

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

<https://doi.org/10.17113/ftb.64.04.26.9500>

original scientific paper

Application of Indigenous Lactic Acid Bacteria in Tulum Cheese Production: Effects on Physicochemical, Microbiological, Volatile and Sensory Properties

Running head: Culture Effect on Tulum Cheese Quality

Tuğba Karabekmez Erdem^{1*} and Kenan Sinan Dayısoylu²

¹Department of Food Processing, Vocational School of Technical Sciences, University of Kahramanmaraş Sutcu Imam, Dulkadiroğlu 46100, Kahramanmaraş, Turkey

²Department of Food Engineering, Faculty of Engineering and Architecture, University of Kahramanmaraş Sütçü İmam, Dulkadiroğlu 46100, Kahramanmaraş, Turkey

Received: 27 November 2025

Accepted: 18 October 2026



Copyright © 2026 Authors retain copyright and grant the FTB journal the right of first publication under CC-BY 4.0 licence that allows others to share the work with an acknowledgement of the work's authorship and initial publication in the journal

SUMMARY

Research background. Tulum cheese, traditionally made from raw milk, serves as a significant source of lactic acid bacteria. These bacteria contribute to the cheese's distinctive properties. By isolating and identifying these bacteria from local sources and incorporating them with starter cultures for Tulum cheese production using pasteurized milk, it is possible to obtain cheeses with more controlled quality characteristics. This study aims to isolate and identify lactic acid bacteria, use them in the production of Tulum cheese, and explore the resulting cheeses' general characteristics, including differences in aromatic profiles and sensory analyses.

Experimental approach. In this study, molecular identification of lactic acid bacteria was performed using the 16S gene sequencing approach. Metabolite characterization of the bacteria was

*Corresponding author:

Phone: +9005532725253

E-mail: tkarabekmez@hotmail.com

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

performed using HPLC. Subsequently, the bacteria were incorporated into the production of Tulum cheese from pasteurized milk in different combinations using a commercial starter culture. All analyses of Tulum cheese samples were performed throughout the storage period (1, 30, 60, and 90 days). Physical, chemical, and microbiological properties were determined for general characterization purposes. Volatile profiles of Tulum cheese samples were determined by HS-GC/MS and evaluated through principal component analysis. The sensory properties of the samples were tested by experienced tasters familiar with Tulum cheese.

Results and conclusions. The results demonstrated the potential of metabolically characterized lactic acid bacteria to produce key aroma compounds associated with pyruvate metabolism. TP2 exhibited the highest titratable acidity and the highest Lactobacilli counts during early and mid-ripening, whereas K2 showed the highest TCA-SN and PTA-SN values at day 90. Different culture combinations significantly affected the physicochemical, microbiological, sensory, and volatile characteristics of Tulum cheese ($p < 0.05$). PCA clearly differentiated cheeses according to culture combination and ripening time. Sensory evaluation showed that TP1, TP3, and TP2 were the most preferred samples on days 1, 30, 60, and 90, respectively.

Novelty and scientific contribution. This study evaluates the application of indigenous lactic acid bacteria, isolated from traditionally produced Tulum cheeses, in combination with commercial starter cultures, for Tulum cheese production. The results highlight innovative approaches to enhance and diversify cheese characteristics through targeted culture selection.

Keywords: indigenous lactic acid bacteria; Tulum cheese; starter culture

INTRODUCTION

Tulum cheese, which is derived from Central Asia, is the most produced and consumed cheese type in Turkey, following white cheese and Kashar cheese. It is a semi-hard artisanal cheese with its white and creamy color, high fat content, crumbly structure, and buttery, slightly tongue-biting characteristics and is economically valuable as well. Tulum cheese is manufactured by fermenting curd without an addition of starter culture, derived from coagulating raw milk from sheep, goats, cows, or a mixture of these milk varieties with rennet, followed by crumbling, salting, and pressing into skin bags made from dairy lamb, sheep, or goat skins. It needs to mature for at least three months before consumption to get its unique characteristics [1].

The diversity of bacteria present in raw milk cheeses, such as Tulum cheese, causes biochemical changes during ripening and contributes to the flavor, aroma, and texture of the final product, which is why lactic acid bacteria (LAB) isolates from raw milk cheeses have been in demand

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

in recent years. Yoon *et al.* [2] reported that cheeses produced from raw milk are more preferred than cheeses produced from pasteurized milk due to their intense and distinctive flavors, and that LABs, which are the dominant flora in the microbial community, direct the flavor development. However, reports indicate that the geographical origin of the samples significantly influences microbial diversity [2]. Therefore, recent studies have demonstrated that traditional cheeses function as a significant reservoir for the isolation of indigenous non-starter lactic acid bacteria (NSLAB), with the isolated NSLAB strains serving as either adjunct or alternative cultures to existing starter cultures [3,4].

On the other hand, pasteurization is necessary to improve the microbial safety and shelf life of cheese by eliminating pathogenic microorganisms present in milk and reducing the number of spoilage microorganisms. So it becomes necessary to use starter cultures to reach desirable acidity as well as cheese's distinctive properties [5]. Vázquez-Velázquez *et al.* [3] compared raw milk cheese with pasteurized milk cheese enriched with selected combinations of natural LAB and reported that there was no significant sensory difference between them. Ruvalcaba-Gómez *et al.* [6] recently reported that autochthonous *Lactobacillus* and *Leuconostoc* strains are a promising starter culture combination for the production of Adobera cheese from pasteurized milk.

In this study, indigenous LAB isolated from traditional Tulum cheeses, including starter (*Streptococcus*) and non-starter (*Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Enterococcus*) bacteria, were used in different combinations with commercial starters (*Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*) to produce Tulum cheese from pasteurized milk. The different indigenous LAB combinations were expected to exert distinct effects on cheese ripening and the development of characteristic quality attributes. Therefore, this study aimed to determine the effects of these culture combinations on the physicochemical, microbiological, sensory, and volatile compound characteristics of Tulum cheese throughout ripening.

MATERIALS AND METHODS

Materials

Traditional Tulum cheese samples ($N=21$), manufactured from raw milk without the addition of commercial starter cultures and ripened in skin bags for at least three months, were collected from local producers and retail markets in the provinces of Erzurum, Erzincan, Elazığ, Malatya, Kayseri, and Kahramanmaraş, Türkiye, for the isolation and identification of indigenous LAB. All samples were transported to the Kahramanmaraş Sütçü İmam University, Food Engineering Department laboratories in a cold chain with a sterile gelatin bag and stored at +4 °C until the analysis day. MRS (Merck KGaA, Darmstadt, Germany; Cat. No. 110661) medium (with 0.5 mg/L cycloheximide) and M17 (Merck KGaA, Darmstadt, Germany; Cat. No. 115029) medium were used for LAB isolation of

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

these cheese samples. The milk and rennet agent used for the production of Tulum cheeses were provided by Akalp Dairy farming (Kahramanmaraş, Türkiye). In the manufacturing of experimental Tulum cheese samples, mesophilic homofermentative lyophilized direct vat set (DVS) commercial culture (R-704, *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*) provided by Chr-Hansen's (Denmark) was used to ensure consistent acidification and standardized fermentation conditions across all treatments.

Identification of lactic acid bacteria strains

All isolates were analyzed with respect to cell morphology, Gram staining, and catalase activity characteristics. Gram-positive, catalase-negative cells were selected and stored in 30 % glycerol at -20 °C for further analysis [7]. Single colonies grown for molecular identification were used for 16S rRNA gene sequencing by polymeric chain reaction (PCR). The primers used for PCR amplification were UEF (5' CCTACGGGNGGCWGCAG 3') and UER (5' GACTACHVGGGTATCTAATCC 3') described by Raju *et al.* [8]. PCR reactions were prepared in a 30 µL volume containing 20.8 µL dH₂O, 1 µL (10 µM) of each primer, 3 µL 10X Buffer, 1.2 µL MgCl₂ (20 mM), 1 µL (200 µM) dNTP, and 1 µL Taq DNA polymerase (5 U/mL) (Solis Biodyne, Estonia), and template DNA. PCR was performed in a thermal cycler (The Applied Biosystems® Veriti® 96-Well Fast Thermal Cycler, USA).

The PCR program was run as follows: 95 °C for 4 min; 35 cycles of 95 °C for 1 min, 58 °C for 1 min, and 72 °C for 1 min; and a final extension at 72 °C for 4 min. The products of the reaction were loaded onto a gel electrophoresis system (Wide Mini-Sub Cell GT Cell, Bio-Rad, USA) in a 1.2 % (m/V) agarose gel stained with ethidium bromide and visualized under UV illumination (UVT 20-M, Herolab, Germany). Nucleotide sequence analysis of the PCR amplification products of the V3-V4 region by the Sanger method was performed by BMLabosis (Ankara, Türkiye). The obtained nucleotide sequences were compared with sequences previously described in the GenBank using the Basic Alignment Search Tool (BLAST) software system [9].

Metabolite characterization of lactic acid bacteria using high performance liquid chromatography

The method used by Akyol *et al.* [10] was modified for determining the amounts of the metabolite products of LAB. Briefly, 1 mL of overnight culture was centrifuged at 14 000×g for five min. The supernatant was filtered (0.45 µm) before transfer to an Eppendorf tube and diluted with 1 % metaphosphoric acid at a 1:1 ratio. The separation process was performed using HPLC (High-Performance Liquid Chromatography; Shimadzu, Kyoto, Japan) with an Intersil ODS 3.5 µm 4.6×250 mm column (Shiseido Co. Ltd., Tokyo, Japan) at a 20 µL injection volume, utilizing continuous gradient separation with 0.5 % metaphosphoric acid (mobile phase) at 30 °C. To evaluate the metabolite

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

production capacities of LABs, sodium pyruvate (10 mg/mL), sodium formate (10 mg/mL), L-lactate (10 mg/mL), acetaldehyde (10 mg/mL), diacetyl (2,3-butanedione) (10 mg/mL), acetoin (10 mg/mL), and 2,3-butanediol (10 mg/mL) metabolite standards (Sigma Aldrich, Germany) were used.

The preparation of bacterial cultures and culture combinations

The cultures that will be used for production of Tulum cheeses were obtained by modifying the procedure mentioned by Guarrasi *et al.* [11]. To achieve this, bacteria were adjusted to $A_{600\text{ nm}}=1$ (Spektromax, USA) and centrifuged (Micro 22R, Hettich, Germany) at $10\,000\times g$ for 3 min at $+4\text{ }^{\circ}\text{C}$, subsequently washed twice with 0.85 % FTS, and suspended in autoclaved ($90\text{ }^{\circ}\text{C}$ for 30 min) reconstituted milk (10 % dry matter). Bacterial inoculations were carried out at a concentration of 1 % (V/V) [12].

