

# Karaman Divle Obruğu Tulum Peyniri: Mikrobiyolojik, Fizikokimyasal ve Tekstürel Özelliklerinin Genel Bir Değerlendirmesi

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## ÖZET

Karaman Divle Obruğu Tulum peyniri, Karaman bölgesine özgü geleneksel bir Türk peyniridir. Bu peynir, Divle Mağarası'ndaki doğal mikrobiyal flora ile gerçekleşen fermentasyon ve olgunlaşma süreçleri sonucunda kendine has özellikler kazanmaktadır. Bu çalışmada, Karaman'daki iki farklı üreticiden temin edilen Divle Tulum peyniri örneklerinin fizikokimyasal, mikrobiyolojik, renk, erime ve tekstürel özellikleri incelenmiştir. Elde edilen sonuçlar, her iki örnekte de laktik asit bakterilerinin (LAB) ve toplam mezofilik aerobik bakterilerin yüksek sayıda ( $>7$  log kob/g) bulunduğunu göstermiştir. Bununla birlikte, maya ve koliform sayılarında da (sırasıyla ortalama 4,50 ve 4,23 log kob/g) yüksek mikroorganizma sayıları gözlemlenmiş olup, bu durum üretim sırasında hijyenik uygulamalara olan ihtiyacı ortaya koymaktadır. Fizikokimyasal analizler sonucunda, örneklerin ortalama pH, asitlik, kuru madde, yağ, protein ve tuz içerikleri sırasıyla  $5,83 \pm 0,00$ ;  $1,12 \pm 0,08$  L.A.;  $61,22 \pm 2,48$ ;  $21,10 \pm 2,33$ ;  $29,52 \pm 2,20$  ve  $4,69 \pm 0,28$  olarak belirlenmiştir. Peynirlerin  $L^*$ ,  $a^*$  ve  $b^*$  renk değerlerinde belirgin farklılıklar olduğu tespit edilmiştir ( $p < 0,05$ ).  $130^\circ\text{C}$ 'de yapılan erime analizlerinde, örneklerin  $58,75 \pm 8,35$  ve  $112,24 \pm 8,26$  oranında eriyebilirlik gösterdiği belirlenmiştir. Tekstür profil analizi, peynirlerin sertlik, sakızimsılık ve çiğnenebilirlik özelliklerinde önemli farklılıklar bulunduğunu göstermiştir ( $p < 0,05$ ). Bu farklılıkların, nem, protein ve yağ içeriği, protein yapısı, LAB aktivitesi ve olgunlaşma koşulları gibi çeşitli faktörlerden kaynaklanabileceği düşünülmektedir. Bu çalışmada elde edilen bulgular, Karaman Divle Obruğu Tulum peynirinin özgün niteliklerini korumak ve gıda güvenliğini sağlayabilmek için standart üretim uygulamalarının önemini vurgulamaktadır.

## Karaman Divle Cave Tulum Cheese: An Overview of Microbiological, Physicochemical, and Textural Properties

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### ABSTRACT

Karaman Divle Obruğu Tulum Cheese is a traditional Turkish cheese unique to the Karaman region. This cheese acquires its distinctive characteristics through fermentation and ripening processes facilitated by the natural microbial flora of Divle Cave. In this study, the physicochemical, microbiological, color, meltability, and textural properties of Divle Tulum cheese samples obtained from two different producers in Karaman were investigated. The results showed that both samples contained high counts of lactic acid bacteria (LAB) and total mesophilic aerobic bacteria ( $>7$  log cfu/g). Additionally, high microbial counts were observed for yeasts and coliforms, with mean values of 4.50 and 4.23 log cfu/g, respectively, highlighting the need for improved hygienic practices during production. As a result of physicochemical analyzes, the average pH, acidity, dry matter, fat, protein and salt contents of the samples were determined as  $5.83 \pm 0.00$ ,  $1.12 \pm 0.08\%$  L.A.,  $61.22 \pm 2.48\%$ ,  $21.10 \pm 2.33\%$ ,  $29.52 \pm 2.20\%$  and  $4.69 \pm 0.28\%$ , respectively. Significant differences were detected in the  $L^*$ ,  $a^*$ , and  $b^*$  color values of the cheeses ( $p < 0.05$ ). Melting analysis at  $130^\circ\text{C}$  showed meltability of  $58.75 \pm 8.35\%$  and  $112.24 \pm 8.26\%$  for the samples. These variations are thought to be influenced by factors such as moisture, protein and fat content, protein structure, LAB activity, and ripening conditions. The findings of this study emphasize the importance of standardized production practices in preserving the unique characteristics of Karaman Divle Obruğu Tulum cheese and ensuring food safety.

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## INTRODUCTION

Due to the increasing health issues associated with modern diets, there has been a growing demand from consumers for more natural and healthier food products [1]. Traditional cheeses, rich in essential nutrients such as calcium, phosphorus, and proteins, play a significant role in a balanced diet. These cheeses not only provide crucial nutrients for bone health and energy metabolism [2], but also promote gut health and improve digestion through the diverse microbial community they harbor, particularly probiotic bacteria [3,4].

'Karaman Divle Obruğu Tulum Peyniri', is a geographically indicated product recognized by the Turkish Patent and Trademark Office, is a traditional Turkish cheese known for its unique production method and distinctive flavor profile. The name "Tulum" translates to "sack" in Turkish, referring to the goat or sheep skin bags traditionally used for ripening the cheese. This method not only influences the texture and taste but also contributes to the cheese's microbiological diversity, as the skins harbor various microorganisms that play a crucial role during the ripening process [5–8].

