

RESEARCH ARTICLE

# The impact on shelf life of Tulum cheese of modified atmosphere packaging

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## ABSTRACT

Tulum cheese is one of the most important conventional fermented dairy products produced in Türkiye. In this study, the effects of modified atmosphere packaging on the microbiological, physicochemical, sensory properties, and shelf life of Tulum cheese were investigated. For this aim, Tulum cheese samples were divided into four groups (B = 100% CO<sub>2</sub>, C = 100% N<sub>2</sub>, D = 70% N<sub>2</sub> + 30% CO<sub>2</sub>, E = 75% N<sub>2</sub> + 25% CO<sub>2</sub>), and the control samples were packaged in the air (A). These samples stored at 4 ± 1 °C were analyzed for the microbiological, chemical, and sensory on days 0, 30, 60, 90, 120, 150, 180, 210, and 240. TMAB, LLP, lactic streptococcus, lipolytic microorganisms, proteolytic microorganisms, and yeast-mold counts increased while the counts of coliform group bacteria, *Enterobacteriaceae*, and *Staphylococcus aureus* continually decreased in all groups during the storage. During the storage period, the E. coli and sulfate-reducing anaerobic bacteria counts were detected below detectable levels (<1.0 log<sub>10</sub> CFU/g) in all groups. Other chemical parameter values increased continuously while pH values continually decreased during the storage in all the groups. It was observed that the total sensory scores of the samples decreased continually during the storage period. However, when the scores of the E group were evaluated within themselves, significant differences were obtained. In conclusion, Tulum cheese samples packaged in MAP showed significant changes in quality, and their shelf life was extended.

**Keywords:** Modified atmosphere packaging; Quality characteristics; Sensory evaluation; Shelf-life extension; Tulum cheese

## INTRODUCTION

Cheese is a fermented dairy product that has different shapes, flavors, and tastes produced in different regions and cultures around the world. The types of cheese produced are well known in that region because they are produced locally. However, it is not well known at the global level. Traditional cheeses are produced in small family businesses far from modern technology. Contrary to popular belief, this situation leads to the emergence of cheeses of different quality and structure (Cicek and Erdogmus, 2023).

Tulum cheese is a popular semi-hard cheese in Türkiye, and according to the Turkish Standard, it is produced from pasteurized cows, buffalo, sheep, goat's milk, or their blends (Anonymous, 2016). In our country, it is generally made in two ways dry and brine (Tekinsen and Tekinsen, 2005). It is estimated that Tulum cheese constitutes 10% of the total cheese produced in Türkiye (Tekinsen and Akar, 2017). According to the Turkish Statistical

Institute, the annual cheese production was approximately 753.000 tons of cheese in 2018, and production of Tulum cheese is estimated at 75.000 tons of cheese in Türkiye (Anonymous, 2019).

There are many factors (such as type of milk, using pasteurized or unpasteurized milk, ripening period and temperatures, type of starter cultures, microbiological and chemical quality of raw milk, etc.) affecting the taste and aroma of Tulum cheese. One of these factors is packaging material (Ucuncu M, 2011). Tulum cheese is traditionally ripened in a goat's or sheep's skin bag ("Tulum" means the bag made of goat's or sheep's skins in Turkish). Because the Tulum bag made from goat or sheep skin may contain animal hair, blood, or fat remnants, it is not preferred by some consumers (Tekinsen and Tekinsen, 2005). Because of that, in recent years, plastic containers (polyethylene jars), which are cheap and easily obtained, have been widely used for the ripening of Tulum cheese. (Tekinsen and Akar, 2017).

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Rapid deterioration and undesirable defects are encountered in traditional cheeses due to their shelf life, sensory properties, and improper packaging techniques. In this case, it leads to food waste (Ghisoni et al., 2022). With the latest legal regulations in the European Union, the ecological activities of food producers have increased. The European Union has introduced new regulations (Directive (EU) 2019/904) to diminish the negative impacts of plastics on the environment. In this way, the recycling rate in the EU has increased (European Union, 2019; Zulawska et al., 2023).