The bacterial combinations used in the production of Tulum cheese are listed below: K1=without bacteria, K2=commercial culture (CC)+*Streptococcus thermophilus*, TP1=CC+S. *thermophilus*+*Lactopantibacillus plantarum*+*Pediococcus acidilactici*+*Enterococcus faecium*, TP2=CC+S. *thermophilus*+*Levilactibacillus brevis*+*Leuconostoc mesenteroides*+*Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, TP3=CC+S. *thermophilus*+*Latilactobacillus curvatus*+*Pediococcus pentaceus*+*Enterococcus durans*.

The production process of Tulum cheeses

Tulum cheese production was carried out by modifying the method described by Tomar *et al.* [13]. The composition of the cow's milk used in cheese production was determined using a LactoFlash milk analyzer (Funke Gerber, Germany) and was as follows: solids-not-fat (SNF) 9.1 %, milk fat 4.3 %, lactose 5.24 %, protein 3.39 %, minerals 0.47 %, density 1.0264 g/mL, freezing point $-0.444\text{ }^{\circ}\text{C}$, pH=6.52, and total aerobic mesophilic bacteria count 8.47 log CFU/mL . For all productions, raw cow's milk was processed at $65\text{ }^{\circ}\text{C}$ for 30 min, then cooled to $(37\pm 1)\text{ }^{\circ}\text{C}$, supplemented with CaCl_2 (0.02 g/L), and then divided into five batches for culture inoculation. Each batch was inoculated with the corresponding starter and adjunct culture combination described in the previous section.

Following culture inoculation, the milk was incubated at $(37\pm 1)\text{ }^{\circ}\text{C}$ until the pH decreased 6.3 (approximately 30 min). Subsequently, milk was left to coagulate with liquid calf rennet (Naturen® Mandra 175, potency 1:16000, Chr-Hansen, Denmark) within 90 min. The whey was drained, and cheese curds were cut into 1 cm^3 cubes and pressed for 18 h (20 kg mass). Afterwards, the curd was manually crumbled and dry salted with 2 % NaCl (*m/m*). Later the curd was transferred to glass jars to minimize variations associated with maturation material and local microbiota and stored at $(8\pm 1)\text{ }^{\circ}\text{C}$ and $(75\pm 5)\text{ %}$ relative humidity and analysed on the 1st, 30th, 60th, and 90th days of ripening.

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Tulum cheese analyses

Physicochemical analyses

The dry matter content of the Tulum cheese samples was determined by drying the samples at (105 ± 2) °C for 16–18 h until a constant mass was obtained. Ash content was determined by burning approximately 10 g of cheese sample at 550–600 °C until white ash was obtained, and the results were expressed as percentage ash. Both dry matter and ash contents were analysed according to the methodology described by Bradley *et al.* [14]. pH of Tulum cheeses was determined using a pH meter (HI 2211, Hanna Instruments, Woonsocket, RI, USA). For titratable acidity, 10 g of cheese sample was mixed with distilled water at 40 °C, filtered, and titrated with 0.1 N NaOH using phenolphthalein as an indicator. The results were expressed as % lactic acid according to Kurt *et al.* [15]. The total nitrogen (TN), water-soluble nitrogen (WSN), trichloroacetic acid-soluble nitrogen (12 %, TCA-SN), and phosphotungstic acid-soluble nitrogen (5 %, PTA-SN) were determined by the Kjeldahl method according to Bütikofer *et al.* [16] using a Kjeldahl digestion unit (DK20, VELP Scientifica, Usmate Velate, Italy) and a Kjeldahl distillation unit (UDK 139, VELP Scientifica, Usmate Velate, Italy). For WSN, 10 g of cheese was homogenized with 50 mL of distilled water, incubated at 40 °C for 1 h, centrifuged, and filtered. The 12 % TCA-SN fraction was obtained by mixing equal volumes of WSN extract and 24 % TCA, followed by filtration after 2 h at room temperature. The 5 % PTA-SN fraction was prepared by adding 7 mL of 3.95 M sulphuric acid and 3 mL of 33 % (*m/V*) phosphotungstic acid to the WSN extract, keeping the mixture at 4 °C overnight, followed by filtration. The nitrogen contents of the resulting fractions were expressed as a percentage of total nitrogen.

Lactocci and lactobacilli counts

A mass of 10 g of cheese sample were aseptically weighed into 90 mL sterile NaCl (0.85 %, *m/V*), and serial dilutions of the suspensions were prepared. To determine lactobacilli counts, MRS agar was used at 37 °C for 48 h and 30 °C for 72 h [17], whereas for lactococci counts, M17 agar was used at 30 °C for 48 h [18], and required inoculations were performed under both aerobic and anaerobic conditions.

Extraction and identification of volatile compounds

To determine the volatile organic compounds (VOCs) in Tulum cheese samples, the method described by Kafkas *et al.* [19] was applied using the headspace solid-phase microextraction/gas chromatography–mass spectrometry (HS-SPME/GC-MS) technique. Briefly, volatile compounds were extracted using a 5 M calcium chloride solution at 40 °C for 30 min with an 85 µm SPME fiber coated with carboxen/polydimethylsiloxane (CAR/PDMS) (Supelco, USA). Analyses were carried out

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

using an HS/GC/MS (Agilent 6890N gas chromatograph coupled with a 5975 mass selective detector; Agilent Technologies, Santa Clara, CA, USA) system equipped with an HP-5 MS fused-silica capillary column (30 m×0.25 mm×0.25 µm; Agilent Technologies, Santa Clara, CA, USA). Helium served as the carrier gas at a flow rate of 1 mL/min, and the injector temperature was maintained at 230 °C. Volatile compounds were identified by comparing their mass spectra with those in the Wiley [20], NIST [21], and FFNSC [22] mass spectral libraries. The relative percentages of the detected compounds were calculated from total ion chromatograms using a computerized integrator.

Sensory analyses

The sensory evaluation of the cheese samples was performed on days 1, 30, 60, and 90 of the ripening period. A trained panel consisting of nine panelists (five females and four males, aged 35–50 years) from Kahramanmaraş Sütçü İmam University (Faculty of Engineering and Architecture, Department of Food Engineering) was selected for the assessment. The panelists were chosen based on their interest, experience, and knowledge in sensory evaluation. All panelists were familiar with Tulum cheese and had previous experience in evaluating cheese characteristics. For this purpose, a 9-point hedonic scale was used to evaluate appearance, odor, texture, taste, and overall acceptability, with scores of 1–2 indicating unacceptable, 4–5 fairly acceptable, 6–7 good, and 8–9 very good [23]. Before the sensory evaluation, all panelists were informed about the purpose of the study, the evaluation procedure, and any potential risks and benefits associated with participation. Participation was voluntary, and panelists were informed that they could withdraw from the evaluation at any time without providing a reason.

Statistical analyses

The experimental design of the study was based on a factorial randomized plot, including five different treatments corresponding to the bacterial culture combinations used in cheese manufacture (K1, control without bacterial inoculation; K2, commercial starter culture; and TP1, TP2, and TP3, commercial starter culture supplemented with different adjunct LAB combinations and four storage periods (1, 30, 60, and 90 days). Cheese manufacture was performed in three independent production trials. All analyses were performed in triplicate. Significant differences identified through analysis of variance (ANOVA) were evaluated using the Tukey multiple comparison test. To determine which volatile organic compounds differed significantly among the samples, principal component analysis (PCA) was applied. All statistical analyses were performed using the SAS JMP 13.02 [24] software package.

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

RESULTS AND DISCUSSIONS

Identified strains

Ten isolates were identified as Gram-positive and catalase-negative, and the consensus sequences were generated from the nucleotide sequences obtained as a result of PCR amplification. The whole genome of LAB strains was compared with the sequences in the GenBank database using the BLAST program, and 98.61–100 % similarity with the reference species was determined (Table 1).

The strains were identified as *S. thermophilus*, *Lpb. plantarum*, *P. acidilactici*, *E. faecium*, *Lv. brevis*, *Leu. mesenteroides*, *Lc. lactis* subsp. *diacetylactis*, *L. curvatus*, *P. pentosaceus*, and *E. durans*.

Metabolite profile of lactic acid bacteria

The distinctive sensory characteristics of traditional cheeses are largely dependent on the metabolic activities of native LAB communities isolated from traditionally produced Tulum cheeses, which represent the authentic cheese microflora and contribute significantly to the development of both flavour and texture throughout the ripening process. Additionally, the strains used in experimental cheese production play a significant role in the formation of key aroma compounds such as L-lactate, formate, acetaldehyde, diacetyl, acetoin, and 2,3-butanediol, which are related to pyruvate metabolism. Table 2 shows the metabolic activities of the strains isolated and identified in the study from traditional Tulum cheese, the components they produce, and the amounts of these components.

Pyruvate is a metabolic intermediate in LAB and is also a precursor to substances like diacetyl, acetoin, and 2,3-butanediol, which play a role in cheese flavor [25]. In the presence of galactose, maltose, or low glucose levels, LAB also produces these products by utilizing pyruvate metabolism [26]. HPLC analysis revealed that the pyruvate production varied depending on the isolates. *Leu. mesenteroides* has the highest pyruvate level (143.22 ± 0.1 mg/L), indicating strong pyruvate accumulation. It has been reported that *Leu. mesenteroides* can increase the formation of aroma compounds by allowing more pyruvate to accumulate inside the cell [27]. On the contrary, *E. faecium* (20.70 ± 8.3 mg/L) and *Lpb. plantarum* (79.24 ± 0.1 mg/L) show lower pyruvate levels. Although *E. faecium* was determined to be the lowest pyruvate-producing strain, *Enterococcus* spp. are generally known to produce pyruvate [28], and the *E. durans* strain was the second highest producer of pyruvate, with (115.81 ± 0.4) mg/L.

Lactate is the primary byproduct of lactose metabolism [26]. As seen in Table 2, the highest L-lactate production (7443.02 ± 6.1 mg/L) was achieved by *L. curvatus*. When lactate production levels were compared, it was determined that *S. thermophilus* produced moderate levels of L-lactate (765.12 ± 5.8 mg/L), while *P. acidilactici* (4430.47 ± 3.9 mg/L) and *Lpb. plantarum* (2279.05 ± 3.1 mg/L)

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

produced lactate at varying rates. These results suggest that these species may contribute to cheese acidity and texture formation.