Divle Tulum Cheese is a semi-hard cheese produced in the Karaman region of Turkey, specifically in Divle Cave. Made from raw ewe's milk, the cheese undergoes a unique ripening process within the cave, which is crucial for developing its characteristic flavor and texture. The cave's microclimate, with temperatures ranging from 5 to 10 °C and high humidity levels (85-90%), provides an optimal environment for natural maturation. Ripening occurs over four months at a depth of 70 meters, during which the goatskin bags used for aging change color from green to red, a distinctive feature of Divle Cave cheese. The absence of starter cultures during this process facilitates spontaneous fermentation, further enhancing the cheese's unique organoleptic properties [9–13].

The ripening process of artisanal raw milk cheeses, such as Divle Tulum cheese, is largely spontaneous and influenced by the interaction of nonstarter lactic acid bacteria, other bacteria, filamentous fungi, and yeasts. These microorganisms, originating from raw milk or introduced from the environment during ripening, play a crucial role in flavor and texture development [12]. Studies have shown that Divle Tulum cheese has a high concentration of aldehydes, ketones, and alcohols, which are crucial for its sensory attributes [9,11,14]. The ripening process is also marked by significant biochemical changes, including lipolysis and proteolysis, which enhance the flavor and texture of the cheese [6,15]. The microbiological profile of Divle Tulum cheese is particularly noteworthy. It is dominated by lactic acid bacteria (LAB), which are essential for fermentation and flavor development. The microbial flora can vary significantly depending on the ripening conditions and the specific characteristics of the milk used [16–18]. However, the lack of standardization in Divle Tulum cheese production presents challenges in ensuring consistent quality. Addressing these challenges is essential to achieving a more uniform and high-quality product while retaining its traditional attributes [13].

Karaman Divle Obruğu Tulum cheese holds significant cultural and economic importance for the Karaman region. Geographical indication certification not only highlights the traditional production methods and unique characteristics of such products but also ensures their preservation and the continuity of their authenticity. These certifications establish a strong connection between the product's distinctive features and its place of origin, benefiting producers by protecting the product's quality and enhancing its market value. Moreover, geographical indications contribute to regional development by promoting tourism and supporting local economies [19]. Considering these factors, this study focuses on Divle Tulum Cheese to explore its unique properties and contribute to the recognition and understanding of this valuable traditional product. In this study, Divle Tulum Cheese samples from two producers in the Karaman market were analyzed, focusing on their biochemical, microbiological, and textural properties. The findings provide a preliminary comparison, offering insights into the characteristics and diversity of this traditional cheese.

## MATERIALS AND METHODS

### Materials

Samples of Divle Tulum cheese, obtained in whole leather casing, were sourced from two different vendors located in Karaman. Both samples were from the same production season, and particular attention was given to selecting similarly sized cheese tulum to ensure comparable ripening times. Based on information obtained from the cheese producers, the cheese production process can be summarized as follows:

1. **Raw Milk:** Sheep's milk is commonly used, though goat and cow's milk can also be mixed. (In this study, only sheep's milk cheese was used.)
2. **Heat Treatment:** The milk is heated to 55 °C for 1-2 minutes and then cooled to 32-35 °C.
3. **Renneting:** 10 mL of rennet is added per 100 kg of milk.
4. **Coagulation:** The coagulation process takes approximately 90 minutes.
5. **Curd Cutting:** The curd is cut to promote whey drainage, heated to 55 °C, and rested for 15-20 minutes.
6. **Pressing/Draining:** The curd is placed in cloth bags and pressed for 15-24 hours to facilitate whey drainage.
7. **Curd Washing:** The curd is cut into pieces, washed three times with cold water at 2-hour intervals, and pressed again for 24 hours.
8. **Salting:** The cheese is salted at a concentration of 2.7-3.0%.
9. **Packaging (Bagging):** The cheese is tightly packed into goat skin bags, perforated with a needle, and stored in a cool environment for 8-10 days.
10. **Ripening:** The cheese is aged in Divle Cave for 5-6 months.



**Figure 1**  
*Samples of Divle Tulum Cheese analyzed in this study.*

Before analysis, the cheese samples were stored briefly at +4 °C. Under sterile laboratory conditions, the cheeses were cut, and homogeneous samples were taken and prepared for analysis. All analyses, except for fat and protein content, were performed immediately after collection, with samples kept at 4 °C during the process. The remaining samples were then stored at -20 °C for fat and protein analyses. Cheese samples were labeled as P1 and P2 for simplicity and clarity throughout the text (Figure 1).

### Methods

#### *Microbiological Analyses*

All microbiological analysis methods used in this study were performed according to Halkman [20]. Further details are provided below. 25 g of cheese samples were homogenized with 225 mL of

sterile Maximum Recovery Diluent (MRD) (Oxoid CM0733B, England), followed by serial dilutions using MRD.

LAB counts in the cheese were determined using the spread plate method on three different agar media: de-Man Rogosa Sharpe (MRS) agar (Conda 1433, Spain), M17 agar (Conda 1318, Spain), and Bifidus Selective agar (BSM) (Sigma-Aldrich G 88517, UK). All these media were supplemented with 50 µg/L cycloheximide (CAS 66-81-9, Sigma-Aldrich, Germany) to inhibit yeast growth [21,22]. MRS agar was incubated under microaerophilic conditions using Anaerocult® (Merck, Germany) for *Lactobacillus* spp., while M17 agar and BSM agar were incubated anaerobically for cocci-shaped LAB and *Bifidobacteria*, respectively. All plates were incubated at 37 °C for 48 hours.

Coliform bacteria were enumerated on Plate Count Agar (PCA) (Conda 1056) using the spread plate method, overlaid with Violet Red Bile (VRB) agar (Merck), and incubated at 35 °C for 24 hours [23–26]. Red colonies were counted as coliforms. *Escherichia coli* enumeration was performed using TBX Agar (Merck) with the pour plate method, incubated at 30 °C for 4 hours and then at 44 °C for 18 hours. Blue-green colonies were counted as *E. coli*. Yeast and mold counts were determined by spreading the sample dilution onto Potato Dextrose Agar (PDA) (Conda 1022) adjusted to pH 3.5 with 10% tartaric acid, followed by incubation at 25 °C for 5 days before counting.