In addition to being a prophylactic mantle against peripheral factors that cause food spoilage, the packaging process increases the quality and safety of the food and extends its shelf life (Rozman et al., 2023). Packaging material is of great importance in affecting the buying preferences of consumers. Modified atmosphere packaging (MAP) is one of these packaging types. In many studies, the MAP technique, which includes different percentages, has been used in cheese (Garabal et al., 2010). The MAP aims to inhibit or decelerate the growth of spoilage microorganisms by modification of the package's internal atmosphere (Spreafico and Russo, 2021; Valle et al., 2023). The MAP at low temperatures is the most used method to increase the shelf life of many products (Ščetar et al., 2019). In the MAP method, after the product is put into a high-barrier gas package, the air is voided, and the package is hermetically sealed by giving a suitable gas mixture ( $\text{CO}_2$ ,  $\text{N}_2$ , and/or  $\text{O}_2$ ). The most commonly used gases in forming the atmospheric composition are  $\text{O}_2$ ,  $\text{CO}_2$ , and  $\text{N}_2$  (Mancuso et al., 2014).

Traditional MAP methods used for dairy products generally produce high concentrations of  $\text{CO}_2$  or  $\text{N}_2$ , between 20% and 100%, and low concentrations of  $\text{O}_2$ , usually below 5% (Ghisoni et al., 2022). Many researchers have noted that the MAP is very influential in extending the shelf life of diverse cheese types (Khoshgozoran et al., 2012; Mastromatteo et al., 2014; Jalilzadeh et al., 2015), and increasing  $\text{CO}_2$  above 20% can result in inhibition or slowing down the growth of many spoilage bacteria (Scott and Smith, 1971; Piergiovanni et al., 1993; Romani et al., 1999; Olarte et al., 2001; Romani et al., 2002; Juric et al., 2003; Erkan et al., 2007; Papaioannou et al., 2007; Garabal et al., 2010; Costa et al., 2017; Semjon et al., 2018; Cabral et al., 2019; Ščetar et al., 2019).

As far as we know and research in the literature, there is no knowledge in the literature on the quality parameters of Tulum cheese stored under MAP. Therefore, the aim of this study was to appraise the impacts of MAP on shelf-life extension, microbiological, physicochemical, and sensory properties of Tulum cheese.

## MATERIALS AND METHODS

### Preparation of cheese samples

Unpasteurized cow's milk (3 times, each time approximately 200 L) was obtained from the Agriculture and Livestock Research Center of Firat University. About 0.5 L of milk was separated for microbiological analysis of the unpasteurized milk. The remaining milk was pasteurized at 71°C for 15-20 sec, and experimental Tulum cheese was produced by adding starter cultures of *Lactococcus lactis subsp. lactis*+*Lactococcus lactis subsp. cremoris* (CM 10 Series, MYStarter Culture, Türkiye). After Tulum cheese was made, these samples (each one is approx. 250 g, 190x144x43 mm) were packaged in a commercially available bag of polyethylene terephthalate/polyethylene/ethylene-vinyl alcohol/polypropylene/antifog (PET/PE/EVOH/PP/AF) with an  $\text{O}_2$  permeability of 1.5cm<sup>3</sup>/m<sup>2</sup> day atm, measured at 23°C and 75% relative humidity (Südpack Amb. Tic., Basaksehir, Istanbul, Türkiye) in MAP machine (Multivac T 200, Australia). Four different gas mixtures (MAP) were used (B=100%  $\text{CO}_2$ , C=100%  $\text{N}_2$ , D=70%  $\text{N}_2$ +30%  $\text{CO}_2$ , E=75%  $\text{N}_2$ +25%  $\text{CO}_2$ ). The control samples were packaged in the air (A) (Fig. 1). Cheese samples stored at 4±1°C was analyzed for microbiological, chemical, and sensory on days 0, 30, 60, 90, 120, 150, 180, 210, and 240. The study was conducted in three independent replicates. The gas percentage used in the packaging of cheese samples was based on the doses given by the researchers conducted on semi-hard cheeses packaged with MAP.

