel and equipment. The absence of *Enterobacteriaceae* in long-term ripened cheeses is attributed to microbial competition, wherein yeasts and moulds inhibit their development.

Color values

Color substantially influences food quality and is as consequential as taste and aroma in shaping consumer preference [47]. In this regard, color is considered a clue to many characteristics of food, such as flavor, cleanliness, naturalness, or ripeness [48]. Research on the color properties of traditional Kars Gravyer is limited [4], and this study reported color values without specifying whether measurements were taken on the inner area or the rind area. Figure 1 illustrates the visible differences between the rind and inner surfaces. For this reason, in the current research, the color values of the inner area (Table 5) and rind area (Table 6) of the cheeses were measured separately and compared statistically (Table 7).

According to the TS 2174 standard for Gravyer cheese [35], the cheese's rind color should be light dirty brown, the inner area should range from ivory to light yellow, and there should be no discoloration caused by mould or other factors. Consistent with literature [49, 50], in this study, it were determined ahat the inner colour of traditional Kars Gravyer was light yellow, whereas the rind varies from golden

Table 5 Color values (L*, a*, b*, C*, h°) of inner area of of traditional Gravyer cheeses (The values given for L*, a* and b* in each sample are the average of 10 measurement)

Sample Number	L*	a*	b*	C*	h°
1	75.69 ± 2.43^{abc}	$-3.50 \pm 0.35^{h-k}$	23.03 ± 0.70^{lm}	23.29 ± 0.71^{op}	98.65 ± 0.83^{a}
2	$66.02 \pm 1.86^{g-j}$	$-3.51 \pm 0.26^{h-k}$	25.40 ± 0.74^{hij}	$25.64 \pm 0.74^{j-m}$	$97.86 \pm 0.55^{a-d}$
3	$67.71 \pm 1.62^{f-i}$	-3.96 ± 0.35^{l}	28.91 ± 0.93^{abc}	29.18 ± 0.94^{abc}	$97.80 \pm 0.65^{a-d}$
4	70.02 ± 3.82^{ef}	$-3.41 \pm 0.39^{h-k}$	28.08 ± 0.93^{cde}	$28.29 \pm 0.95^{c-f}$	$96.92 \pm 0.68^{d-g}$
5	$67.25 \pm 6.77^{f-j}$	$-3.37 \pm 1.03^{g-k}$	$27.64 \pm 1.94^{cf.}$	$27.86 \pm 1.95^{d-g}$	$96.94 \pm 2.13^{d-g}$
6	73.22 ± 1.21^{cd}	-2.31 ± 0.32^{ab}	26.10 ± 0.55^{ghi}	$26.20 \pm 0.55^{h-k}$	95.06 ± 0.69^{j}
7	68.02 ± 2.30^{fgh}	$-3.48 \pm 0.15^{h-k}$	27.15 ± 0.82^{efg}	$27.38 \pm 0.82^{f-i}$	$97.31 \pm 0.39^{c-f}$
8	69.96 ± 0.65^{ef}	$-3.33 \pm 0.34^{f-k}$	26.05 ± 0.47^{ghi}	$26.27 \pm 0.48^{h-k}$	$97.29 \pm 0.73^{c-f}$
9	$67.39 \pm 1.85^{f-j}$	-3.97 ± 0.17^{l}	27.10 ± 1.34^{efg}	$27.39 \pm 1.32^{f-i}$	98.36 ± 0.62^{ab}