### ***Textural Profile Analysis***

The texture profile analysis (TPA) of Tulum cheese samples was carried out at room temperature using a TA-XT2 Texture Analyzer (TA.XT Plus, Stable Micro Systems, Surrey, UK) featuring a 30 kg load cell. The Tulum cheeses were diced into small cubes measuring approximately 10 mm. The TPA was executed with a cylindrical stainless steel compression probe (36 mm diameter) under the following parameters: a crosshead speed of 1.0 mm/s and a compression level set to 40% of the original height, with a 5-second pause between two compressions. The instrument recorded values for hardness, adhesiveness, cohesiveness, springiness, chewiness, and gumminess [27].

### ***Total solid content***

The total solid content of cheeses was analyzed using the oven-drying method according to IDF 4 standard [28]. In this method, approximately 2–3 g of the sample, mixed with sand, is dried by heating in a drying oven at 102 °C. After drying, the test portion is weighed to determine the loss of mass.

### ***Acidity Determination***

The pH of the cheese samples was measured by blending 10 g of the sample with 50 mL of distilled water for 30 seconds. The pH values were determined using a Mettler-Toledo pH meter (Seven Compact Duo, Columbus, USA).

The titratable acidity values of the samples were measured according to the IDF/RM 150 method [29]. A 10 g sample was dissolved in 100 mL of water at 38 °C. The solution was then titrated with a standardized 0.1 N NaOH solution, using phenolphthalein as an indicator to determine the endpoint. The volume of NaOH consumed during the titration was recorded and used to calculate the titratable acidity as a percentage, following the equation:

$$\text{Titratable Acidity (\%)} = \frac{(V_{\text{NaOH}} \times N_{\text{NaOH}} \times 0.009 \times 100)}{m_{\text{sample}}}$$

where;

$V_{\text{NaOH}}$  is the volume of NaOH used in mL,  $N_{\text{NaOH}}$  is the normality of NaOH (0.1 N), 0.009 is the lactic acid equivalent value,  $m_{\text{sample}}$  is the mass of the sample in grams.

### ***Salt Content Determination***

The salt content of the cheese samples was measured according to the IDF Standard 17A method [30]. A 10 g sample was extracted with water, and the solution was then titrated with a standardized 0.1 N AgNO<sub>3</sub> solution, using 5% K<sub>2</sub>CrO<sub>4</sub> as an indicator to determine the endpoint. A blank titration was also performed. The volume of AgNO<sub>3</sub> consumed during the titration was recorded and used to calculate the salt content as a percentage, following the equation:

$$\%Salt (g) = \frac{(0.00585 \times V)}{m} \times DF \times 100$$

where;

$V$  is the volume of AgNO<sub>3</sub> used (mL),  $N$  is the concentration of AgNO<sub>3</sub>,  $m$  is the mass of the sample (g),  $DF$  is the dilution factor (if diluted)

### ***Crude Fat Content***

The crude fat content of cheese samples was determined according to Nielsen and Carpenter [31] using a Soxhlet extractor (Velp Scientifica, Monza-Brianza, Italy). The dried and finely milled cheese samples were extracted with n-hexane, and the results were expressed as a percentage, adjusted for moisture content.

### ***Crude Protein Content***

The determination of crude protein ( $N \times 6.38$ ) of samples was performed using the Kjeldahl method, as described by Nielsen [32]. The analysis was performed with a Kjelttec 8200 distillation unit (FOSS, Sweden).

### ***Meltability***

The meltability of Divle Tulum cheese samples was assessed using a modified version of the Schreiber test, similar to the method outlined by Kapoor et al. [33]. Each sample was cut into discs measuring 30 mm in diameter and 10 mm in height. Three discs, each weighing 5 grams, were placed on aluminum plates measuring 100 mm x 100 mm with a thickness of 0.3 mm. The plates were then promptly transferred to an air convection oven set at 90 °C and 130 °C. After 15 minutes of heating, the plates containing the melted cheese discs were allowed to cool to room temperature. Meltability was expressed as the diameter of the melted cheese in millimeters, with average measurements taken from at least four points and calculated using the formula below.

$$Melting Capacity (\%) = \frac{(\text{Final Diameter} - \text{Initial Diameter})}{\text{Initial Diameter}} \times 100$$

### ***Color Analysis***

Color analysis of the samples was conducted using a Konica Minolta CR-400 instrument. Following calibration with a standard calibration plate,  $L^*$ ,  $a^*$ , and  $b^*$  color values were measured in both the cheese and its casing, with measurements taken from 10 different areas in each section.

### ***Statistical Analysis***

Statistical analyses were performed using IBM SPSS Statistics 22. Group differences were evaluated with an independent samples t-test, considering a significance level of  $p < 0.05$ . Results are presented as means  $\pm$  standard deviation (SD).