### Microbiological analysis

Decimal dilutions of experimental cheese samples were made for microbiological cultivation and incubated Plate Count agar (Merck, Darmstadt/Germany) for 48 hrs at 35°C for enumeration of TMAB (Maturin and Peeler, 2001); De Man Rogosa Sharpe agar (Biokar, France) for 72 hrs at 30°C for enumeration of *LLP* (APHA, 1995); M17 Agar (Liofilchem, Italy) for 72 hrs at 30°C for enumeration of *Lactic streptococcus* (Halkman, 2005); Violet Red Bile Agar (Sharlav, Spain) for 24 hrs at 30°C for enumeration of



Fig 1. Cheese samples packaged in MAP.

coliforms (Halkman, 2005); Violet Red Bile Glucose Agar (Biokar, France) for 24 hrs at 37°C for enumeration of *Enterobacteriaceae* counts (ISO 21528-2:2004(en)); Tributyrin Agar (Liofilchem, Italy) for 48 hrs at 30°C for enumeration of lipolytic microorganisms (Halkman, 2005); Calcium Caseinat Agar (Conda Pronadisa, Spain) for 48 hrs at 30°C for enumeration of proteolytic microorganisms (Halkman, 2005); DRBC Agar (LAB, Lancashire, UK) for 5 d at 25°C for census of yeast and mold counts (ICMSF, 1982); Tryptone Bile X-Glucuronide Medium (Merck, Darmstadt, Germany) for 4 hrs at 30°C and then at for 18 hrs 44°C for enumeration of *E. coli* (ISO 16649-2:2001(en)). Baird Parker Agar (Biokar, France) supplemented with Egg Yolk Tellurite Emulsion (Himedia, India) for 30 hrs at 36°C for enumeration of coagulase (+) *Staphylococcus aureus* (ISO 6888-1:1999(en); Lancette and Bennett, 2001), and Anaerobic Agar (Conda Pronadisa, Spain) for 18-48 hrs at 35°C for sulfate-reducing anaerobic bacteria (Baron et al., 1994).

### Physicochemical analysis

The pH values the samples were determined by a pH meter (P Selecta- pH 2001) (Case et al., 1985). Titration method for the determination of the acidity (% l.a) (Demirci and Gunduz, 1994), the gravimetric method for the determination of the dry matter content (AOAC, 1984), Mohr method for the determination of salt amounts (Demirci and Gunduz, 1994), TSE recommended method for determination of ash content (Anonymous, 2016), Gerber Method (Anonymous, 2016) for oil determination was used. In addition,  $a_w$  values were measured at  $a_w$  meters (Testo - 650) (Lang and Sternberg, 1998). Protein analyses were performed by the Kjeldahl method (LECO FP 528, USA), and the protein values were calculated by multiplying the % N<sub>2</sub> concentration by a factor of 6.40. (ISO, 14891:2002(en)). Determination of SC-N, degree of ripening (Kuchroo and Fox, 1982), 12% TCA-N ratio (Polchroiadou et al., 1999), and 5% PTA-N ratio (Jarret et al., 1982) were used as standard micro-Kjeldahl method (IDF, 1993).

### Sensory analysis

Sensory analysis were performed by at least 5 panelists. "Sensory Evaluation Form of Modified Atmosphere Packaged Tulum Cheese Samples" was used for the evaluation (Uysal and Kavas, 2004). According to this form, the results were evaluated on a total of 100 points for packaging (8), appearance (28), structure (12), smell (20), and taste (32). Sensory analysis were performed on days 30, 60, 90, 120, 150, 180, 210, and 240, except on day 0 of the storage period.

### Statistical analysis

In the statistical evaluation of the study, the significance of the intra-group and inter-group differences was analyzed

with the SPSS22 (IBM SPSS, IBM Corporation, USA) (Version 22) package program. According to the results of the normality analysis, it was determined that the data met the nonparametric test assumptions. In the comparison of microbiological, chemical, and sensory parameters for the MAP groups, which were repeated 3 times, and on days 0, 30, 60, 90, 120, 150, 180, 210, 240. Kruskal-Wallis H (K Independent Samples) and Mann-Whitney U (2 Independent Samples) tests were used for evaluation in terms of days 240 and 240. Data are presented as mean  $\pm$  standard error. The statistical significance is considered when  $P \leq 0.05$  (Ozdamar, 2009).