10	65.38 ± 3.39^{hij}	$-3.41 \pm 0.56^{h-k}$	22.10 ± 1.83^{m}	22.37 ± 1.84^{p}	98.80 ± 1.37^{a}
11	74.96 ± 2.18^{abc}	$-2.90 \pm 0.33^{c-f}$	25.05 ± 0.88^{ijk}	$25.22 \pm 0.90^{k-n}$	$96.60 \pm 0.62^{e-h}$
12	64.68 ± 2.60^{j}	-2.26 ± 0.72^{a}	24.91 ± 2.58^{ijk}	$25.03 \pm 2.52^{k-n}$	95.33 ± 2.09^{ij}
13	71.12 ± 3.47^{de}	$-3.35 \pm 0.49^{g-k}$	28.73 ± 0.81^{bc}	28.93 ± 0.78^{bcd}	$96.67 \pm 1.09^{e-h}$
14	69.10 ± 2.74^{ef}	$-3.09 \pm 0.23^{d-h}$	25.97 ± 1.19^{ghi}	$26.16 \pm 1.20^{i-l}$	$96.77 \pm 0.38^{e-h}$
15	68.36 ± 1.41^{efg}	$-3.26 \pm 0.14^{e-j}$	$27.59 \pm 0.85^{c-f}$	$27.79 \pm 0.84^{d-g}$	$96.75 \pm 0.45^{e-h}$
16	64.93 ± 1.79^{ij}	$-3.39 \pm 0.31^{h-k}$	23.98 ± 1.07^{kl}	24.22 ± 1.09^{no}	98.05 ± 0.57^{abc}
17	65.03 ± 4.14^{ij}	-3.65 ± 0.45^{jkl}	$27.28 \pm 1.53^{d-g}$	$27.52 \pm 1.55^{e-h}$	$97.62 \pm 0.81^{b-e}$
18	75.83 ± 1.26^{abc}	$-3.15 \pm 0.33^{e-i}$	22.27 ± 2.08^{m}	22.50 ± 2.07^{p}	98.09 ± 0.95^{abc}
19	$67.17 \pm 2.74^{f-j}$	$-3.50 \pm 0.19^{h-k}$	27.15 ± 0.64^{efg}	$27.37 \pm 0.65^{f-i}$	$97.34 \pm 0.29^{c-f}$
20	76.44 ± 2.22^{ab}	$-2.92 \pm 0.59^{c-g}$	$27.87 \pm 1.15^{c-f}$	$28.03 \pm 1.16^{c-g}$	$95.99 \pm 1.21^{g-j}$
21	74.85 ± 1.02^{abc}	$-3.49 \pm 0.36^{h-k}$	24.63 ± 0.45^{jk}	24.87 ± 0.47^{lmn}	98.06 ± 0.77^{abc}
22	77.66 ± 1.97^{a}	-2.52 ± 0.43^{abc}	24.65 ± 0.65^{jk}	24.78 ± 0.63^{mn}	95.85 ± 1.06^{hij}
23	71.05 ± 2.73^{de}	-3.65 ± 0.37^{jkl}	28.73 ± 1.08^{bc}	28.96 ± 1.04^{bcd}	$97.26 \pm 0.91^{c-f}$
24	69.72 ± 2.04^{ef}	$-3.60 \pm 0.22^{i-l}$	28.51 ± 1.31^{cd}	28.73 ± 1.30^{cde}	$97.14 \pm 0.62^{c-f}$
25	76.41 ± 2.37^{ab}	-2.83 ± 0.44^{cde}	26.54 ± 0.78^{fgh}	$26.70 \pm 0.78^{g-j}$	96.08 ± 0.96^{ghi}
26	74.13 ± 3.75^{bc}	$-2.89 \pm 0.71^{c-f}$	26.17 ± 1.07^{ghi}	$26.34 \pm 1.08^{h-k}$	$96.29 \pm 1.54^{f-i}$
27	77.56 ± 4.08^{a}	-2.70 ± 0.52^{bcd}	$27.62 \pm 2.45^{c-f}$	$27.75 \pm 2.45^{d-g}$	95.60 ± 1.15^{ij}
28	73.66 ± 3.32^{bcd}	-3.79 ± 0.31^{kl}	29.81 ± 1.42^{ab}	30.05 ± 1.43^{ab}	$97.24 \pm 0.45^{c-f}$
29	75.90 ± 4.82^{abc}	$-3.52 \pm 0.43^{h-k}$	30.08 ± 1.15^{a}	30.29 ± 1.10^{a}	$96.70 \pm 1.06^{e-h}$
30	75.08 ± 2.26^{abc}	$-3.20 \pm 0.35^{e-j}$	24.84 ± 1.82^{ijk}	$25.04 \pm 1.85^{k-n}$	$97.31 \pm 0.38^{c-f}$
Minimum	64.68 ± 2.60	-3.97 ± 0.17	22.10 ± 1.83	22.37 ± 1.84	95.06 ± 0.67
Maximum	77.66 ± 1.97	-2.26 ± 0.72	30.08 ± 1.15	30.29 ± 1.10	98.80 ± 1.37
Mean ± SD	71.14 ± 5.03	-3.26 ± 0.60	26.46 ± 2.39	26.67 ± 2.39	97.06 ± 1.32
P	**	**	**	**	**