## RESULTS AND DISCUSSIONS

### Microbial analyses

The results of the microbiological analyses, presented in Table 1, revealed a notably high density of LAB in both samples. Measurements on MRS agar showed that the LAB count in P1 cheese was 7.63 log cfu/g, while for P2, the count on M17 agar was 7.62 log cfu/g. These results indicate that both cheeses possess a rich LAB flora during their fermentation processes. The LAB counts in Tulum cheeses are influenced by packaging methods, milk sources, and ripening conditions. Ceylan et al. [34] reported an average LAB count of 6.14 log cfu/g in Tulum cheeses, influenced by different packaging types and the use of ewe's milk. Karagözlü et al. [35] found a LAB count of 6.5 log cfu/g in Cimi Tulum cheese, while Öksüztepe et al. [36] documented counts of 5.56 log cfu/g for *Lactobacillus* and 7.00 log cfu/g for *Lactococcus* in Şavak Tulum cheese. Öner et al. [37] observed LAB counts of 7.22 log cfu/g for *Lactobacillus* and 6.93 log cfu/g for *Lactococcus* in Tulum cheese. In a study by Akgül et al. [38] detected 6.04 log cfu/g LAB in Erzincan Tulum cheese. Additionally, Levkov et al. [39] reported LAB counts in traditional ewe's cheese ranging from 6.00-7.00 log cfu/g. Gürses and Erdoğan [17] found that *Lactobacillus* species dominated microbiota of Tulum cheese, increasing during ripening, while other genera remained relatively stable. Similarly, Çakmakçı et al. [16] observed that *Lactococcus* species rapidly decreased after three months of ripening in Tulum cheese. In other types of cheese, Şenel et al. [40] reported LAB counts of 6.64 log cfu/g in Urfa cheese and 4.95 log cfu/g in Van herbed cheese. Kırdar et al. [41] analyzed 90-day matured Keş cheese and found *Lactobacillus* and *Lactococcus* counts of 7.16 log and 7.54 log cfu/g, respectively.

*Bifidobacteria* counts in P2 were 12.5% higher than in P1, reaching 7.58 log cfu/g. This difference may be influenced by multiple factors, including variations in raw milk composition, fermentation conditions, or potential microbial contributions from the production environment. While traditional Divle Tulum cheese is typically characterized by spontaneous fermentation, the potential use of starter cultures in certain market-available products cannot be entirely excluded. In a study conducted by Güley et al. [42], the presence of *Bifidobacteria*, specifically *Bifidobacterium mongoliense*, was reported in all samples of traditional Izmir Tulum and Izmir Brined Tulum cheeses.

The total mesophilic aerobic bacterial (TMAB) counts were also quite high in both samples: 7.80 log cfu/g in P1 and 7.61 log cfu/g in P2. These high values indicate an active fermentation process. According to Ceylan et al. [34], the total aerobic mesophilic bacteria counts in Tulum cheeses were 7.0 log cfu/g for those packed in goat skin (combining results from raw milk cheese and pasteurized milk cheese with starter culture), 6.82 log cfu/g for cheeses stored in plastic barrels, and 5.92 log cfu/g for those kept in ceramic pots. Karagözlü et al. [35] reported 8.85 log cfu/g in Cimi Tulum cheese, while Öksüztepe et al. [36] documented a count of 7.34 log cfu/g in Şavak Tulum cheese. Additionally, Akgül et al. [38] found the TMAB counts in Erzincan Tulum cheese to be approximately 6.63 log cfu/g. Similar findings have been observed in other studies on different traditional cheeses. In a study by Levkov et al. [39] it was found that ewe's milk cheese produced using traditional methods had TMAB counts exceeding 7 log cfu/g after 45 days of maturation. Additionally, Şenel et al. [40], reported that the TMAB levels in Urfa cheese reached 7.3 log cfu/g, while Van herbed cheese exhibited 6.30 log cfu/g. Kırdar et al. [41] reported a TMAB level of 7.69 log cfu/g in Keş cheese after a 90-day maturation period.

Yeast growth was observed in the cheese samples, whereas mold growth was not within the countable range. Notably, the P1 sample exhibited a higher yeast count, measured at 5.90 log cfu/g. Yeast and mold counts in various Tulum cheese types show notable variability depending on the cheese type, ripening period, and production conditions. Çakır and Çakmakçı [43] reported a decrease in yeast and mold counts from 6.14 to 4.13 log cfu/g in Erzincan Tulum cheese during a 90-day ripening period, emphasizing the effect of maturation on microbial populations. Similarly, Gürsoy et al. [44] found

**Table 1**

Microbial counts (log cfu/g) in P1 and P2 cheese samples.

Microorganisms		P1	P2	Average	Difference (%)
Lactic acid bacteria	<i>MRS agar</i>	7.63 ± 0.30	7.41 ± 0.17	7.52 ± 0.24	3.02
	<i>MI7 agar</i>	7.03 ± 0.35	7.62 ± 0.39	7.33 ± 0.46	8.48
<i>Bifidobacteria</i>		6.65 ± 0.56	7.58 ± 0.23	7.12 ± 0.64	12.25
TMAB		7.80 ± 0.14	7.61 ± 0.41	7.71 ± 0.28	2.53
Yeast-Mold		5.90 ± 0.38	3.10 ± 0.23	4.50 ± 1.64	90.36
Total Coliform		4.00 ± 0.60	4.46 ± 0.27	4.23 ± 0.47	11.64
<i>E. coli</i>		nd	4.10 ± 0.37	-	-

ranging from 1.00 to 4.80 log cfu/g in Söğle Tulum cheese. Higher yeast and mold counts of 6.44 log cfu/g were observed in Kargı Tulum cheese [45]. Ceylan et al. [34] reported an average count of 5.44 log cfu/g in Tulum cheese made from ewe's milk, with variations depending on the packaging method. Karagözlü [35] documented a count of 1.9 log cfu/g in Cimi Tulum cheese, while Öksüztepe [36] observed approximately 4.43 log cfu/g in Şavak Tulum cheese.

The coliform bacterial load in P2 was 4.46 log cfu/g, which was 11.64% greater than that of P1. Additionally, the presence of *E. coli* in the P2 sample was detected at 4.10 log cfu/g. This finding suggests that hygiene conditions may not have been adequately maintained during the production process of the P2 cheese. In the literature, the fecal *Enterobacteria* count in Erzincan Tulum cheese was found to be 2.04 log cfu/g [38]. The coliform count in Kargı Tulum cheese was measured at 3.48 log cfu/g [45]. Ceylan et al. [34] found coliform counts of less than 1.00 log in Tulum cheese made from ewe's milk. On the other hand, higher coliform counts were observed, with 7.30 log cfu/g in Cimi Tulum cheese [35] and 5.79 log cfu/g in Şavak Tulum cheese [36]. In other studies on traditional cheeses, *Enterobacteria* counts in Urfa and Van herby cheeses were reported as 4.12 log and 2.48 log, respectively [40], while the coliform count in 90-day ripened Keş cheese was found to be 1.99 log cfu/g [41].