When formate production levels of bacterial strains were evaluated, *Lc. diacetylactis* (224.09 ± 4.1 mg/L) and *Lv. brevis* (205.62 ± 1.1 mg/L) stood out with their high levels of formate production. This is considered a feature that may reduce the complexity of the fermentation process and balance the flavor profile. On the other hand, the low formate production of *P. pentosaceus* (7.17 ± 1.8 mg/L) indicates that the effect of this metabolite on flavor is limited.

Acetaldehyde, the main compound responsible for fresh, green, and slightly fruity aromas [29], was determined to be (825.28 ± 2.8) mg/L in *Lpb. plantarum* and (779.25 ± 14.4) mg/L in *Lv. brevis*. Specifically, the acetyltransferase enzyme catalyzes the formation of diacetyl, an intermediate in citrate metabolism, by converting acetyl-CoA to diacetyl. Furthermore, the acetolactose synthase enzyme directly synthesizes acetoin from pyruvate or acetaldehyde via α -acetolactose; the simultaneous conversion of diacetyl to acetoin occurs via the reduction of NAD^+ to NADH. Diacetyl and its derivative, acetoin, are known to impart soft and butter-like aromatic properties to cheese [30]. In terms of diacetyl production, *P. pentosaceus* (137.32 ± 0.1 mg/L), *E. faecium* (119.92 ± 0.3 mg/L), and *Leu. mesenteroides* (135.31 ± 0.1 mg/L) were determined as the prominent species, while *L. curvatus* showed only (21.28 ± 0.3) mg/L production. As shown in Table 2, *Lc. diacetylactis* (2439.20 ± 4.4 mg/L), *S. thermophilus* (2147.61 ± 3.2 mg/L), and *Lv. brevis* (1864.86 ± 0.3 mg/L) stand out as strong acetoin producers. This suggests that these species can enhance the development of buttery and creamy flavor characteristics. On the other hand, acetoin production was not detected in several strains, such as *Lpb. plantarum*, *P. acidilactici*, *E. faecium*, *L. curvatus*, *P. pentosaceus*, and *E. durans*. This finding suggests that the contribution of these species to the creamy flavor formation is limited.

Some LAB can synthesize 2,3-butanediol from the processes of glycolysis or citrate metabolism via the reduction of acetoin. Among the strains tested, *Lv. brevis* (1897.21 ± 0.1 mg/L) and *P. acidilactici* (3611.84 ± 3.6 mg/L) produced high levels of 2,3-butanediol, which significantly contributed to the development of creamy flavor [29]. In contrast, *L. curvatus* (385.32 ± 5.4 mg/L) and *S. thermophilus* (96.25 ± 0.5 mg/L) showed significantly lower production levels.

The physicochemical composition of Tulum cheese samples

Dry matter content, one of the physicochemical properties of cheese, significantly affects the flavor, aroma, and texture of the final product by regulating the biochemical reactions in the cheese during ripening. In addition, lactic acid, which develops as a result of the fermentation of lactose using starter cultures responsible for acid production in cheese production, increases the dry matter value

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

by increasing the amount of whey and syneresis removed from the environment during cheese production [30]. Tulum cheese, the main material of this study, was subjected to similar biochemical reactions during certain periods of ripening, and the resulting metabolites affected the flavor, aroma, and texture profile of the product.

During the ripening process, the percentage of dry matter in all cheese samples ranged from 42.57 % (K1) to 53.78 % (TP1) (Table 3). It was obtained as an expected finding that the dry matter value of the K1 sample, which did not use starter culture in its production and had the lowest value, was lower than that of the other samples. In addition, the results presented by Gürsoy *et al.* [31], and Varol [32], as 46.5–55.0 %, and 53.22–54.82 %, respectively, are similar to the results of this study. The ash content can vary due to the use of NaCl in cheese manufacturing, the solubility of minerals in the environment, interactions with whey proteins, pH levels during coagulation, and the size or pressing of the curd [33]. The ash value of the cheeses varied between 4.13 % (K1) and 4.71 % (K2) on the last day of ripening, as shown in Table 3. Among the different culture combinations used in terms of ash values, K1 exhibited a situation distinct from the others. When evaluated independently, the initial ash value showed similar findings at 30, 60, and 90 days of storage and was statistically grouped together. The K2 culture combination, however, belonged to a different statistical group regarding initial storage values compared to other storage stages. The TP1 combination displayed statistically different values only on the 90th day of storage, while the other time points were in the same group. In evaluating the ash values of TP2 and TP3 samples, all values except the initial storage value of TP3 were statistically grouped together. It is noteworthy that some culture combinations (TP1, TP2, and TP3) exhibited similar performance regarding the changes in the ash values of the samples due to storage. This change is thought to result from the dynamics of mineral metabolism and bacterial activity in the cheese mass, which affect solubility.

In a study by Rençber and Çelik [34], the ash content of Tulum cheeses from various producers, aged on the skin for 4 months, ranged from 4.07 % to 5.32 %. The reported values align with those found in this study.

The pH level of cheese affects ripening by directly influencing the proteolytic activity of coagulants and natural milk proteinases [35]. The common point in terms of pH values of all experimental samples depending on the storage process was that the initial values of each combination were statistically significantly different from the other stages of storage, and almost all of the culture combinations were in different statistical groups in terms of pH change within themselves. The K1 sample without any starter culture or NSLAB had a pH value of 5.57 at the end of ripening, while the samples with commercial culture (K2) and combinations of commercial culture and NSLAB (TP1, TP2, TP3) had pH values ranging from 5.06. to 5.25. Thus, the different acidifying abilities of

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

NSLABs show a similar effect, with the starter culture contributing to the lowering of pH in cheeses. However, Sheehan [36] stated that NSLABs exhibit greater tolerance to salts that inhibit bacteria compared to starter lactococci used in commercial culture. It was an expected result that the K2 sample produced by using only starter culture had a higher pH value than the cheeses produced by using NSLAB with starter culture. According to Öztürk *et al.* [37], the pH values of traditional Tulum cheese samples from the Ereğli region of Konya province ranged between 5.05 and 5.17, which are in good agreement with the pH values determined in the present study. Comparable pH values were reported by Rençber *et al.* [38], who found pH values ranging from 4.98 to 5.36 in five different Muş Tulum cheeses after 90 days of ripening. Titration acidity values of Tulum cheeses may vary depending on the microorganisms present during ripening, storage conditions, packaging material, lactose, and salt content. The lowest and highest TA % values observed in the samples at the end of the storage period were determined as 0.77 % (K1) and 1.92 % (TP2). However, no statistically significant difference was observed between the titration acidity values of TP1, TP2, and TP3 cheese samples in which different NSLAB combinations were used with starter culture. A statistically significant difference was observed between the storage processes of the same applications ($p < 0.01$). Drainage of whey is greatly affected by the production of the right amount of acid, which determines the growth of bacteria, the activity of enzymes in the cheese, the ripening rate, structure, and therefore the general quality of the cheese, and also helps to control the moisture content of the whey [35,39]. Rençber *et al.* [38] determined the TA% values of the samples ranged from 0.19 to 0.23 % on the 90th day of storage that are produced with raw milk. This difference is believed to arise from several factors, such as the use of starter and non-starter lactic acid bacteria cultures in our study, as well as variations in fermentation dynamics, milk composition, and production practices.

Salt (NaCl) affects lactose metabolism, pH value, degradation of fats and casein, and the formation of flavor compounds such as peptides, free amino acids, and free fatty acids; it indirectly contributes to cheese flavor through its controlling effect on microbial and enzymatic activities [40]. When looking at related data of this study, the salt in dry matter of the cheese samples at the end of ripening ranged between 3.99 % (TP3) and 4.37 % (K1) (Table 3). It is known that the moisture content and pH value of cheese affect the rate of salt absorption and diffusion. In contrast to other experimental cheese samples, the K1 sample, which did not utilize starter cultures or NSLAB during production, exhibited an inadequate reduction in initial pH value, resulting in water retention within the curd due to limited whey drainage. Over time, the amount of salt in the cheese increased as a result of the penetration of NaCl, which is soluble in the liquid phase [41]. The salt content of Tulum cheeses at 90 days of storage was examined by Beykaya [42], who reported values between 2.93 and 3.05 %. The salt value obtained in this study was consistent with that reported in a similar study.

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Fat, another one of the components, has an important function in cheese, along with protein (casein) and moisture. Cheese is mainly composed of fat globules and water droplets trapped within the cheese casein micelles. Therefore, increases and decreases in fat content cause changes in moisture, protein, and cheese yield, and this component is also one of the most important factors affecting cheese quality. Additionally, fat contributes to the formation of flavor compounds like fatty acids and derivatives and determines the rheology and texture of cheese [43]. Cheese samples produced using starter cultures and NSLAB combinations were statistically grouped together in terms of fat content, whereas K1 cheese fell into a statistically different group ($p < 0.05$) and exhibited lower fat content. When examining K2 and TP1, TP2, and TP3 samples regarding fat change, a notable observation was that the values obtained on the 60th day for all samples, except TP2, were statistically different. It is stated that fat globules during serum separation in cheeses using starter cultures in production have a higher fat content in the pores in the paracasein matrix as a result of the stopping effect on the starter cells leaking from the curd and the retained starter cells settling around the fat globules [44].