±; Standard Deviation, **; $P<0.01$

a-j: Means of the same column having the same letters are not significantly different at $P > 0.05$ (Duncan's test) between the means of samples for L* and h° values

a-l: Means of the same column having the same letters are not significantly different at $P > 0.05$ (Duncan's test) between the means of samples for a* values

a-m: Means of the same column having the same letters are not significantly different at $P > 0.05$ (Duncan's test) between the means of samples for b* values

a-p: Means of the same column having the same letters are not significantly different at $P > 0.05$ (Duncan's test) between the means of samples for C* values

yellow to brownish. Significant differences were found in the inner area ($P<0.01$, Table 5) and rind area ($P<0.01$, Table 6) colors of the Gravyer cheeses examined. The differences between inner and rind area colors were also found to be significant ($P<0.01$, Table 7). The rind area a* value was positive in samples 1, 2, 4–9, 11, 15–17, and 20 (Table 6). When examining the yeast and mould counts of these samples, it was observed that the counts were particularly high in samples 4, 8, 9, and 16 (Table 3). This can be due to yeasts facilitating the growth of bacteria, particularly those that produce red-orange pigments, in the surface microflora. The highest rind area b* was observed in sample 4 and the lowest in sample 10 ($P<0.05$) (Table 6). For the inner surface, sample 29 exhibited the highest b* ($P<0.05$), and samples 18 and 10 the lowest (Table 5). Çavuş and Beykaya [4] reported L*, a*, and b* values of 70.75 ± 2.70, -2.80 ± 0.72, and 23.51 ± 2.28 for Kars Gravyer—lower than those for Swiss Gravyer (77.15 ± 3.96, -2.42 ± 0.72, and 24.40 ± 3.13). As seen in Table 7, there are statistically significant differences ($p<0.01$) in all color values between

Table 6 Color values (L*, a*, b*, C*, h°) of rind area of of traditional Gravyer cheeses (The values given for L*, a* and b* in each sample are the average of 10 measurement)