In a study conducted by Morul and İşleyici [46], 50 Divle Tulum cheese samples were evaluated chemically and microbiologically. The microbiological analyses revealed average counts for aerobic mesophilic bacteria at  $6.78 \pm 1.42$  log cfu/g in 50 samples, *E. coli* at  $3.61 \pm 0.87$  log cfu/g in 9 samples, coliforms at  $3.04 \pm 1.52$  log cfu/g in 20 samples, *Lactobacillus-Leuconostoc-Pediococcus* group microorganisms at  $6.93 \pm 1.17$  log cfu/g in 50 samples, and yeast/mold at  $6.36 \pm 1.43$  log cfu/g in 50 samples. While our study observed slightly lower yeast and mold counts, the other microbial parameters were generally higher. However, when compared with the minimum and maximum bacterial counts reported in Morul and İşleyici's study, our findings fall within the range of those values [46].

Ozturkoglu-Budak et al. [12] have reported that in Divle cheeses, microbial diversity is mainly introduced through raw milk and production-related contamination. Additionally, in a different study, it has been reported that 23 bacterial species were identified in the inner and outer parts of Divle Cave Cheese at 60 and 120 days of ripening using molecular techniques. The microbiota displayed similar community structures across batches, but significant diversity was observed between different parts of the cheese during ripening [13].

### Physicochemical properties of cheeses

Tulum cheese, exhibits distinct physicochemical properties that are crucial for assessing its quality and safety. The data on some physical and chemical properties are presented in Table 2. Moisture content, a critical parameter for Tulum cheese, is typically reported to have a maximum limit of 45% as per Turkish standards [47]. However, studies have documented total solids (TS) contents in ripened

Tulum cheese (aged for a minimum of 90 days) ranging from 46.5% to 69.4%, depending on factors such as ripening conditions, the type of milk used, specific starter cultures, and packaging methods. [14,44,48–51]. Our results align with the literature, as the moisture content of P2 (62.96%) was found to be higher compared to P1 (59.47%). The variation in moisture content may have been influenced by the composition of the milk used in cheese production and microbial activity. Higher protein levels, particularly casein, contribute to increased moisture and protein content in cheese, while an elevated somatic cell count has been linked to higher moisture and water-soluble nitrogen levels [52]. Additionally, EPS-producing bacteria have been reported to enhance moisture in cheese [53].

**Table 2**  
*Physicochemical, color, and melting properties of divle tulum cheeses.*

Parameters		P1	P2	$\bar{X} \pm SD$
<i>Physicochemical Properties</i>				
Total Solid (%) <sup>†</sup>		59.47 ± 0.78	62.97 ± 1.90	61.22 ± 2.30
pH <sup>†</sup>		5.91 ± 0.06	5.76 ± 0.01	5.83 ± 0.09
Acidity (% L.A.) <sup>†</sup>		1.81 ± 0.08	1.36 ± 0.09	1.58 ± 0.26
Fat (%) <sup>†</sup>		19.14 ± 1.06	23.05 ± 0.95	21.10 ± 2.33
Fat in Dry Matter (%) <sup>†</sup>		32.17 ± 1.78	36.62 ± 1.50	34.39 ± 2.84
Protein (%) <sup>†</sup>		31.45 ± 0.68	27.59 ± 0.68	29.52 ± 2.20
Salt Content (%)		4.64 ± 0.34	4.74 ± 0.29	4.69 ± 0.29
Salt in Dry Matter (%)		7.80 ± 0.58	7.53 ± 0.46	7.66 ± 0.49
<i>Colour</i>				
Inner	<i>L</i> * <sup>†</sup>	78.02 ± 1.16	63.47 ± 2.59	70.74 ± 7.70
	<i>a</i> * <sup>†</sup>	-4.35 ± 0.24	-4.71 ± 0.37	-4.53 ± 0.35
	<i>b</i> * <sup>†</sup>	14.80 ± 0.47	16.97 ± 0.74	15.89 ± 1.26
Skin	<i>L</i> * <sup>†</sup>	52.95 ± 4.22	59.01 ± 4.43	55.98 ± 5.22
	<i>a</i> * <sup>†</sup>	5.74 ± 1.43	11.46 ± 1.78	8.60 ± 3.32
	<i>b</i> * <sup>†</sup>	14.41 ± 1.59	24.04 ± 2.26	19.23 ± 5.28
<i>Melting Properties</i>				
90 °C		45.00 ± 4.30	Not observed	
130 °C <sup>†</sup>		58.75 ± 8.35	112.24 ± 8.26	85.50 ± 30.27

$\bar{X} \pm SD$ : mean ± standard deviation of the two cheese samples, L.A.: lactic acid equivalent. The difference between samples in parameters marked with <sup>†</sup> is statistically significant ( $p < 0.05$ ).

During the ripening process, the pH of Tulum cheese generally increases, primarily due to the assimilation of acids by yeasts and mold, along with the deamination of amino acids [50]. This increase in pH can influence the flavor and texture of the cheese, contributing to its characteristic taste profile. The pH values reported for Tulum cheese typically range from approximately 4.5 to 5.5, depending on the specific production methods and ripening conditions [11,43,44,50,54]. The pH values of Tulum cheeses in this study are slightly higher than those reported in the existing literature.