Proteolysis has been reported as a series of biochemical reactions catalyzed by the action of coagulating enzymes, proteinases present in milk, enzymes of starter and non-starter, and secondary microorganisms [45]. It seemed that when compared to the K1 sample without starter culture and culture combination, the cheeses made with starter culture and NSLAB combinations had a substantially higher total nitrogen content (Table 3). Water-soluble nitrogen content, which indicates ripening, includes plasmin enzymes, microorganisms, and the amount of chymosin retained in the cheese curd, as well as whey proteins, soluble peptides, and free amino acids [46]. Water-soluble nitrogen (WSN) content ranged from 12.87 % (TP2) to 17.17 % (K1) after 90 days of ripening. It was observed that WSN% increased during ripening in all cheese samples and reached its highest level at 90 days (Table 3). This increase is thought to be due to the breakdown of the cultures in the cheese samples during ripening and the increase in proteolytic and peptidolytic enzyme activities in the environment [12]. The starter-free K1 sample had a low percentage of WSN at the beginning of the ripening period, but this quantity reached its highest value after 90 days. The nitrogen fractions that are soluble in 12 % trichloroacetic acid (TCA-SN) include medium and small peptides, along with free amino acids, which are produced by the hydrolysis of medium-sized peptides through the proteinase and peptidase activities of both starter and non-starter LABs [47]. In this study, at the end of the ripening period, the 12 % (TCA-SN) content of K2 cheese (8.57 %) was higher than the other cheeses. It is thought that the increase in the 12 % TCA-SN content of K2 cheese, in which only starter culture was used in production, is due to the *Lactococcus* bacteria with rich proteinase and peptidase enzyme activities in the starter culture content. Unlike TP1, TP2, and TP3, K2 contained only the starter

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

culture, without microbial competition from adjunct cultures, which may have contributed to the higher 12 % TCA-SN value. At the beginning of the ripening process, starter LAB begin to break down after their numbers reach approximately 10^9 cfu/g [35]. Thus, NSLABs develop and become the dominant microbiota. NSLABs are known to produce peptides with a similar profile to *Lactococcus* but with different levels of proteolysis. Another cheese sample, TP1, was found to have the second highest 12 % TCA-SN ratio (7.29 %); this proportion may be due to the *Lpb. plantarum* used in the bacterial combination, which is responsible for rapid ripening by metabolizing β -casein [48]. The nitrogen fraction acquired with 5 % phosphotungstic acid (PTA-SN) consists only of extremely short peptides, amino acids, and smaller dibasic amino acids (Arg, Cit, Lys), apart from components other than ammonia. The highest 5 % PTA-SN ratio was recorded in K2 cheese as 1.83 % at the end of the ripening. The Tukey multiple comparison results showed statistically insignificant differences in the 5 % PTA-SN ratios of the TP1 and TP3 cheese samples at the end of ripening (Table 3). This suggests that the NSLAB bacterial combinations used in the cheese samples have similar enzyme systems. As a matter of fact, some researchers reported that *P. acidilactici* and *P. pentosaceus* were used in cheese ripening [49].

Changes in lactocci and lactobacilli counts during ripening

The lactobacilli and lactococci cell counts were significantly affected by ripening in all Tulum cheese samples (Table 4). The initial lactobacilli counts were in K1, K2, TP1, TP2, and TP3 cheese samples, noted as 6.69 log CFU/g, 7.34 log CFU/g, 7.09 log CFU/g, 7.94 log CFU/g, and 8.06, respectively. Similarly, at the beginning of ripening, lactococci counts were recorded as 8.46 log CFU/g for K1, 6.51 log CFU/g for K2, 6.48 log CFU/g for TP1, 8.04 log CFU/g for TP2, and 7.92 log CFU/g for TP3.

In all lactobacilli and lactococci bacterial groups, increases were observed until the 30th day of ripening. After 30 days, dynamic fluctuations were recorded in all these bacterial counts in the cheese samples. This trend can be explained by the fact that autolysis of the starter bacterial cells resulted in a decrease in the number of bacteria and an increase in the number of NSLABs due to the utilization of small molecule nutrients released from the lysed cells into the cheese medium by the bacteria [50]. On the 90th day of ripening, when the cheese samples, including starter and NSLAB, were compared in terms of lactobacilli counts, it was observed that there was no statistically significant difference between TP1, TP2, and TP3, while K2 cheese was significantly different from these cheese groups with lower lactobacilli counts. These findings are in agreement with the information given above by Czárán *et al.* [50].

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Furthermore, on the 90th day of ripening, K2 cheese had the lowest amounts of lactobacilli ((7.98 ± 0.02) log CFU/g) and lactococci ((8.21 ± 0.01) log CFU/g). It is thought that the presence of different flora was suppressed due to possible bacteriocin production of the starter culture used.

It was determined that the NSLAB combinations used in TP1, TP2, and TP3 samples significantly changed the lactobacilli and lactococci counts of the cheeses, which resulted in significant ($p < 0.01$) differences between the samples. Ortigosa *et al.* [51] reported that *Pediococcus* species showed rapid growth in the first stages of fermentation, when the pH was between 6.5 and 5.0, and with this feature, they dominated the first stages of inoculation and promoted the pH decrease, and it was stated that the lactobacilli species, which became more active and grew rapidly with the pH decrease, supported the predominance of the microflora.

The high rate of *Lactobacillus* sp. increase between the 1st day of ripening (7.09 log CFU/g) and the 30th day (8.54 log CFU/g) of the TP1 sample containing *P. acidilactici* in the culture combination seems to be in accordance with the relevant literature. In the first 30-day period of the ripening process, logarithmic increases in Lactococci numbers were obtained. Wishah [52] stated that the Lactococci numbers of cheese samples decreased during the storage period, but the number of lactococci was higher in the cheeses with additional culture compared to the control, and concluded that the synergistic effect of lactobacilli and lactococci caused this. The LAB counts obtained in this study were comparable to those reported for Divle Tulum cheese, in which lactobacilli and lactococci counts of 7.63 and 7.62 log CFU/g were determined [53]. The slightly different LAB populations observed among the experimental cheeses may be attributed to the different starter and adjunct culture combinations used, as well as differences in milk source, packaging material, and ripening conditions [54].

Correlation coefficients of volatile compounds in Tulum cheese samples

The relationship between samples and variables (aroma components) is elucidated by principal component analysis, which is employed for the classification and clustering of chemical patterns. In the study, a principal component analysis (PCA) was performed to determine which were the most important volatile compounds on each ripening day of cheeses produced using different combinations of cultures (Fig. 1). In this study, the PCA biplot on day 1 of storage (Fig. 1a) accounted for more than 71.5 % of the total variability in the data set. PC1 and PC2 accounted for 46.7 and 24.8 % of the total variance, respectively. K1D1 and K2D1 were located on the negative PC1, and TP1D1, TP2D1, and TP3D1 were located on the positive PC1. PCA plots showed significant differences when different culture combinations were considered at the beginning of storage. In PC2, it was positive for sample K1D1 and negative for samples K2D1, TP1D1, TP2D1, and TP3D1. K1D1 is related to

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

variables such as 2-propanone, acetic acid, and D-limonene, differentiating it from other samples. Variables such as oleic acid and nonadecane relate to TP1D1. K2D1 is associated with variables such as hexane and 2-butanone. TP3D1 seems to be closer to variables such as nonanal, hexanoic acid, and dodecanoic acid.

At day 30 of storage (Fig. 1b), the PC1 and PC2 components account for the majority of the data set and the total variance of approximately 81.4 %, where PC1 accounts for 52.1 % and PC2 accounts for 29.3 % of the total variance. The PCA plots showed that the use of non-starter LAB strains in combination with starter culture on day 30 of the storage period resulted in significant differences in the volatile component profile compared to cheese samples that only used starter culture. TP1D2, TP2D2, and TP3D2 were located on negative PC1, while K1D2 and K2D2 were located on positive PC1. In other words, the use of NSLABs as adjunct culture in cheese samples resulted in negative PC1 values, which distinguished them from K1 and K2 samples, suggesting that a different metabolic mechanism occurs during storage. In PC2, K1D2 and TP3D2 are located in positive PC2, while TP1D2, K2D2, and TP2D2 are located in negative PC2. The PCA plot reveals a close relationship between the TP2D2 sample and compounds like oleic acid and hexane, while TP1D2 is associated with butanoic acid. TP3D2 is shifted towards acetic acid, octadecanoic acid, and nonanal.

The PCA plot for day 60 of storage (Fig. 1c) accounted for approximately 85.7 % of the total variability in the data set. PC1 and PC2 accounted for 45 % and 40.7 % of the total variance, respectively. In PC2, it is positive for K1D3 and K2D3 samples and negative for TP1D3, TP2D3, and TP3D3. The K2D3 sample exhibits a profile associated with aromatic hydrocarbons such as α -methylstyrene and cumene, as well as hexacosane, isopropyl palmitate, and butanoic acid, which are products of fat metabolism. The PCA plot reveals the dominance of saturated and unsaturated fatty acids in the TP1D3, TP2D3, and TP3D3 samples and their close relationship with compounds like oleic acid, pentadecanoic acid, octadecanoic acid, dodecanoic acid, and decanoic acid.

The PCA biplot at the end of ripening (Fig. 1d) accounted for approximately 63 % of total variability in the data set. PC1 and PC2 accounted for 36.3 and 26.7 % of the total variance, respectively. The TP1D4 cheese was associated with medium- and long-chain fatty acids, including hexanoic, octanoic, decanoic, and octadecanoic acids, whereas TP3D4 showed a closer association with oleic and dodecanoic acids. In contrast, K2D4 was characterized by ketones (2-propanone and 2-butanone) and the aromatic hydrocarbon cumene, while TP2D4 was mainly associated with hexacosane.

PCA plots indicated that particular compounds were prevalent in Tulum cheese samples produced with different combinations and during storage. It was established that NSLAB strains

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

utilized as adjunct cultures actively contributed to the fermentation process and the formation of volatile compounds. Similar to previous findings for Muş Tulum cheese, acetic acid, butanoic acid, hexanoic acid, and D-limonene were identified among the characteristic volatile compounds associated with cheese ripening [38].

Sensory properties

The sensory properties of five different Tulum cheese samples were evaluated on days 1, 30, 60, and 90 of ripening. Sensory scores changed throughout ripening as a consequence of biochemical changes associated with proteolysis, lipolysis, and the formation of aroma compounds. On day 1 (Fig. 2a), cheeses produced with starter and adjunct cultures generally received higher scores for odor, texture, flavor, and overall acceptability than K1. On day 30 (Fig. 2b), TP1 exhibited the highest overall acceptability and flavor scores, whereas K1 remained the lowest-rated sample for all sensory attributes. At day 60 (Fig. 2c), differences among treatments became less pronounced; however, TP1 and TP2 maintained comparatively higher flavor and overall acceptability scores. At the end of ripening (Fig. 2d), TP1 received the highest scores for flavor and odor, while TP1 and TP3 showed the highest overall acceptability and appearance scores. In general, cheeses produced with starter and adjunct cultures were consistently preferred over K1, indicating that the selected indigenous adjunct cultures positively influenced sensory quality during ripening.