Sample Number	L*	a*	b*	C*	h°
1	$60.99 \pm 3.69^{f-k}$	$0.85 \pm 0.81^{c-f}$	$19.60 \pm 3.01^{h-m}$	$19.64 \pm 3.00^{i-n}$	$87.51 \pm 2.53^{i-l}$
2	55.82 ± 2.31^{lmn}	$0.09 \pm 0.71^{e-i}$	$23.01 \pm 2.35^{d-h}$	$23.02 \pm 2.35^{d-i}$	$89.92 \pm 1.81^{f-i}$
3	70.43 ± 4.85^{abc}	-1.94 ± 0.87^{lm}	$17.64 \pm 5.28^{k-n}$	$17.76 \pm 5.30^{l-o}$	96.31 ± 2.26^{bc}
4	50.66 ± 4.37^{o}	4.02 ± 2.26^{b}	33.08 ± 4.17^{a}	33.41 ± 4.05^{a}	82.89 ± 4.38^{m}
5	56.88 ± 3.80^{klm}	1.32 ± 0.90^{cde}	$24.48 \pm 3.70^{b-e}$	24.53 ± 3.72^{cde}	$87.05 \pm 2.03^{i-l}$
6	64.37 ± 1.69^{efg}	$0.54 \pm 1.70^{d-g}$	$24.15 \pm 1.07^{c-f}$	$24.21 \pm 1.12^{cf.}$	88.81 ± 3.89^{ij}
7	56.25 ± 6.06^{lm}	5.23 ± 1.20^{a}	27.33 ± 2.04^{b}	27.85 ± 2.11^{b}	79.17 ± 2.31^{n}
8	$64.78 \pm 5.02^{d-h}$	4.16 ± 3.16^{ab}	$20.74 \pm 7.78^{g-l}$	$21.21 \pm 8.26^{e-l}$	79.50 ± 3.52^{n}
9	$62.63 \pm 2.77^{e-i}$	$0.26 \pm 0.53^{e-h}$	15.79 ± 1.87^{no}	15.80 ± 1.87^{op}	89.17 ± 1.99^{hij}
10	71.90 ± 8.77^{ab}	-1.83 ± 1.51^{klm}	13.39 ± 2.28^{o}	13.60 ± 2.15^{p}	98.42 ± 6.89^{b}
11	69.24 ± 6.61^{bcd}	0.42 ± 1.25^{efg}	$19.54 \pm 3.72^{i-m}$	$19.58 \pm 3.70^{i-n}$	88.16 ± 3.73^{ijk}
12	$63.62 \pm 4.14^{e-h}$	$-0.29 \pm 2.06^{f-j}$	$20.82 \pm 4.92^{g-l}$	$20.90 \pm 4.94^{f-m}$	89.74 ± 5.55^{ghi}
13	$60.02 \pm 0.92^{g-l}$	$-0.61 \pm 1.70^{g-k}$	$21.09 \pm 1.57^{f-k}$	$21.16 \pm 1.53^{e-l}$	$92.08 \pm 4.63^{e-h}$
14	$57.35 \pm 4.06^{j-m}$	-1.68 ± 1.11^{klm}	$18.53 \pm 1.35^{i-n}$	$18.65 \pm 1.31^{j-o}$	95.23 ± 3.53^{cde}
15	64.17 ± 3.83^{efg}	1.70 ± 1.16^{cd}	$19.86 \pm 2.50^{h-m}$	$19.95 \pm 2.55^{h-n}$	85.35 ± 3.09^{klm}
16	$57.26 \pm 6.81^{j-m}$	1.71 ± 0.81^{cd}	17.48 ± 3.06^{lmn}	17.57 ± 3.07^{mno}	84.42 ± 2.70^{lm}
17	51.72 ± 3.47^{no}	4.06 ± 0.67^{b}	$22.98 \pm 3.39^{d-h}$	$23.36 \pm 3.25^{d-h}$	79.60 ± 3.07^{n}
18	66.28 ± 3.78^{cde}	-2.15 ± 0.42^{lm}	$21.23 \pm 2.91^{e-j}$	$21.34 \pm 2.90^{e-k}$	95.84 ± 1.29^{bcd}
19	70.80 ± 11.30^{abc}	-1.72 ± 0.91^{klm}	$17.76 \pm 5.77^{j-n}$	$17.86 \pm 5.80^{k-o}$	95.42 ± 2.17^{cd}
20	$61.61 \pm 3.74^{e-j}$	1.31 ± 1.21^{cde}	26.10 ± 3.29^{bcd}	26.16 ± 3.30^{bcd}	$87.06 \pm 2.60^{i-l}$
21	69.15 ± 1.93^{bcd}	$-0.94 \pm 0.49^{h-l}$	$20.16 \pm 1.81^{h-m}$	$20.19 \pm 1.80^{h-n}$	$92.74 \pm 1.43^{d-g}$
22	$62.69 \pm 3.83^{e-i}$	-2.00 ± 0.80^{lm}	$21.96 \pm 3.32^{e-i}$	$22.06 \pm 3.38^{e-j}$	95.01 ± 1.45^{cde}
23	$62.03 \pm 4.75^{e-i}$	$-1.34 \pm 1.71^{j-m}$	$19.22 \pm 1.47^{i-m}$	$19.33 \pm 1.55^{j-n}$	93.72 ± 4.89^{cde}
24	$59.36 \pm 5.57^{h-l}$	-2.59 ± 0.68^{m}	17.12 ± 2.67^{mn}	17.32 ± 2.70^{no}	98.63 ± 1.85^{b}
25	65.27 ± 3.95^{def}	$-0.02 \pm 1.19^{f-i}$	$19.26 \pm 1.60^{i-m}$	$19.30 \pm 1.61^{j-n}$	$90.01 \pm 3.44^{f-i}$
26	$58.41 \pm 1.63^{i-l}$	-1.69 ± 0.58^{klm}	$23.71 \pm 1.92^{d-g}$	$23.78 \pm 1.90^{d-g}$	94.14 ± 1.57^{cde}
27	65.02 ± 2.44^{def}	1.87 ± 0.67^{c}	26.94 ± 2.13^{bc}	27.01 ± 2.15^{bc}	86.07 ± 1.23^{jkl}
28	66.44 ± 2.43^{cde}	$-1.09 \pm 0.53^{i-l}$	$19.25 \pm 3.19^{i-m}$	$19.29 \pm 3.17^{j-n}$	93.39 ± 1.79^{cde}
29	53.18 ± 0.59^{mno}	-3.92 ± 0.17^{n}	17.15 ± 0.53^{mn}	17.59 ± 0.53^{mno}	102.88 ± 0.53^{a}
30	73.80 ± 0.44^{a}	$-1.05 \pm 0.30^{i-l}$	$20.56 \pm 0.63^{g-m}$	$20.59 \pm 0.62^{g-n}$	92.91 ± 0.86^{def}
Minimum	50.66 ± 4.37	-3.92 ± 0.17	13.39 ± 3.78	13.60 ± 2.15	79.17 ± 2.31
Maximum	73.80 ± 0.44	5.23 ± 1.20	33.08 ± 4.17	33.41 ± 4.05	102.88 ± 0.53
Mean ± SD	62.40 ± 7.35	0.09 ± 2.49	21.00 ± 4.98	21.13 ± 5.02	90.37 ± 6.52
P	**	**	**	**	**

±; Standard Deviation, **; $P < 0.01$

a-o: Means of the same column having the same letters are not significantly different at $P > 0.05$ (Duncan's test) between the means of samples for L* and b* values

a-n: Means of the same column having the same letters are not significantly different at $P > 0.05$ (Duncan's test) between the means of samples for a* and h° values

a-p: Means of the same column having the same letters are not significantly different at $P > 0.05$ (Duncan's test) between the means of samples for C* values

Table 7 Descriptive statistics and paired comparisons (inner and rind area)