## RESULTS AND DISCUSSION

Samples of Tulum cheese packed in modified gas conditions were not analyzed because of sensory deterioration of the products in the A and C groups after on day 90, the B group after on day 120, the D group after on day 150, and the E group after on day 240. Because of the formation of expansion (swelling) in packages of the A (control) and C (100% N<sub>2</sub>) groups after day 90, those groups were not analyzed on day 120. As for the B group, the formation of collapse in packages was observed after day 120, and this group of cheese was not analyzed on day 150.

Microbiological, chemical, and sensory analysis results are shown in Table 1, Table 2, and Table 3, respectively.

### Microbiological results

The TMAB counts gradually increased during the storage in all the groups, and statistically significant differences were sighted between storage days in MAP groups ( $p < 0.05$ ). The findings showing the increase in TMAB counts in the present study are compatible with the results of the previous studies reporting the increase in TMAB counts in various types of cheese packaged (Olarde et al., 2002; Juric et al., 2003; Papapioannou et al., 2007; Stephanie et al., 2018). However, it should be emphasized that there are other studies reporting that MAP has no significant effect on TMAB numbers in Lor whey cheese and Stracciatella cheese (30/65/5: CO<sub>2</sub>/N<sub>2</sub>/O<sub>2</sub>) (Erkan et al., 2007). These discrepancies between the studies may be due to the cheese type, starter cultures, and gas mixtures used in the studies. There are some researchers noted that using a high amount of N<sub>2</sub> in the MAP gives better results in preserving hard and semi-hard cheeses (Juric et al., 2003). Tulum cheese is categorized as hard or semi-hard cheese. The outcomes acquired in this study agree with the information given in the literature and displayed that 70 N<sub>2</sub> - 30 CO<sub>2</sub> and 75 N<sub>2</sub> - 25 CO<sub>2</sub> mixtures have a slowing down effect on TMAB in Tulum cheese, and 75 N<sub>2</sub> - 25 CO<sub>2</sub> mixture was better.

Table 1: The microbiological analysis results of Tulum cheese samples ( $\log_{10}$  CFU/g  $\pm$  standard error)