CONCLUSIONS

The results demonstrated that the adjunct use of indigenous *Lpb. plantarum*, *P. acidilactici*, *E. faecium*, *Lv. brevis*, *Leu.mesenteroides*, *Lc. lactis* subsp. *lactis* biovar *diacetylactis*, *L. curvatus*, *P. pentosaceus*, and *E. durans*, together with the commercial starter culture (*Lc. lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris*, and *S. thermophilus*), successfully improved the quality characteristics of Tulum cheese. Culture combinations significantly influenced physicochemical, microbiological, sensory, and volatile properties during ripening ($p < 0.05$). The adjunct cultures promoted the persistence of LAB and modulated acidification and proteolysis, resulting in distinct ripening patterns among the experimental cheeses. TP2 exhibited the highest titratable acidity, whereas K2 showed the highest TCA-SN and PTA-SN values at day 90. PCA clearly differentiated cheeses according to culture combination and ripening stage, confirming that adjunct NSLAB strains actively contributed to volatile compound development. Sensory evaluation showed that culture-containing cheeses were generally preferred over the control, with TP1, TP3, and TP2 receiving the highest overall acceptance at different ripening stages. Overall, the incorporation of selected indigenous NSLAB as adjunct cultures represents a promising strategy for enhancing flavor complexity, maintaining the

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

characteristic properties of traditional Tulum cheese, and supporting its controlled and standardized industrial production.

FUNDING

This study was funded by the Scientific Research Projects Coordination Unit of Kahramanmaraş Sütçü İmam University under the project entitled “Determination of the Effects of Different Culture Combinations and Milk Types on the Ripening and Qualitative Properties of Tulum Cheese” (Project No. 2016/6-48D).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTION

K.S. Dayısoylu supervised the entire study, designed and planned the research, and contributed to data interpretation. T. Karabekmez Erdem conducted the main experiments, performed data analysis, conducted statistical analysis, prepared the tables and figures, and finalised the manuscript for submission. All authors read and approved the final version of the manuscript.

ORCID ID

T. Karabekmez Erdem <https://orcid.org/0000-0001-6361-4796>

K.S. Dayısoylu <https://orcid.org/0000-0002-0673-3526>

REFERENCES

1. Tekinşen KK, Akar D. Erzincan Tulum cheese. Atatürk Univ J Vet Sci. 2017; 2(2):218-26 (in Turkish).
2. Yoon Y, Lee S, Choi KH. Microbial benefits and risks of raw milk cheese. Food Control. 2016;63:201-15.
<https://doi.org/10.1016/j.foodcont.2015.11.013>
3. Vázquez-Velázquez R, Salvador-Figueroa M, Adriano-Anaya L, DeGyves-Córdova G, Vázquez-Ovando A. Use of starter culture of native lactic acid bacteria for producing an artisanal Mexican cheese safe and sensory acceptable. CyTA - J Food. 2018;16(1): 460-8.
<https://doi.org/10.1080/19476337.2017.1420694>

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

4. Reyes-Díaz R, González-Córdova AF, del Carmen Estrada-Montoya M, Méndez-Romero JI, Mazorra-Manzano MA, Soto-Valdez H, *et al.* Volatile and sensory evaluation of Mexican Fresco cheese as affected by specific wild *Lactococcus lactis* strains. *J Dairy Sci.* 2020;103(1):242-53.
<https://doi.org/10.3168/jds.2019-17188>
5. Leboš Pavunc A, Beganović J, Kos B, Uroić K, Blažić M, Šušković J. Characterization and application of autochthonous starter cultures for fresh cheese production. *Food Technol Biotechnol.* 2012;50(2):141-51.
6. Ruvalcaba-Gómez JM, Ruiz-Espinosa H, Méndez-Robles MD, Arteaga-Garibay RI, Anaya-Esparza LM, Villagrán Z, *et al.* Use of Autochthonous lactic acid bacteria as starter culture of pasteurized milk adobera cheese. *Fermentation.* 2022;8(5):234.
<https://doi.org/10.3390/fermentation8050234>
7. Gezginç Y, Topcal F, Comertpay S, Akyol I. Quantitative analysis of the lactic acid and acetaldehyde produced by *Streptococcus thermophilus* and *Lactobacillus bulgaricus* strains isolated from traditional Turkish yogurts using HPLC. *J Dairy Sci.* 2025;98(3):1426-34.
<https://doi.org/10.3168/jds.2014-8447>
8. Raju SC, Lagström S, Ellonen P, De Vos WM, Eriksson JG, Weiderpass E, *et al.* Reproducibility and repeatability of six high-throughput 16S rDNA sequencing protocols for microbiota profiling. *J Microbiol Methods.* 2018;147:76-86.
<https://doi.org/10.1016/j.mimet.2018.03.003>
9. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol.* 1990;215(3):403–10.
[https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
10. Akyol İ, Serdaroğlu K, Gezginç Y, Dayısoylu KS, Ekinci MS, Özkose E. Redirection of pyruvate pathway of lactic acid bacteria to improve cheese quality. *Food Biotechnol.* 2009; 23(3):200-13.
<https://doi.org/10.1080/08905430903102562>
11. Guarrasi V, Sannino C, Moschetti M, Bonanno A, Di Grigoli A, Settanni L. The individual contribution of starter and non-starter lactic acid bacteria to the volatile organic compound composition of Caciocavallo Palermitano cheese. *Int J Food Microbiol.* 2017;259:35-42.
<https://doi.org/10.1016/j.ijfoodmicro.2017.07.022>
12. Hayaloğlu AA. Influence of the strains of lactococcus used as a starter on the characteristics and ripening properties of Turkish white cheese [Phd Thesis]. Adana, Turkey: University of Çukurova; 2003 (in Turkish).

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

13. Tomar O, Akarca G, Beykaya M, Çağlar A. Some characteristics of Erzincan Tulum cheese produced using different probiotic cultures and packaging material. *Kafkas Univ Vet Fak Derg.* 2018;24(5):647-54.

<https://doi.org/10.9775/kvfd.2018.19596>

14. Bradley RL, Arnold E, Barbano DM, Semerad RG, Smith DE, Vines BK. Chemical and physical methods. In: Marshall RT (editor). *Standard Methods For The Examination of Dairy Products*, Am. Publ. Health Assoc, Washington DC;1992. pp. 433-529.

15. Kurt, A, Çakmakçı, S, Çağlar, A. Guide to milk and dairy products examination and analysis methods. *Erzurum: Atatürk Univ. Faculty of Agriculture Press*; 2012. pp. 254 (in Turkish).

16. Bütikofer U, Rüegg M, Ardö Y. Determination of nitrogen fractions in cheese: Evaluation of a collaborative study. *LWT - Food Sci. Technol.*1993;26(3): 271-5.

<https://doi.org/10.1006/fstl.1993.1056>

17. Ceylan ZG, Çağlar A, Çakmakçı S. Some physicochemical, microbiological, and sensory properties of Tulum cheese produced from ewe's milk via a modified method. *Int J Dairy Technol.* 2007;60(3):191-7.

<https://doi.org/10.1111/j.1471-0307.2007.00326.x>

18. Albenzio M, Corbo MR, Rehman SU, Fox PF, De Angelis M, Corsetti A, *et al.* Microbiological and biochemical characteristics of Canestrato Pugliese cheese made from raw milk, pasteurized milk or by heating the curd in hot whey. *Int J Food Microbiol.* 2001;67(1-2):35-48.

[https://doi.org/10.1016/S0168-1605\(00\)00533-X](https://doi.org/10.1016/S0168-1605(00)00533-X)

19. Kafkas E, Kafkas S, Koch-Dean M, Schwab W, Larkov O, Lavid N, *et al.* Comparison of methodologies for the identification of aroma compounds in strawberry. *Turk J Agric For.* 2005;29(5):383-90.

20. Wiley Registry of Mass Spectral Data, 7th ed. Hoboken (NJ): John Wiley & Sons, Inc.; 2010.

Available from: <https://sciencesolutions.wiley.com/solutions/technique/gc-ms/>

21. National Institute of Standards and Technology (NIST). NIST/EPA/NIH Mass Spectral Library (NIST 14). Gaithersburg (MD): NIST; 2014. Available from:

<https://chemdata.nist.gov/dokuwiki/doku.php>

22. FFNSC Database: Flavor and Fragrance Natural and Synthetic Compounds, 2020 edition. Shimadzu Corporation; Kyoto, Japan. Available from:

<https://sciencesolutions.wiley.com/solutions/technique/gc-ms/mass-spectra-of-flavors-and-fragrances-of-natural-and-synthetic-compounds-3rd-edition/>

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

23. Frélin M, Martin B, Buchin S, Desserre B, Lavigne R, Tixier E, *et al.* Milk fat composition modifies the texture and appearance of Cantal-type cheeses but not their flavor. *J Dairy Sci.* 2019;102(2):1131-43.

<https://doi.org/10.3168/jds.2018-15534>

24. SAS Institute Inc. JMP® Statistics and Graphics Software, v. 13.0.2. Cary (NC): SAS Institute Inc.; 2015. Available from: <https://www.jmp.com/en/home>

25. Ferrer Valenzuela J, Pinuer LA, Garcia Cancino A, Borquez Yanez R. Metabolic fluxes in lactic acid bacteria - a review. *Food Biotechnol.* 2015;29(2):185-217.

<https://doi.org/10.1080/08905436.2015.1027913>

26. Bintsis T. Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. *AIMS Microbiol.* 2018;4(4):665.

<https://doi.org/10.3934/microbiol.2018.4.665>

27. Ricciardi A, Storti LV, Giavalisco M, Parente E, Zotta T. The effect of respiration, pH, and citrate co-metabolism on the growth, metabolite production and enzymatic activities of *Leuconostoc mesenteroides* subsp. *cremoris* E30. *Foods.* 2022;11(4):535.

<https://doi.org/10.3390/foods11040535>

28. Silva LF, Sunakozawa TN, Monteiro DA, Casella T, Conti AC, Todorov SD, *et al.* Potential of cheese-associated lactic acid bacteria to metabolize citrate and produce organic acids and acetoin. *Metabolites.* 2023;13(11):1134.

<https://doi.org/10.3390/metabo13111134>

29. Krastanov, A, Yeboah, PJ, Wijemanna, ND, Eddin, AS., Ayivi, RD. Ibrahim, SA. Volatile aromatic flavor compounds in yogurt: A review. In: Salam AA., Bronze, MR, editors. *Current issues and advances in the dairy industry.* London: IntechOpen;2023.