Titrateable acidity is another key indicator of the fermentation process in Tulum cheese, affecting its flavor, texture, and preservation. Studies show that acidity increases during ripening, with levels typically ranging from around 0.5% to 2.0%, depending on production conditions [38,43–45,48,54]. The titrateable acidity values for P1 (1.81 ± 0.08%) and P2 (1.36 ± 0.39%) fall within the range reported for typical Tulum cheeses. Akarca [15] found that adding probiotic bacteria and specific starter cultures increased acidity and lowered pH during ripening, significantly shaping the cheese's sensory characteristics. Similarly, Çelik and Tarakçı [55] reported that certain starter cultures enhance lactic acid production, further improving acidity and flavor development. These findings highlight the importance of starter culture in Tulum cheese, which contains various types of lactic acid bacteria and probiotic bacteria.

The analysis results showed that the fat content of the cheese samples was  $19.14 \pm 1.06\%$  for P1 and  $23.05 \pm 0.95\%$  for P2, while the fat in dry matter content was  $32.17 \pm 1.78\%$  for P1 and  $36.62 \pm 1.50\%$  for P2. Based on these values, both samples are classified as semi-fat cheese according to the Turkish Food Codex [47]. Morul and İşleyici [46] reported that the fat content of Divle tulum cheese varies widely, ranging from 13% to 32%. They attributed this variation to the lack of a standardized production method for this cheese type and the occasional use of skimmed milk in its production. In the same study, the protein content of Divle tulum cheese was reported to range between 16.79% and 31.62%. The findings of this study revealed protein contents of  $32.17 \pm 0.68\%$  (P1) and  $27.26 \pm 0.68\%$  (P2), which were found to be consistent with the previously reported range. An analysis of 50 randomly selected tulum cheese samples from the Konya and Erzurum regions revealed an average fat content of 25.9% and a protein content of 22.72% [56]. Research on Erzincan tulum cheese has reported fat content between 27.76% and 31.48% and protein content ranging from 17.91% to 20.03% [57,58,38,59]. In comparison, Divle tulum cheese exhibits lower fat content and higher protein content. Similarly, Çimi cheese, which has been reported to contain 30.63% fat and 22.79% protein after a 90-day maturation period [35], is comparable to Erzincan tulum cheese. In contrast, Söğle cheese has a significantly lower fat content (average 3.75%) but a remarkably high protein content (average 38.08%) [44].

Salt, in addition to enhancing the flavor of cheese, represents one of the oldest preservation methods [60], providing critical functions such as microbial stability, moisture regulation, maturation control, and extended shelf life. In this study, similar salt levels were detected in both cheese samples (P1: 2.76%, P2: 2.82%). Salt content is a critical aspect of Tulum cheese, with salt-in-dry matter (SDM) levels significantly higher than those in many other cheese varieties. For Tulum cheeses aged for 90 days, SDM levels are typically reported to range from approximately 3.7% to 8% [38,43,45,50,54], playing a crucial role in the preservation and sensory qualities of the cheese. While the salt levels identified in our study were considerably lower than those reported by Akgül et al [38], Çakır and Çakmakçı [43], and Demirci et al. [50], they were found to be closer to the results reported by Yıldırım and Özbey [45] and Sert et al.[54]. This high salt concentration is essential for microbial safety and flavor enhancement [40].

Morul and İşleyici [46] reported that, for 50 Divle Tulum cheese samples, the mean values for pH, acidity, dry matter, fat, protein and salt content were  $5.42 \pm 0.61$ ,  $1.074 \pm 0.425\%$  L.A.,  $56.27 \pm 7.59\%$ ,  $23.46 \pm 4.48$ ,  $25.90 \pm 3.40$  and  $3.99 \pm 0.75\%$ , respectively. Our study observed slightly higher values for these parameters (except protein content). However, considering the differences in sample size and standard deviations, there appears to be no substantial discrepancy between the two studies, suggesting a similar overall chemical profile.

### **Color Characteristics of Tulum Cheeses**

Tulum cheese is typically characterized by a white to cream color, which can vary with the presence of carotenoids in the milk [54,61]. The color values of the cheeses investigated in this study are presented in Table 2. The color analysis revealed significant differences between the two cheese samples. The lightness value ( $L^*$ ) for P1 was 78.02, while P2 had a lower  $L^*$  value of 63.47, indicating that P1 exhibited a lighter color. The redness-greenness index ( $a^*$ ) was negative for both samples, with P1 at -4.35 and P2 at -4.71, showing a predominance of greenish hues in both cheeses. The yellowness-blueness index ( $b^*$ ) was higher for P2 (16.97) compared to P1 (14.80), suggesting that P2 had a more yellowish tint. Tekin and Güler [51] noted that Tulum cheese ripened in goat skin bags exhibited distinct color parameters compared to those ripened in plastic containers, with the former often displaying a more appealing appearance to consumers. This suggests that the ripening medium plays a crucial role in shaping the cheese's aesthetic qualities, potentially enhancing consumer preference based on its color and overall visual appeal.

In the study, the color values of the goatskin bags were also determined. Based on the color parameters, P1 showed an  $L^*$  value of 52.95, which was lower than P2's  $L^*$  value of 59.01, indicating a lighter skin bag color in P2.  $a^*$  value for the bag also differed significantly between the two samples, with P1 (5.74) showing a lower value than P2 (11.46), suggesting a more reddish hue in the bag of P2. Additionally, the  $b^*$  value for P2 (24.04) was notably higher than P1 (14.41), indicating a stronger yellowish tone on the bag of P2. These color differences are thought to be influenced by both the animal and the ripening environment. The surface of the goatskin bags changes from green to red during ripening in the cave, and this red hue is a distinguishing characteristic of Divle Cave cheese [11,12]. During the ripening process, the cheese bags become covered with a distinctive red mold due to the natural flora of the cave [19]. As a result, a specific red mold species influences the color of the outer skin.