Microorganisms	Groups	Storage Days											
		0	30	60	90	120	150	180	210	240			
Total Mesophilic Aerobic Bacteria	A	7.16 $\pm$ 0.71	7.67 $\pm$ 0.53	8.32 $\pm$ 0.28 <sup>WY</sup>	8.60 $\pm$ 0.34 <sup>WY</sup>	NA	NA	NA	NA	NA	NA	NA	NA
	B	7.27 $\pm$ 0.10 <sup>c</sup>	7.87 $\pm$ 0.12 <sup>b</sup>	8.18 $\pm$ 0.09 <sup>Wb</sup>	8.65 $\pm$ 0.13 <sup>Wa</sup>	9.00 $\pm$ 0.18 <sup>Wa</sup>	NA	NA	NA	NA	NA	NA	NA
	C	7.31 $\pm$ 0.16 <sup>b</sup>	7.55 $\pm$ 0.07 <sup>b</sup>	7.70 $\pm$ 0.10 <sup>xb</sup>	7.91 $\pm$ 0.05 <sup>xa</sup>	NA	NA	NA	NA	NA	NA	NA	NA
	D	7.47 $\pm$ 0.09 <sup>d</sup>	7.73 $\pm$ 0.08 <sup>c</sup>	7.92 $\pm$ 0.04 <sup>yc</sup>	8.09 $\pm$ 0.02 <sup>yb</sup>	8.34 $\pm$ 0.05 <sup>xa</sup>	8.44 $\pm$ 0.05 <sup>Wa</sup>	8.17 $\pm$ 0.18 <sup>ab</sup>	8.42 $\pm$ 0.16 <sup>a</sup>	8.52 $\pm$ 0.14 <sup>a</sup>	8.52 $\pm$ 0.14 <sup>a</sup>	8.52 $\pm$ 0.14 <sup>a</sup>	8.52 $\pm$ 0.14 <sup>a</sup>
	E	6.62 $\pm$ 0.10 <sup>g</sup>	6.85 $\pm$ 0.05 <sup>fg</sup>	6.98 $\pm$ 0.06 <sup>zef</sup>	7.22 $\pm$ 0.13 <sup>zde</sup>	7.47 $\pm$ 0.14 <sup>ycd</sup>	7.79 $\pm$ 0.17 <sup>xbc</sup>	8.17 $\pm$ 0.18 <sup>ab</sup>	8.42 $\pm$ 0.16 <sup>a</sup>	8.52 $\pm$ 0.14 <sup>a</sup>	8.52 $\pm$ 0.14 <sup>a</sup>	8.52 $\pm$ 0.14 <sup>a</sup>	8.52 $\pm$ 0.14 <sup>a</sup>
<i>Lactobacillus</i> - <i>Leuconostoc</i> - <i>Pediococcus</i>	A	6.92 $\pm$ 0.50	7.26 $\pm$ 0.55	7.55 $\pm$ 0.43 <sup>W</sup>	7.87 $\pm$ 0.35 <sup>W</sup>	NA	NA	NA	NA	NA	NA	NA	NA
	B	6.86 $\pm$ 0.21	7.05 $\pm$ 0.22	7.45 $\pm$ 0.29 <sup>W</sup>	7.73 $\pm$ 0.32 <sup>W</sup>	8.19 $\pm$ 0.22	NA	NA	NA	NA	NA	NA	NA
	C	6.40 $\pm$ 0.11 <sup>d</sup>	6.54 $\pm$ 0.09 <sup>cd</sup>	6.78 $\pm$ 0.03 <sup>xb</sup>	7.12 $\pm$ 0.06 <sup>xa</sup>	NA	NA	NA	NA	NA	NA	NA	NA
	D	6.78 $\pm$ 0.55	6.90 $\pm$ 0.52	7.01 $\pm$ 0.50 <sup>WX</sup>	7.18 $\pm$ 0.57 <sup>WX</sup>	7.52 $\pm$ 0.56	7.66 $\pm$ 0.52 <sup>W</sup>	7.66 $\pm$ 0.52 <sup>W</sup>	7.66 $\pm$ 0.52 <sup>W</sup>	7.66 $\pm$ 0.52 <sup>W</sup>	7.66 $\pm$ 0.52 <sup>W</sup>	7.66 $\pm$ 0.52 <sup>W</sup>	7.66 $\pm$ 0.52 <sup>W</sup>
	E	5.19 $\pm$ 0.09 <sup>e</sup>	5.36 $\pm$ 0.03 <sup>e</sup>	5.55 $\pm$ 0.04 <sup>yd</sup>	5.75 $\pm$ 0.04 <sup>yc</sup>	5.81 $\pm$ 0.05 <sup>c</sup>	6.30 $\pm$ 0.07 <sup>xb</sup>	6.52 $\pm$ 0.06 <sup>b</sup>	6.82 $\pm$ 0.04 <sup>a</sup>	6.97 $\pm$ 0.04 <sup>a</sup>	6.97 $\pm$ 0.04 <sup>a</sup>	6.97 $\pm$ 0.04 <sup>a</sup>	6.97 $\pm$ 0.04 <sup>a</sup>