<https://doi.org/10.5772/intechopen.109034>

30. Fox PF, McSweeney PLH. Chapter 1-Cheese: An Overview. In: McSweeney PLH, Fox P, Cotter PD, Everett DW, editors. *Cheese Chemistry, Physics and Microbiology* Springer US: New York, NY, USA; 2017. pp. 5–21.

31. Gürsoy O, Küçükçetin A, Gökçe Ö, Ergin F, Kocatürk K. Physicochemistry, microbiology, fatty acids composition and volatile profile of traditional Söğle Tulum (goat's skin bag) cheese. *An Acad Bras Ciênc.* 2018;90(4):3661-74.

<https://doi.org/10.1590/0001-3765201820180310>

32. Varol S. Use of casein hydrolysate in tulum cheese production [MSc Thesis]. Niğde, Turkey: University of Niğde Ömer Halisdemir; 2019 (in Turkish).

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

33. Manuelian CL, Currò S, Penasa M, Cassandro M, De Marchi M. Characterization of major and trace minerals, fatty acid composition, and cholesterol content of Protected Designation of Origin cheeses. *J Dairy Sci.* 2017;100(5):3384-95.

<https://doi.org/10.3168/jds.2016-12059>

34. Rençber F, Çelik S. Some characteristic properties of Muş Tulum cheese ripened in different packing materials. *AUZFD.* 2021;52(1):1-10.

<https://doi.org/10.17097/ataunizfd.712037>

35. Fox PF. Cheese-Overview. In: Fuguay JW, Fox PF, McSweeney PLH, editors. *Encyclopedia of Dairy Sciences*. UK: Elsevier Academic Press; 2011. pp. 534-43.

36. Sheehan JJ. How does NaCl affect the microbiology of cheese? In: McSweeney PLH, editor. *Cheese problems solved*. USA: CRC Press; 2007. pp. 96.

37. Öztürk Hİ, Orac A, Akın N. Characterization of bioactive peptides derived from goatskin Tulum cheese of the Ereğli region at different stages of ripening. *Food Res Int.* 2022;162,112124.

<https://doi.org/10.1016/j.foodres.2022.112124>

38. Rençber F, Önlü H, Akgül FY, Atasoy AF. Ripening dynamics in Muş Tulum cheese: physicochemical changes, free fatty acid composition and aroma profile evolution. *Int Dairy J.* 2025;106408.

<https://doi.org/10.1016/j.idairyj.2025.106408>

39. Erceyes Ö, Yıldırım M, Yıldırım Z. Total carbonyl compound and some chemical and microbiological properties of tulum cheese. *JASP.* 2018;1(1):67-83 (in Turkish).

40. Fox PF, Guinee TP, Cogan TM, McSweeney PLH. Salting of Cheese Curd. In: Fox PF, Guinee TP, Cogan TM, McSweeney PLH editors. *Fundamentals of Cheese Science*. New York, USA: Springer; 2017. pp. 251-75.

41. Guinee TP. Salting and the role of salt in cheese. *Int J Dairy Technol.* 2004;57(2-3):99-109.

<https://doi.org/10.1111/j.1471-0307.2004.00145.x>

42. Beykaya M. quality properties of Erzincan Tulum cheese produced with different packaging materials and probiotic culture during storage period [PhD Thesis]. Afyonkarahisar, Turkey: University of Afyonkarahisar; 2018 (in Turkish).

43. Hill AR, Kethireddipalli P. Chapter 8, Dairy products: Cheese and yoghurt In: Eskin NAM, Shahidi F, editors. *Biochemistry of foods*. UK: Elsevier Academic Press; 2013. pp. 319-62.

44. Yanachkina P, McCarthy C, Guinee T, Wilkinson M. Effect of varying the salt and fat content in Cheddar cheese on aspects of the performance of a commercial starter culture preparation during ripening. *Int J Food Microbiol.* 2016;224:7-15.

<https://doi.org/10.1016/j.jfoodmicro.2016.02.006>

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

45. Hayaloğlu AA, McSweeney PLH. Chapter 7, Primary biochemical events during cheese ripening. In: Özer BH, Akdemir-Evrendilek G, editors. Dairy microbiology and biochemistry-recent developments. USA: CRC Press; 2015. pp.134-66.

46. Zamora A, Juan B, Trujillo AJ. Compositional and biochemical changes during cold storage of starter-free fresh cheeses made from ultra-high-pressure homogenised milk. Food Chem. 2025;176:433-40.

<https://doi.org/10.1016/j.foodchem.2014.12.070>

47. Ardö Y. Evaluating proteolysis by analysing the N content of cheese fractions. International Dairy Federation. 1999;337:4-9.

48. Mangia NP, Murgia MA, Garau G, Sanna MG, Deiana P. Influence of selected LAB cultures on the evolution of free amino acids, free fatty acids and Fiore Sardo cheese microflora during the ripening. Food Microbiol. 2008;25(2):366-77.

<https://doi.org/10.1016/j.fm.2007.09.009>

49. Raccach M. *Pediococcus*. In: Batt CA, Tortorello ML, editors. Encyclopedia of Food Microbiology. UK: Elsevier Academic Press; 2014. pp. 1630.

50. Czárán T, Rattray FP, Cleide ODA, Christensen BB. Modelling the influence of metabolite diffusion on non-starter lactic acid bacteria growth in ripening Cheddar cheese. Int Dairy J. 2018;80:35-45.

<https://doi.org/10.1016/j.idairyj.2017.12.010>

51. Ortigosa M, Torre P, Izco JM. Effect of pasteurization of ewe's milk and use of a native starter culture on the volatile components and sensory characteristics of Roncal cheese. J Dairy Sci. 2001;84(6):1320-30.

[https://doi.org/10.3168/jds.S0022-0302\(01\)70161-0](https://doi.org/10.3168/jds.S0022-0302(01)70161-0)

52. Wishah R. Utilization of some adjunct bacteria strains in cheese production in addition to starter culture and their effects on the cheese properties [MSc Thesis]. Turkey, Ankara: University of Hacettepe; 2007 (in Turkish).

53. Üçök G, Jararaa R. Karaman Divle Cave Tulum Cheese: An Overview of Microbiological, Physicochemical, and Textural Properties. NEJSE. 2025;7(3):440-57.

<https://doi.org/10.47112/neufmbd.2026.105>

54. Montel MC, Buchin S, Mallet A, Delbes-Paus C, Vuitton DA, Desmasures N, Berthier F. Traditional cheeses: Rich and diverse microbiota with associated benefits. Int J Food Microbiol. 2014;177:136–54.

<https://doi.org/10.1016/j.ijfoodmicro.2014.02.019>

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

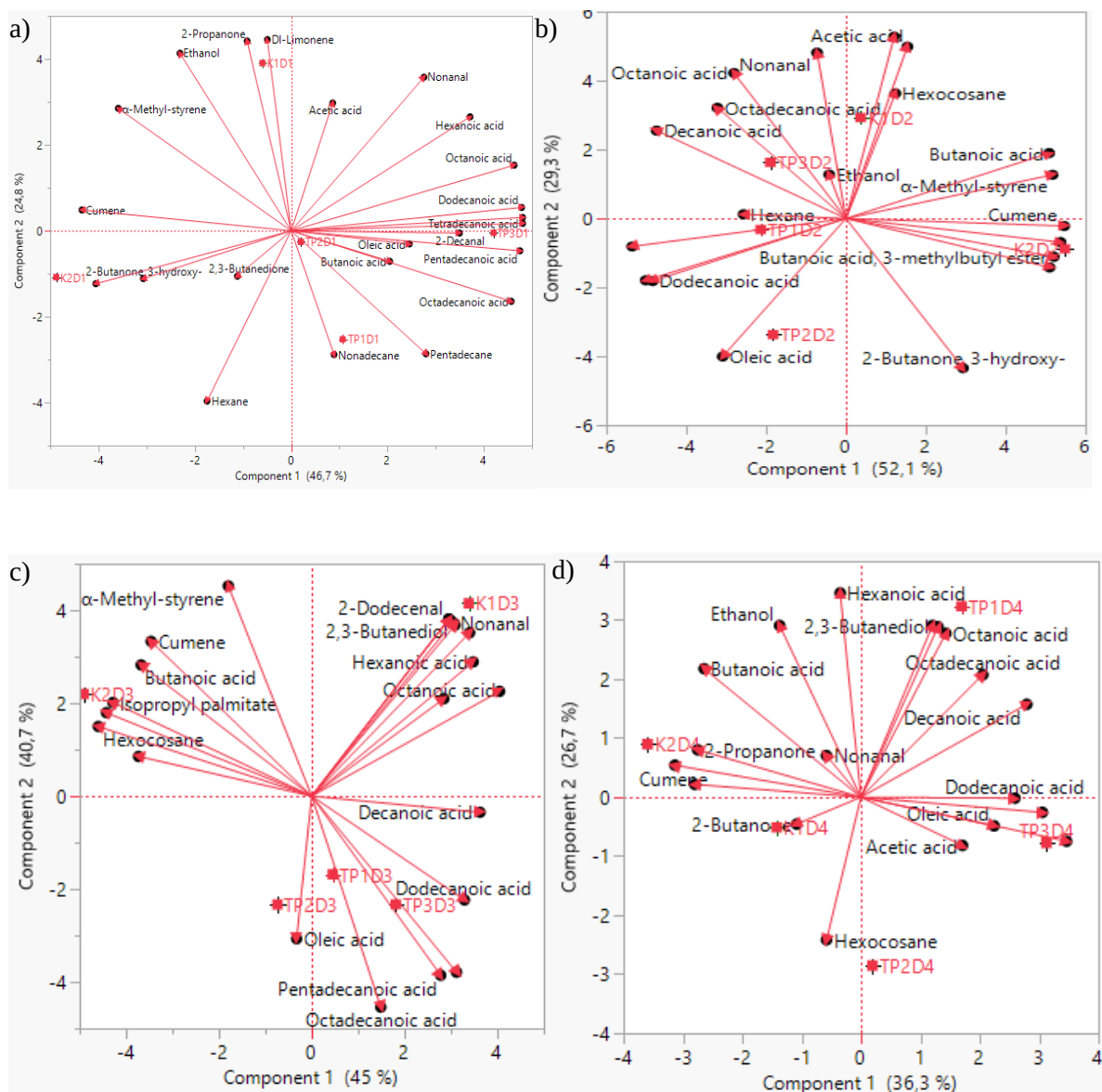


Fig. 1. Principal component analysis (PCA) biplots showing the distribution of Tulum cheese samples in the rotated component space at different storage times: a) day 1, b) day 30, c) day 60, and d) day 90