Color Properties	*N*	Surface	Minimum	Maximum	Mean	*P* values
L*	30	Inner area	64.68	77.66	71.14 ± 0.78^{a}	**
	30	Rind area	50.66	73.80	62.40 ± 1.09^{b}	
a*	30	Inner area	-3.97	-2.26	-3.26 ± 0.08^{b}	**
	30	Rind area	-3.92	5.23	0.09 ± 0.40^{a}	
b*	30	Inner area	22.10	30.08	26.46 ± 0.38^{a}	**
	30	Rind area	13.39	33.08	21.00 ± 0.72^{b}	
C*	30	Inner area	22.37	30.29	26.67 ± 0.38^{a}	**
	30	Rind area	13.60	33.41	21.13 ± 0.73^{b}	
h°	30	Inner area	95.06	98.80	97.06 ± 0.17^{a}	**
	30	Rind area	79.17	102.88	90.37 ± 1.08^{b}	

±; Standard Error, ** $P < 0.01$

a–b: Means of the same column and in the section having the same letters are significantly different at $P < 0.05$ (Duncan's test)

the inner and outer/rind surfaces of Gravyer cheeses. This difference is thought to be due to the presence of *D. hansenii*, which reduces the acidity of the cheese surface and promotes the growth of acid-sensitive, pigment-forming bacteria. In a model of Munster cheese, Leclercq-Perlat et al. [51] determined that *Brevibacterium linens* pigment development accelerated during deacidification in the presence of *D. hansenii*, with all colorimetric parameters except L* rising more rapidly. In the current results, inner surface L*, b*, C*, and h° were significantly higher than rind area values ($p<0.01$), whereas rind a* values were higher than inner area a* ($p<0.05$). All inner surfaces displayed negative a* (Table 5), while 14 rinds showed positive a* (Table 6), consistent with drier rind matter and microbially mediated coloration. During ripening, black spots may appear on the rind due to *Cladosporium* spp [52]. In surface-ripened cheeses such as Munster, Livarot, and Taleggio, *D. hansenii* and other yeasts stimulate *Corynebacterium* growth, driving orange–red pigment formation and rind color shifts [53]. In the current study, a correlation was found between the inner surface L* value and dry matter ($P<0.05$) and total aerobic mesophilic bacteria count ($P<0.05$) and lactic acid bacteria count ($P<0.01$) (Table 4). Similarly, strong correlations between inner and rind, particularly C* and h° values, were observed. Significant ($p<0.01$) coefficients included inner area b* with inner C* ($r=1.000$); inner a* with inner h°; rind area a* with rind area b*; rind area a* with rind area C*; rind area a* with rind area h°; rind area b* with rind area C*; rind area b* with rind area h°; and rind area C* with rind area h°. Additionally significant ($p<0.05$) relationships included inner area L* with inner a*; inner h° with rind area b* and rind area C*; and rind area L* with rind area b* and rind area C*. In a Munster-style model, Leclercq-Perlat et al. [51] reported C* increasing from 15 to 30–35 during the first 12 days of incubation and remaining stable thereafter, while h° decreased from 88 to 78 by day 12.

Conclusions

In this survey of 30 traditional Gravyer cheeses, *Debaryomyces hansenii*, present in 66.7% of all samples, was clearly the predominant yeast. The other yeast species is *Cryptococcus laurentii* (26.7%). The identification of both individual and combined yeast species (6.6%) in traditional Gravyer cheeses is a significant finding of this research. The dominance of *D. hansenii* over *C. laurentii* in cheeses where both yeasts were present indicates that production, ripening, and storage conditions are more suitable for *D. hansenii*. The presence of this yeast species in cheese varieties is important for classifying Gravyer cheeses traditionally produced in Kars and demonstrating its relationship with traditionally produced European cheeses. However, there is no comprehensive literature on the potential effects of *C. laurentii* yeast detected in Kars Gravyer cheeses on quality. Therefore, comprehensive research is needed to determine the growth conditions of *C. laurentii* in Gravyer cheese and to evaluate its positive or negative effects on product quality.

The microbial, chemical, and color analysis results and images (Fig. 1) of the examined cheeses showed that the cheeses were marketed at different stages of ripening or that there was no standardized production among manufacturers. Thus, the results of the current study, particularly concerning the identification of the yeast species, can be assessed to inform future technological research, product standardization, and the cultivation of new cultures specific to traditional Kars Gravyer cheese production. Given the functional properties of *D. hansenii*, commonly found in cheeses produced from raw cow's milk, its potential to improve the ripening process and serve as a viable candidate for an auxiliary industrial culture should be confirmed by experimental findings in detailed research. Furthermore, comprehensive research into the application of the identified yeast strain as a commercial yeast could provide advantages for both producers and consumers. For these reasons, in future studies, the isolated dominant strain, *D. hansenii*, should also be used in Gravyer cheese production via a controlled inoculation method, and its effects on final product quality should be more clearly revealed through experimental findings.