<i>Lactic</i> <i>Streptococcus</i>	A	7.22 $\pm$ 0.12 <sup>W</sup>	7.91 $\pm$ 0.27 <sup>W</sup>	8.08 $\pm$ 0.28 <sup>W</sup>	8.25 $\pm$ 0.35 <sup>W</sup>	NA	NA	NA	NA	NA	NA	NA	NA
	B	6.79 $\pm$ 0.16 <sup>Wxc</sup>	7.15 $\pm$ 0.18 <sup>xc</sup>	7.37 $\pm$ 0.20 <sup>xbc</sup>	7.77 $\pm$ 0.15 <sup>WVab</sup>	8.07 $\pm$ 0.13 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA
	C	6.35 $\pm$ 0.15 <sup>xb</sup>	6.54 $\pm$ 0.12 <sup>yb</sup>	6.73 $\pm$ 0.09 <sup>yab</sup>	6.95 $\pm$ 0.03 <sup>xa</sup>	NA	NA	NA	NA	NA	NA	NA	NA
	D	6.68 $\pm$ 0.37 <sup>WX</sup>	6.80 $\pm$ 0.34 <sup>XY</sup>	7.02 $\pm$ 0.38 <sup>XY</sup>	7.20 $\pm$ 0.38 <sup>XY</sup>	7.50 $\pm$ 0.38	7.65 $\pm$ 0.36 <sup>W</sup>	7.65 $\pm$ 0.36 <sup>W</sup>	7.65 $\pm$ 0.36 <sup>W</sup>	7.65 $\pm$ 0.36 <sup>W</sup>	7.65 $\pm$ 0.36 <sup>W</sup>	7.65 $\pm$ 0.36 <sup>W</sup>	7.65 $\pm$ 0.36 <sup>W</sup>
	E	5.18 $\pm$ 0.06 <sup>Ye</sup>	5.39 $\pm$ 0.11 <sup>zde</sup>	5.53 $\pm$ 0.12 <sup>zd</sup>	5.76 $\pm$ 0.14 <sup>zcd</sup>	5.90 $\pm$ 0.10 <sup>bc</sup>	6.17 $\pm$ 0.16 <sup>xbc</sup>	6.47 $\pm$ 0.24 <sup>ab</sup>	6.76 $\pm$ 0.12 <sup>a</sup>	6.87 $\pm$ 0.09 <sup>a</sup>	6.87 $\pm$ 0.09 <sup>a</sup>	6.87 $\pm$ 0.09 <sup>a</sup>	6.87 $\pm$ 0.09 <sup>a</sup>
Coliform	A	2.50 $\pm$ 0.42	2.15 $\pm$ 0.42	1.69 $\pm$ 0.21	1.12 $\pm$ 0.05	NA	NA	NA	NA	NA	NA	NA	NA
	B	2.04 $\pm$ 0.21 <sup>a</sup>	1.68 $\pm$ 0.19 <sup>ab</sup>	1.36 $\pm$ 0.18 <sup>b</sup>	1.20 $\pm$ 0.12 <sup>b</sup>	<1.0	NA	NA	NA	NA	NA	NA	NA
	C	1.54 $\pm$ 0.05 <sup>a</sup>	1.47 $\pm$ 0.08 <sup>ab</sup>	1.30 $\pm$ 0.06 <sup>b</sup>	1.00 $\pm$ 0.00 <sup>c</sup>	NA	NA	NA	NA	NA	NA	NA	NA
	D	2.23 $\pm$ 0.13 <sup>a</sup>	2.05 $\pm$ 0.07 <sup>ab</sup>	1.60 $\pm$ 0.31 <sup>bc</sup>	1.00 $\pm$ 0.00 <sup>c</sup>	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
	E	1.97 $\pm$ 0.12 <sup>a</sup>	1.73 $\pm$ 0.12 <sup>a</sup>	1.00 $\pm$ 0.00 <sup>b</sup>	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
<i>Enterobacteriaceae</i>	A	3.08 $\pm$ 0.24 <sup>Wa</sup>	2.13 $\pm$ 0.25 <sup>ab</sup>	1.63 $\pm$ 0.32 <sup>bc</sup>	1.00 $\pm$ 0.00 <sup>c</sup>	NA	NA	NA	NA	NA	NA	NA	NA