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

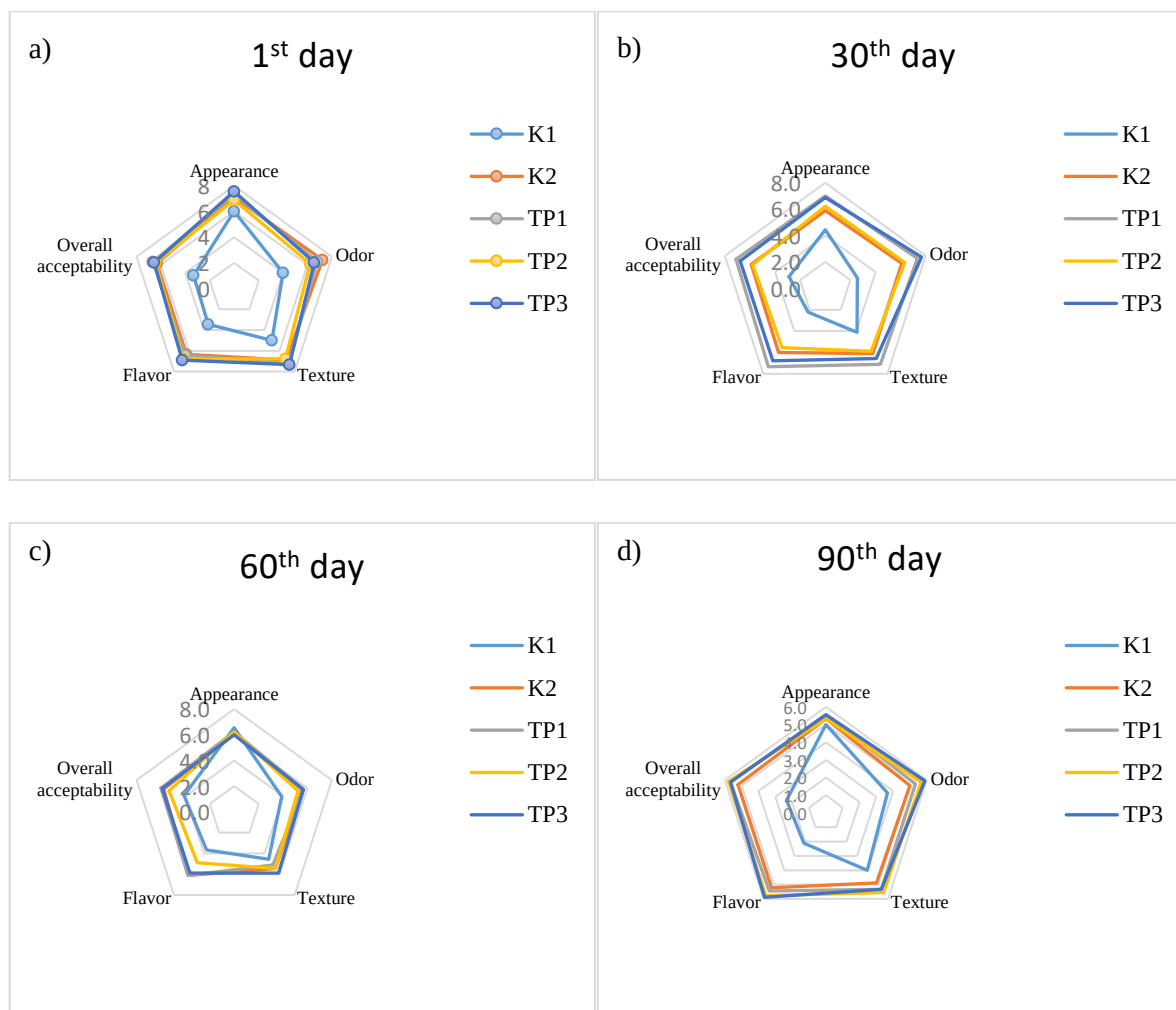


Fig. 2. Sensory evaluation scores of Tulum cheese samples (K1, K2, TP1, TP2, and TP3) at different ripening stages: a) day 1, b) day 30, c) day 60, and d) day 90

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Table 1. Results of comparison of consensus sequences of LAB isolates from Tulum cheeses with reference species in the database

Isolate no.	Bacteria	Similarity/%	GenBank no.
1	<i>S. thermophilus</i>	99.76	KX926522.1
2	<i>Lpb. plantarum</i>	99.53	KC538945.1
3	<i>P. acidilactici</i>	98.84	KU376395.1
4	<i>E. faecium</i>	99.30	MK333716.1
5	<i>Lv. brevis</i>	99.52	MG722901.1
6	<i>L. mesenteroides</i>	98.61	MT597708.1
7	<i>Lc lactis</i> subsp. <i>diacetylactis</i>	99.30	AY9204469.1
8	<i>L. curvatus</i>	99.06	MT597551.1
9	<i>P. pentosaceus</i>	99.53	JN851781.1
10	<i>E. durans</i>	99.53	LT900388.1

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Table 2. Quantities of metabolites produced by LAB isolates (mg/L)

Bacteria	Pyruvate	L-lactate	Formate	Acetaldehyde	Diacetyl	Acetoin	2,3-butanediol
<i>S. thermophilus</i>	(84.4±0.1) ^C	(765.12±5.8) ^G	(4.40±1.1) ^H	(631.8±3.3) ^D	(173.23±1.1) ^A	(2147.61±3.2) ^B	(96.25±0.5) ^F
<i>Lpb. plantarum</i>	(79.24±0.1) ^D	(2279.05±3.1) ^D	(113.11±0.8) ^D	(825.28±2.8) ^A	(28.71±0.3) ^{DE}	n.d.	(903.22±6.8) ^C
<i>P. acidilactici</i>	(31.94±5.5) ^H	(4430.47±3.9) ^B	(173.51±0.2) ^C	(422.30±9.8) ^E	(109.77±0.2) ^C	n.d.	(3611.84±3.6) ^A
<i>E. faecium</i>	(20.70±8.3) ^I	(254.37±13.9) ^H	(45.80±0.7) ^F	(352.01±0.5) ^F	(119.92±0.3) ^{BC}	n.d.	(547.67±2.3) ^D
<i>Lv. brevis</i>	(30.49±0.1) ^H	(4292.36±1.8) ^B	(205.62±1.1) ^B	(779.25±1.4) ^B	(48.2±0.1) ^D	(1864.86±0.3) ^C	(1897.21±0.1) ^B
<i>Leu. mesenteroides</i>	(143.22±0.1) ^A	(3580.12±1.5) ^C	(24.02±0.5) ^G	(130.31±1.1) ^H	(135.31±0.1) ^B	(131.93±0.1) ^D	(838.44±0.6) ^C
<i>Lc. diacetylactis</i>	(44.50±2.3) ^G	(4218.7±4.3) ^B	(224.09±4.1) ^A	(32.675±3.3) ^I	(129.23±0.1) ^{BC}	(2439.20±4.4) ^A	(141.03±3.7) ^F
<i>L. curvatus</i>	(52.64±0.3) ^F	(7443.02±6.1) ^A	(108.48±1.3) ^D	(679.93±2.02) ^C	(21.28±0.3) ^E	n.d.	(385.32±5.4) ^E
<i>P. pentaceus</i>	(64.54±4.3) ^E	(1502.345±10.3) ^F	(7.17±1.8) ^{GH}	(195.98±1.21) ^G	(137.32±0.1) ^B	n.d.	(158.40±0.1) ^F
<i>E. durans</i>	(115.81±0.4) ^B	(1879.495±9.8) ^E	(67.81±5.3) ^E	(4.075±0.3) ^I	(49.65±1.3) ^D	n.d.	(391.09±4.7) ^E

Values are expressed as mean±standard deviation (S.D.) (N=3). Different uppercase letters within the same column indicate statistically significant differences among bacterial strains (p<0.05). n.d.=not detected

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Table 3. Physicochemical characteristics of Tulum cheese samples during ripening