### **Meltability of Tulum Cheeses**

Cheese meltability is significantly influenced by several factors, including moisture content, fat and protein composition, and ripening conditions. As the moisture content increases, the fluidity of the cheese matrix improves, leading to enhanced meltability [62]. Additionally, the proteolytic breakdown of casein proteins during ripening softens the cheese matrix, further promoting meltability. The type of milk used and the ripening time also play key roles in determining the meltability of Tulum cheese [63].

In this study, the melting properties of the Tulum cheeses were assessed at two different temperatures, 90 °C and 130 °C (Table 2). At 90 °C, P1 exhibited a melting value of 45.00%, while P2 showed no observable melting behavior. This suggests that the protein matrix of P2 is more resistant to melting at this temperature compared to P1, possibly due to the formation of a tighter and firmer protein network [64]. At 130 °C, the melting behavior differed significantly between the samples. P1 had a melting value of 58.75%, whereas P2 demonstrated a much higher value of 112.24%. This indicates that P2 had a greater ability to melt at higher temperatures, which could be attributed to a higher level of protein denaturation or a more fluid matrix in P2 compared to P1. This melting behavior can also be explained by the relatively lower fat and higher protein content of P2. Cheeses with higher fat content, such as Mozzarella, tend to have better meltability [65]. The melting behavior at lower temperatures (90 °C) may be associated with the moisture content, as higher moisture levels increase the flexibility of the protein matrix, facilitating melting. However, the absence of melting in P2 at 90 °C suggests that factors beyond moisture content, such as protein network integrity, may also play a role [66]. In contrast, melting at higher temperatures (130 °C) is likely linked to the thermal denaturation and breakdown of the protein structure, influenced by factors such as higher total solids content and lower acidity.

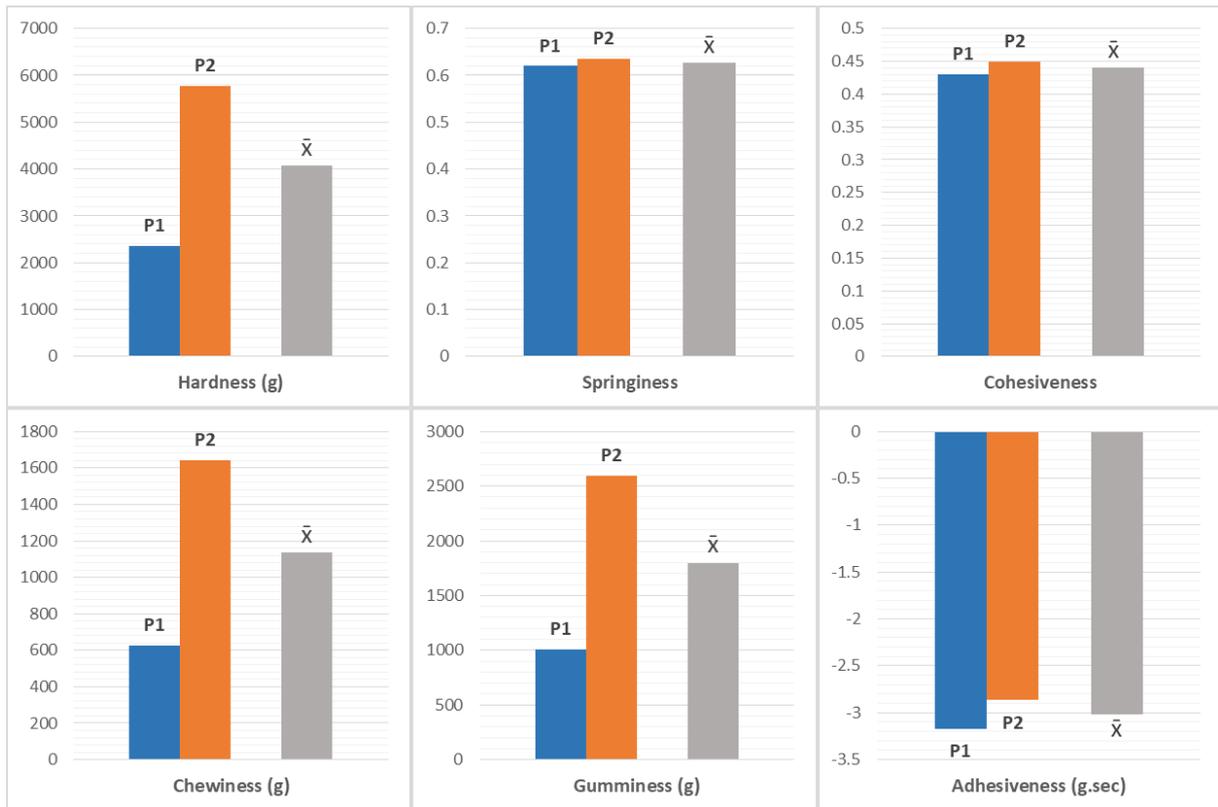
### **Textural Characterization**

Cheese texture is influenced by various factors, including moisture content, fat levels, protein structure, and processing methods. Moisture acts as a plasticizer, enhancing properties like springiness and cohesiveness, while higher fat content generally softens the texture by weakening the protein matrix. Conversely, low moisture and fat levels can increase hardness and gumminess, making the cheese denser [67,68]. Protein content also plays a significant role in cheese texture. A higher protein content is positively correlated with chewiness and firmness but negatively with gumminess [69]. Proteolysis and other biochemical changes during ripening further modify texture by breaking down proteins and fats, which can either soften or firm the cheese depending on the extent of these processes [70]. Additionally, proteolysis, lipolysis, and glycolysis are critical biochemical processes during ripening that significantly impact the composition and texture of cheese. These processes, driven by specific microbial activities, influence the breakdown of proteins and fats, leading to notable changes in textural attributes [70,71]. Nevertheless, establishing direct cause-and-effect relationships between specific biochemical changes and textural properties is complex, as multiple factors often interact synergistically [70]. Thus, cheese

texture is a complex interplay of compositional and processing factors, each contributing uniquely to its sensory and mechanical properties.