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Declarations

Conflict of interests The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

1. S. Duru, A. Seçer, Consumer's purchase behaviour and attitudes ın traditional food products: Mersin province. Atatürk Univ. J. Agric. Fac. **50**(1), 1–10 (2019). https://doi.org/10.17097/ataunizfd.407116
2. K. Arınç, The Gruyere cheese production in Bogatepe village and its importance in terms of sustainable development (Kars/Turkey). Turkish Geograph Rev. **70**, 7–18 (2018). https://doi.org/10.17211/tcd.349760
3. U. Kamber, M. Elmalı, H. Yaman, Microbiological and chemical properties of Gravier cheeses. Atatürk Univ. J. Vet. Sci. **3**(1), 1–7 (2008)
4. M. Çavuş, M. Beykaya, Investigation of some quality properties of Kars Gravyer, Gruyere, and Emmental Cheese. Eur. J. Sci. Technol. **35**, 187–194 (2022). https://doi.org/10.31590/ejosat.1067172
5. P.L.H. McSweeney, P.F. Fox, P.D. Cotter, D.W. Everett, Cheese: Chemistry, Physics & Microbiology, Elsevier. (2017)
6. P.L.H. McSweeney, G. Ottogalli, P.F. Fox, *Diversity and Classification of Cheese Varieties: An Overview, Cheese* (Fourth Edition), Elsevier. (2017)
7. M.T. Fröhlich-Wyder, E. Arias-Roth, E. Jakob, Cheese yeasts. Yeast. **36**, 129–141 (2019). https://doi.org/10.1002/yea.3368
8. F. Irlinger, S. Helinck, J.L. Jany, *Secondary and Adjunct Cultures, Cheese: Chemistry, Physics and Microbiology. 1, General Aspects* (Elsevier Academic, London, 2017), pp. 273–300
9. A. Çetinkaya, F. Öz, Composition and microbiological analysis for quality evaluation of Kars Gravyer cheese: influence of ripening period. Food Sci. Technol. **39**(4), 1052–1058 (2019). https://doi.org/10.1590/fst.18218
10. Z. Ulutaş, A. Çağlar, A. Kurt, A Study for manufacturing and organoleptic, physical and chemical properties of Kars Gravyer Cheese. J. FOOD **18**(3), 197–202 (1993). https://dergipark.org.tr/tr/download/article-file/79218
11. Ş. Topuk, Ç. Sezer, Some quality characteristics of Kars Gravyer cheese. J. FOOD **40**(2), 69–75 (2015). https://doi.org/10.15237/gida.GD14053
12. A. Çetinkaya, F. Öz, Changes in cholesterol and free fatty acid content of Kars Gravyer Cheese (A Turkish dairy product produced by the traditional method). Ukrainian Food J. **7**(4), 409–420 (2018). https://doi.org/10.24263/2304-974X-2018-7-3-6
13. R. Vasdinyei, T. Deák, Characterization of yeast isolates originating from Hungarian dairy products using traditional and molecular identification techniques. Int. J. Food Microbiol. **86**(1–2), 123–130 (2003). https://doi.org/10.1016/S0168-1605(03)00251-4
14. B. Esteve-Zarzoso, C. Belloch, F. Uruburu, A. Querol, Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. Int. J. Syst. Evol. Bacteriol. **49**(1), 329–337 (1999). https://doi.org/10.1099/00207713-49-1-329
15. M. Nei, S. Kumar, *Molecular Evolution and Phylogenetics* (Oxford University Press, New York. USA, 2000)
16. S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, Molecular Evolutionary Genetics Analysis across computing platforms. Mol. Biol. Evol. **35**, 1547–1549 (2018). https://doi.org/10.1093/molbev/msy096
17. D.J. Appel, T.R. Gordon, Intraspecific variation within a population of *Fusarium oxysporum* based on the RFLP analysis of the intergenic spacer region of the rDNA. Exp. Mycol. **19**, 120–128 (1995). https://doi.org/10.1006/emyc.1995.1014
18. J. Sambrock, E.F. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press (Cold Spring Harbor, New York, 1989)
19. R Core Team. A language and environment for statistical computing, Viyana, Avusturya: R Foundation for Statistical Computing, (2019). https://www.R-project.org