Cheese sample	t(storage)/day	w(dry matter)/%	w(ash)/%	pH	TA/%	w(salt in dry matter)/%	w(fat)/%	w(TN)/%	w(WSN)/%	w(TCA-SN)/%	w(PTA-SN)/%
K1	1	(42.57±0.06) ^{Bb}	(3.90±0.06) ^{Ca}	(6.49±0.01) ^{Aa}	(0.47±0.06) ^{Bc}	(5.04±0.01) ^{Aa}	(18.33±1.57) ^{Bc}	(3.50±0.02) ^{Ca}	(2.28±0.02) ^{Cb}	(0.89±0.20) ^{Cc}	(0.13±0.03) ^{Cb}
	30	(48.27±0.01) ^{Ba}	(4.15±0.01) ^{Ba}	(5.63±0.13) ^{Bb}	(0.45±0.05) ^{Dc}	(4.49±0.00) ^{ABa}	(19.66±0.57) ^{Bc}	(3.92±0.54) ^{Aa}	(3.33±0.47) ^{Db}	(2.01±0.07) ^{Bb}	(0.21±0.02) ^{Cab}
	60	(48.58±0.64) ^{Ba}	(4.12±0.07) ^{Ba}	(5.60±0.02) ^{Ab}	(0.63±0.03) ^{Cb}	(4.18±0.01) ^{Bb}	(22.66±0.57) ^{Bb}	(3.63±0.05) ^{Ca}	(13.49±0.22) ^{Aa}	(2.05±0.07) ^{Cb}	(0.32±0.04) ^{Cab}
	90	(48.67±0.63) ^{Ba}	(4.13±0.28) ^{Ba}	(5.57±0.00) ^{Ab}	(0.77±0.05) ^{Ca}	(4.37±0.13) ^{Ba}	(25.00±0.00) ^{Aa}	(3.57±0.02) ^{Da}	(17.17±0.35) ^{Aa}	(5.57±0.5) ^{Ba}	(0.44±0.03) ^{Ba}
K2	1	(53.43±0.33) ^{Aa}	(4.63±0.06) ^{ABa}	(5.30±0.00) ^{Dc}	(1.08±0.00) ^{Ac}	(3.83±0.01) ^{Bb}	(23.00±1.73) ^{Ab}	(4.06±0.01) ^{Aa}	(3.03±0.18) ^{BCd}	(1.79±0.03) ^{Ad}	(0.50±0.02) ^{Ad}
	30	(53.49±1.40) ^{Aa}	(4.68±0.07) ^{Aa}	(5.67±0.00) ^{ABa}	(0.86±0.02) ^{Bd}	(4.05±0.19) ^{ABa}	(22.66±0.57) ^{Ab}	(4.01±0.13) ^{Aa}	(5.75±0.68) ^{BCc}	(4.10±0.04) ^{Ac}	(0.85±0.03) ^{Ac}
	60	(53.52±0.03) ^{Aa}	(4.69±0.03) ^{Aa}	(5.34±0.00) ^{Bb}	(1.29±0.00) ^{Bb}	(4.09±0.08) ^{Aa}	(23.66±1.52) ^{ABab}	(4.08±0.20) ^{Aa}	(11.01±0.12) ^{Bb}	(5.66±0.07) ^{Ab}	(1.17±0.05) ^{Ab}
	90	(53.74±0.08) ^{Aa}	(4.71±0.15) ^{Aa}	(5.25±0.00) ^{Bd}	(1.66±0.049) ^{Ba}	(4.10±0.09) ^{ABa}	(26.66±0.57) ^{Aa}	(4.02±0.08) ^{ABa}	(13.74±0.16) ^{Aa}	(8.57±0.29) ^{Aa}	(1.83±0.03) ^{Aa}
TP1	1	(53.72±0.10) ^{Aa}	(4.71±0.15) ^{Aa}	(5.37±0.00) ^{Ba}	(1.10±0.08) ^{Ab}	(3.92±0.01) ^{ABb}	(24.00±0.00) ^{Aa}	(3.75±0.01) ^{Ba}	(2.48±0.15) ^{Cd}	(1.30±0.1) ^{Bd}	(0.14±0.04) ^{Cc}
	30	(53.30±0.08) ^{Aa}	(4.69±0.01) ^{Aa}	(5.40±0.01) ^{Ca}	(0.89±0.02) ^{Bc}	(4.22±0.13) ^{Aa}	(24.33±2.08) ^{Aa}	(3.79±0.01) ^{Aa}	(5.35±0.08) ^{Cc}	(4.14±0.06) ^{Ac}	(0.34±0.03) ^{BCa}
	60	(53.67±0.38) ^{Aa}	(4.70±0.03) ^{Aa}	(5.20±0.00) ^{Db}	(1.75±0.07) ^{ABa}	(4.15±0.02) ^{Aa}	(24.66±0.57) ^{ABa}	(3.80±0.26) ^{BCa}	(11.05±1.06) ^{Bb}	(5.63±0.02) ^{Ab}	(0.45±0.01) ^{BCb}
	90	(53.78±0.20) ^{Aa}	(4.69±0.09) ^{ABa}	(5.07±0.05) ^{Cc}	(1.80±0.09) ^{Aa}	(4.22±0.07) ^{Aa}	(26.66±0.57) ^{Aa}	(3.82±0.03) ^{BCa}	(15.07±0.09) ^{Aa}	(7.29±0.52) ^{Aa}	(0.74±0.03) ^{Aa}
TP2	1	(53.11±0.10) ^{Aa}	(4.51±0.07) ^{ABa}	(5.33±0.00) ^{Cb}	(1.14±0.15) ^{Ac}	(3.91±0.01) ^{ABb}	(23.33±0.57) ^{Ab}	(3.77±0.11) ^{Ba}	(3.71±0.13) ^{Bc}	(1.78±0.29) ^{Ab}	(0.11±0.03) ^{Cb}
	30	(52.90±0.01) ^{Aa}	(4.36±0.07) ^{ABa}	(5.82±0.05) ^{Aa}	(0.57±0.01) ^{Cd}	(4.08±0.00) ^{Ba}	(23.66±0.57) ^{Ab}	(3.87±0.13) ^{Aa}	(7.52±0.68) ^{ABb}	(4.92±0.20) ^{Aa}	(0.43±0.08) ^{Ba}
	60	(52.88±0.41) ^{Aa}	(4.36±0.14) ^{ABa}	(5.37±0.01) ^{Bb}	(1.38±0.09) ^{Bb}	(4.14±0.13) ^{Aa}	(25.66±0.57) ^{Aa}	(3.89±0.03) ^{ABa}	(9.15±0.23) ^{Cb}	(4.67±0.14) ^{Ba}	(0.42±0.05) ^{BCa}
	90	(52.93±0.22) ^{Aa}	(4.39±0.03) ^{ABa}	(5.08±0.02) ^{Cc}	(1.92±0.01) ^{Aa}	(4.13±0.21) ^{ABa}	(26.66±0.57) ^{Aa}	(4.04±0.14) ^{Aa}	(12.87±0.41) ^{Aa}	(5.57±0.25) ^{Ba}	(0.47±0.03) ^{Ba}
TP3	1	(53.20±0.82) ^{Aa}	(4.51±0.1) ^{Ba}	(5.37±0.01) ^{Ba}	(1.08±0.06) ^{Ad}	(3.94±0.00) ^{ABa}	(23.66±2.30) ^{Aa}	(3.42±0.09) ^{Cc}	(5.84±0.07) ^{Ad}	(1.84±0.10) ^{Ad}	(0.26±0.03) ^{Bc}
	30	(53.21±0.00) ^{Aa}	(4.50±0.07) ^{ABa}	(5.32±0.00) ^{Cb}	(1.28±0.02) ^{Ac}	(3.96±0.17) ^{Bb}	(24.00±0.00) ^{Aa}	(3.56±0.02) ^{Ab}	(8.24±0.14) ^{Ac}	(4.17±0.03) ^{Ac}	(0.42±0.05) ^{Bb}
	60	(53.23±0.26) ^{Aa}	(4.51±0.19) ^{ABa}	(5.30±0.00) ^{Cc}	(1.63±0.03) ^{Ab}	(4.15±0.20) ^{Ab}	(25.00±1.00) ^{ABa}	(3.82±0.02) ^{Ba}	(12.03±0.07) ^{Bb}	(6.07±0.06) ^{Aa}	(0.55±0.02) ^{Bab}
	90	(53.34±0.05) ^{Aa}	(4.53±0.06) ^{ABa}	(5.06±0.01) ^{Cd}	(1.80±0.00) ^{ABa}	(3.99±0.13) ^{Bb}	(26.00±1.00) ^{Aa}	(3.81±0.06) ^{Ca}	(16.43±0.18) ^{Aa}	(5.43±0.17) ^{Bb}	(0.67±0.02) ^{Ba}

Values are means±S.D. (N=5). Different uppercase superscript letters indicate significant differences between samples on the same storage day (p<0.05), different lowercase superscript letters indicate significant differences for the same sample within different days of storage (p<0.05). Treatments were as follows: K1=cheese produced from pasteurized milk without the addition of any starter or adjunct culture, K2=cheese produced with the starter culture (*Lactococcus lactis* subsp. *lactis*+*Lc. lactis* subsp. *cremoris*+*Streptococcus thermophilus*), TP1=K2 supplemented with *Lactiplantibacillus plantarum*, *Pediococcus acidilactici* and *Enterococcus faecium*,

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

TP2=K2 supplemented with *Levilactobacillus brevis*, *Leuconostoc mesenteroides* and *Lc. lactis* subsp. *lactis* biovar *diacetylactis*, TP3=K2 supplemented with *Latilactobacillus curvatus*, *Pediococcus pentosaceus* and *Enterococcus durans*. TA=titratable acidity, TN=total nitrogen, WSN=water-soluble nitrogen, TCA-SN=trichloroacetic acid-soluble nitrogen, PTA-SN=phosphotungstic acid-soluble nitrogen

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Table 4. Microbial changes of Tulum cheese samples during ripening

Variable	t(storage)/day	K1	K2	TP1	TP2	TP3
Lactobacilli	1	(6.69±0.02) ^{Dd}	(7.34±0.01) ^{Bd}	(7.09±0.03) ^{Cb}	(7.94±0.00) ^{Ad}	(8.06±0.00) ^{Ac}
	30	(7.26±0.02) ^{Dc}	(8.34±0.01) ^{Ca}	(8.59±0.01) ^{Ba}	(8.97±0.01) ^{Aa}	(8.76±0.02) ^{Ba}
	60	(8.17±0.02) ^{Bb}	(8.15±0.01) ^{Bb}	(8.47±0.00) ^{Aa}	(8.67±0.00) ^{Ab}	(8.08±0.02) ^{Bc}
	90	(8.58±0.03) ^{Aa}	(7.98±0.02) ^{Bc}	(8.42±0.00) ^{Aa}	(8.45±0.02) ^{Ac}	(8.32±0.02) ^{Ab}
Lactococci	1	(8.46±0.02) ^{Ab}	(6.51±0.01) ^{Cc}	(6.48±0.00) ^{Cd}	(8.04±0.00) ^{Bc}	(7.92±0.02) ^{Bc}
	30	(8.73±0.02) ^{Ca}	(9.05±0.01) ^{Ba}	(9.14±0.01) ^{Ba}	(9.45±0.02) ^{Aa}	(9.08±0.01) ^{Ba}
	60	(8.75±0.02) ^{Ba}	(8.36±0.01) ^{Cb}	(8.77±0.02) ^{Bb}	(9.39±0.03) ^{Aa}	(8.30±0.02) ^{Cb}
	90	(8.86±0.02) ^{Aa}	(8.21±0.01) ^{Cb}	(8.23±0.02) ^{Cc}	(8.35±0.01) ^{Bb}	(8.44±0.02) ^{Bb}

Values are means±S.D. (N=5). Different uppercase superscript letters indicate significant differences between samples on the same storage day ($p<0.05$), different lowercase superscript letters indicate significant differences for the same sample within different days of storage ($p<0.05$). Treatments were as follows: K1=cheese produced from pasteurized milk without the addition of any starter or adjunct culture, K2=cheese produced with the starter culture (*Lactococcus lactis* subsp. *lactis*+*Lc. lactis* subsp. *cremoris*+*Streptococcus thermophilus*), TP1=K2 supplemented with *Lactiplantibacillus plantarum*, *Pediococcus acidilactici*, and *Enterococcus faecium*; TP2, K2 supplemented with *Levilactobacillus brevis*, *Leuconostoc mesenteroides* and *Lc. lactis* subsp. *lactis* biovar *diacetylactis*, TP3=K2 supplemented with *Latilactobacillus curvatus*, *Pediococcus pentosaceus* and *Enterococcus durans*