The textural profile analysis results of the cheeses are presented in Figure 2. The textural parameters of P1 and P2 showed significant differences ( $p < 0.05$ ) in hardness, gumminess, and chewiness, which are influenced by the structural and compositional properties of the cheeses. P2 showed a significantly higher hardness value ( $5780.93 \pm 692.73$  g) compared to P1 ( $2353.31 \pm 175.34$  g), likely due to its higher total solids content and lower moisture, resulting in a firmer protein matrix. It has been reported that there is a direct correlation between moisture content and cheese hardness, where a reduction in water content promotes a higher concentration of caseins within the matrix, thereby increasing hardness [72]. As fat content decreases, the protein network becomes denser, resulting in a firmer and harder texture [73]. However, in the present study, the P1 sample, which had relatively lower fat and higher protein content, exhibited a lower hardness value compared to P2. Additionally, the moisture content in cheese is inversely related to fat content; lower-fat cheeses often have higher moisture levels, which can further influence texture by altering protein interactions [74]. In agreement with this, the P1 sample had a relatively higher moisture content. Studies have shown that as moisture content increases, the activity of proteolytic enzymes also enhances, leading to increased proteolysis [75,76]. The lower hardness value observed in P1 can be attributed to this phenomenon. This structural rigidity of P2 also correlates with its enhanced melting performance at  $130\text{ }^{\circ}\text{C}$  ( $112.24 \pm 8.26\%$ ), as the breakdown of its robust protein network under higher thermal conditions requires more energy. Springiness values were similar between the samples ( $p > 0.05$ ), with P1 measured at  $0.620 \pm 0.051$  and P2 at  $0.635 \pm 0.039$ , suggesting no significant difference in their elastic properties. Cheeses with higher moisture content tend to have better springiness, as the water acts as a plasticizer within the protein matrix [67]. However, P2 demonstrates slightly higher springiness despite its lower moisture content, which can be attributed to its well-structured and denser protein matrix. Adhesiveness values were negative and similar for both samples, with P1 ( $-3.17$  g·sec) and P2 ( $-2.87$  g·sec), indicating no significant differences in surface interaction properties ( $p > 0.05$ ). Cohesiveness values showed minimal variation ( $p > 0.05$ ), with P1 and P2 being  $0.430 \pm 0.080$  g and  $0.450 \pm 0.080$  g, respectively, suggesting similar internal bonding strength. However, P2 exhibited significantly higher gumminess ( $2593.53 \pm 519.43$  g) and chewiness ( $1643.61 \pm 316.37$  g) compared to P1 ( $p > 0.05$ ), highlighting its dense and firm structure, which resists deformation and requires greater effort to compress and chew. Gumminess and chewiness values showed a similar trend to hardness, as these properties are derived from hardness and are directly influenced by moisture and protein content [72]. Exopolysaccharide (EPS)-producing bacteria present in Tulum cheese can influence its texture by modifying the structure and enhancing characteristics such as chewiness and gumminess [4]. These variations emphasize the firmer and more resilient structure of P2, making it potentially better suited for applications requiring a robust texture.

Ensuring that a geographically indicated product meets the specified production standards, maintains certain characteristics, and achieves consistent quality is crucial for the sustainability of its designation. However, in the production of Karaman Divle Tulum Cheese, challenges such as insufficient adherence to hygiene practices, reduced livestock farming due to increasing drought, and the absence of standardized production processes have led to variations in chemical properties and quality issues [77]. These factors may also explain the differences observed in the samples analyzed in this study.



**Figure 2**  
Comparison of key textural attributes between tulum cheese samples.

## CONCLUSIONS

The findings of this study highlight the distinctive characteristics of Divle Tulum cheese, including its microbiological richness, typical physicochemical properties, and specific textural attributes. The high levels of lactic acid bacteria and mesophilic aerobic bacteria emphasize its microbial diversity, which is integral to its flavor and quality. However, variations in coliform and yeast counts between samples highlight the importance of implementing standardized hygienic practices during production to ensure consistent quality and safety.

Physicochemical, textural, and color distinctions between the samples, driven by factors such as moisture content, ripening duration, and raw material quality, emphasize the importance of preserving traditional production methods. At the same time, integrating these artisanal practices with modern standardization techniques is crucial to maintaining the authenticity and quality of this geographically indicated product.

These findings provide a strong foundation for improving quality control strategies and safeguarding the heritage of Divle Tulum cheese. Future research should focus on standardizing production parameters, particularly hygiene protocols, raw material quality, and ingredient composition, while adhering to stringent food safety standards. This approach will sustain the cultural and gastronomic significance of Divle Tulum cheese, enhancing its recognition in global markets.

### **Ethical Statement**

The present study is an original research article designed and produced by the authors and did not involve human or animal experimentation.

### **Author Contributions**

Research Design (CRediT 1): G.Ü. (80%) – R.J. (20%)

Data Collection (CRediT 2): G.Ü. (30%) – R.J. (70%)

Research – Data Analysis – Validation (CRediT 3-4-6-11): G.Ü. (80%) – R.J. (20%)

Manuscript Writing (CRediT 12-13): G.Ü. (90%) – R.J. (10%)

Text Revision and Improvement (CRediT 14): G.Ü. (90%) – R.J. (10%)

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### **Conflict of Interest**

The authors have no conflicts of interest to disclose for this study.

### **Sustainable Development Goals (SDG)**

Sustainable Development Goals: 12 Responsible Consumption and Production (12a and 12b).

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