20. M.C. Rea, S. Görges, R. Gelsomino, N.M. Brennan, J. Mounier, M. Vancanneyt, T.M. Cogan, Stability of the biodiversity of the surface consortia of Gubbeen, a red-smear cheese. J. Dairy. Sci. **90**(5), 2200–2210 (2007). https://doi.org/10.3168/jds.2006-377
21. N. Banjara, M.J. Suhr, H.E. Hallen-Adams, Diversity of yeast and mold species from a variety of cheese types. Cur Microbiol. **70**, 792–800 (2015). https://doi.org/10.1007/s00284-015-0790-1
22. A. Ceugniez, D. Drider, P. Jacques, F. Coucheney, Yeast diversity in a traditional French cheese Tomme d'orchies reveals infrequent and frequent species with associated benefits. Food Microbiol. **52**, 177–184 (2015). https://doi.org/10.1016/j.fm.2015.08.001
23. A.C. Freitas, A.E. Pintado, M.E. Pintado, F.X. Malcata, Role of dominant microflora of Picante cheese on proteolysis and lipolysis. Int. Dairy. J. **9**(9), 593–603 (1999). https://doi.org/10.1016/S0958-6946(99)00129-6
24. P. Banerjee, M. Haider, V. Trehan, B. Mishra, A. Thakur, V. Dogra, P. Loomba, *Cryptococcus laurentii* fungemia. Indian J. Med. Microbiol. **31**(1), 75–77 (2013). https://doi.org/10.4103/0255-0857.108731
25. A.D. Ferreira, B.C. Viljoen, Yeasts as adjunct starters in matured Cheddar cheese. Int. J. Food Microbiol. **86**, 131–140 (2003). https://doi.org/10.1016/s0168-1605(03)00252-6
26. C. Huang, L. Zhang, P.G. Johansen, M.A. Petersen, N. Arneborg, L. Jespersen, *Debaryomyces hansenii* strains isolated from Danish cheese brines act as biocontrol agents to inhibit germination and growth of contaminating molds. Front. Microbiol. 12, 662785. (2021). https://doi.org/10.3389/fmicb.2021.662785
27. S.Q. Liu, M. Tsao, Biocontrol of dairy moulds by antagonistic dairy yeast *Debaryomyces hansenii* in yoghurt and cheese at elevated temperatures. Food Cont. **20**, 852–855 (2009). https://doi.org/10.1016/j.foodcont.2008.10.006
28. C. Bartschi, J. Berthier, G. Valla, Inventaire et evolution des flores fongiques de surface du Reblochon de Savoie. Lait. **74**, 105–114 (1994). https://doi.org/10.1051/lait:199429
29. A.C. Freitas, C. Pais, F.X. Malcata, T.A. Hogg, Microbiological characterization of Picante da Beira Baixa cheese. J. Food Protect. **59**(2), 155–160 (1996). https://doi.org/10.4315/0362-028X-59.2.155
30. C. Biagiotti, M. Ciani, L. Canonico, F. Comitini, Occurrence and involvement of yeast biota in ripening of Italian Fossa cheese. Eur. Food Res. Technol. **244**(11), 1921–1931 (2018). https://doi.org/10.1007/s00217-018-3104-6
31. G.K. Zerfiridis, A. Vafopoulou-Mastrogiannaki, E. Litopoulou-Tzanetaki, Changes during ripening of commercial Gruyere cheese. Int. Dairy. J. **67**(7), 1397–1405 (1984). https://doi.org/10.3168/jds.S0022-0302(84)81454-X
32. J. Mounier, M. Coton, F. Irlinger, S. Landaud, P. Bonnarme, *Smear-Ripened Cheeses*. Cheese (Fourth Edition), Chemistry, Physics and Microbiology, 2017, Chepter 38, Academic Press
33. M. Montel, S. Buchin, A. Mallet, C. Delbes-Paus, D.A. Vuitton, N. Desmasures, F. Berthier, Traditional cheeses: Rich and diverse microbiota with associated benefits. Int. J. Food Microbiol. **177**, 136–154 (2014). https://doi.org/10.1016/j.ijfoodmicro.2014.02.019
34. C.F.V. Souza, T.D. Rosa, M.A.Z. Ayub, Changes in the microbiological and physicochemical characteristics of Serrano cheese during manufacture and ripening. Braz J. Microbiol. **34**, 260–266 (2003). https://doi.org/10.1590/S1517-83822003000300016
35. T.S. Anonymous, Institute, *TS 2174, Gravyer Cheese Standard* (Ankara, TÜRKİYE, 1989)
36. M. Nuñez, *Micrococcus*. Encyclopedia of Food Microbiology (Second Edition), Elsevier Academic Press, 2014, pp. 1344–1350

37. P.F. Fox, P.L.H. McSweeney, *Cheese: An Overview, Cheese (Fourth Edition)* (Academic, 2017)
38. S. Burri, Microbiology and biochemistry of the Gram-positive microflora of the surface of typical Swiss cheeses. Doctoral thesis, ETH Zürich, 1999, p. 102
39. P. Rodríguez-Alonso, J.I. Garabal, J. Centeno, Preliminary characterization of staphylococcal, micrococcal and yeast isolates obtained from raw cow milk cheeses currently produced in Galicia (NW Spain). Italian J. Food Sci. **20**, 161 (2008)
40. F. Irlinger, A. Morvan, N.E. Solh, J.L. Bergere, Taxonomic characterization of coagulase-negative staphylococci in ripening flora from traditional French cheeses. Syst. Appl. Microbiol. **20**(2), 319–328 (1997). https://doi.org/10.1016/S0723-2020(97)80079-3
41. R.B. Place, D. Hiestand, H.R. Gallmann, M. Teuber, *Staphylococcus equorum* subsp. *linens*, subsp. nov., a starter culture component for surface ripened semi-hard cheeses. Syst. Appl. Microbiol. 26 (1), 12747407. (2003). https://doi.org/10.1078/072320203322337281
42. N.M. Quijada, S. Schmitz-Esser, B. Zwirzitz, C. Guse, C.R. Strachan, M. Wagner, M. Dzieciol, Austrian raw-milk hard-cheese ripening involves successional dynamics of non-inoculated bacteria and fungi. Foods. **9**(12), 1851 (2020). https://doi.org/10.3390/foods9121851
43. J.M. Banks, A.G. Williams, The role of the nonstarter lactic acid bacteria in Cheddar cheese ripening. Int. J. Dairy. Technol. **57**, 145–152 (2004). https://doi.org/10.1111/j.1471-0307.2004.00150.x
44. M.G. Casey, J.P. Häni, J. Gruskovnjak, W. Schaeren, D. Wechsler, Characterisation of the non-starter lactic acid bacteria (NSLAB) of Gruyère PDO cheese. Lait. **86**(6), 407–414 (2006). https://doi.org/10.1051/lait:2006020
45. W. Bockelmann, *Secondary Cheese Starter Cultures, Technology of Cheesemaking* (John Wiley & Sons, Ltd., 2010)
46. T.P. Beresford, N.A. Fitzsimons, N.L. Brennan, T.M. Cogan, Recent advances in cheese microbiology. Int. Dairy. J. **11**, 259–274 (2001). https://doi.org/10.1016/S0958-6946(01)00056-5
47. T.N. McCaig, Extending the use of visible/near-infrared reflectance spectrophotometers to measure colour of food and agricultural products. Food Res. Int. **35**(8), 731–736 (2002). https://doi.org/10.1016/S0963-9969(02)00068-6
48. R. Gómez-Ladrón, de J.E. Guevara, R. Pardo-González, F. Varón-Castellanos, Navarro Albaladejo, Evolution of color during the ripening of selected varieties of paprika pepper (*Capsicum annuum* L). J. Agric. Food Chem. **44**(8), 2049–2052 (1996). https://doi.org/10.1021/jf950465m
49. R. Fernandes, Microbiology Handbook Dairy Products. Leatherhead Publishing. a Division of Leatherhead Food International Ltd, Randalls Road, Leatherhead, Surrey KT22 7RY, 2008, UK
50. P. Lavanchy, U. Bütikofer, Sensory characterization of Swiss hard and semi-hard cheeses. Mitt Lebensmittelunters Hyg. **90**(6), 670–683 (1999)
51. M.N. Leclercq-Perlat, G. Corrieu, H.E. Spinnler, The color of *Brevibacterium linens* depends on the yeast used for cheese deacidification. J. Dairy. Sci. **87**(5), 1536–1544 (2004). https://doi.org/10.3168/jds.S0022-0302(04)73305-6
52. A. Messini, A. Buccioni, S. Minieri, F. Mannelli, L. Mugnai, C. Comparini, M. Venturi, C. Viti, A. Pezzati, S. Rapaccini, Effect of chestnut tannin extract (*Castanea sativa* Miller) on the proliferation of *Cladosporium cladosporioides* on sheep cheese rind during the ripening. Int. Dairy. J. **66**, 6–12 (2017). https://doi.org/10.1016/j.idairyj.2016.10.012
53. L. Dufossé, P. Galaup, E. Carlet, C. Flamin, A. Valla, Spectrocolorimetry in the CIE L*a*b* color space as useful tool for monitoring the ripening process and the quality of PDO red-smear soft cheeses. Food Res. Int. **38**(8), 919–924 (2005). https://doi.org/10.1016/j.foodres.2005.02.013

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