	B	2.53 $\pm$ 0.27 <sup>WX</sup>	2.14 $\pm$ 0.47	1.97 $\pm$ 0.49	1.40 $\pm$ 0.23	<1.0	NA	NA	NA	NA	NA	NA	NA
	C	1.73 $\pm$ 0.15 <sup>XY</sup>	1.53 $\pm$ 0.14	1.27 $\pm$ 0.18	1.00 $\pm$ 0.00	NA	NA	NA	NA	NA	NA	NA	NA
	D	1.86 $\pm$ 0.07 <sup>Ya</sup>	1.77 $\pm$ 0.06 <sup>a</sup>	1.59 $\pm$ 0.07 <sup>ab</sup>	1.33 $\pm$ 0.16 <sup>b</sup>	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
	E	1.75 $\pm$ 0.06 <sup>Ya</sup>	1.35 $\pm$ 0.07 <sup>b</sup>	1.00 $\pm$ 0.00 <sup>c</sup>	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Lipolytic Bacteria	A	3.28 $\pm$ 1.02	4.18 $\pm$ 0.78	4.56 $\pm$ 0.68	5.02 $\pm$ 0.63	NA	NA	NA	NA	NA	NA	NA	NA
	B	4.33 $\pm$ 0.33 <sup>c</sup>	4.77 $\pm$ 0.28 <sup>bc</sup>	5.17 $\pm$ 0.12 <sup>bc</sup>	5.94 $\pm$ 0.45 <sup>ab</sup>	6.55 $\pm$ 0.29 <sup>Wa</sup>	NA	NA	NA	NA	NA	NA	NA
	C	4.82 $\pm$ 0.06 <sup>c</sup>	4.97 $\pm$ 0.11 <sup>bc</sup>	5.24 $\pm$ 0.12 <sup>ab</sup>	5.47 $\pm$ 0.15 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA
	D	4.51 $\pm$ 0.14 <sup>c</sup>	4.66 $\pm$ 0.14 <sup>bc</sup>	4.86 $\pm$ 0.17 <sup>bc</sup>	5.00 $\pm$ 0.12 <sup>ab</sup>	5.13 $\pm$ 0.12 <sup>xa</sup>	5.23 $\pm$ 0.12 <sup>Wa</sup>	4.53 $\pm$ 0.07 <sup>b</sup>	4.94 $\pm$ 0.06 <sup>a</sup>	5.07 $\pm$ 0.08 <sup>a</sup>	5.07 $\pm$ 0.08 <sup>a</sup>	5.07 $\pm$ 0.08 <sup>a</sup>	
	E	3.39 $\pm$ 0.05 <sup>g</sup>	3.66 $\pm$ 0.02 <sup>f</sup>	3.86 $\pm$ 0.03 <sup>e</sup>	4.09 $\pm$ 0.05 <sup>d</sup>	4.27 $\pm$ 0.06 <sup>ycd</sup>	4.44 $\pm$ 0.07 <sup>xbc</sup>	4.53 $\pm$ 0.07 <sup>b</sup>	4.94 $\pm$ 0.06 <sup>a</sup>	5.07 $\pm$ 0.08 <sup>a</sup>	5.07 $\pm$ 0.08 <sup>a</sup>	5.07 $\pm$ 0.08 <sup>a</sup>	
Proteolytic Microorganisms	A	3.70 $\pm$ 0.92 <sup>b</sup>	4.44 $\pm$ 0.64 <sup>b</sup>	5.90 $\pm$ 0.11 <sup>Wa</sup>	6.13 $\pm$ 0.12 <sup>Wa</sup>	NA	NA	NA	NA	NA	NA	NA	NA
	B	4.74 $\pm$ 0.39 <sup>c</sup>	5.18 $\pm$ 0.34 <sup>bc</sup>	5.54 $\pm$ 0.19 <sup>Wbc</sup>	6.10 $\pm$ 0.23 <sup>Web</sup>	6.79 $\pm$ 0.33 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA
	C	4.18 $\pm$ 0.09 <sup>c</sup>	4.49 $\pm$ 0.10 <sup>b</sup>	4.72 $\pm$ 0.08 <sup>xb</sup>	4.97 $\pm$ 0.03 <sup>xa</sup>	NA	NA	NA	NA	NA	NA	NA	NA
	D	4.20 $\pm$ 0.06 <sup>e</sup>	4.42 $\pm$ 0.08 <sup>de</sup>	4.52 $\pm$ 0.10 <sup>xcd</sup>	4.71 $\pm$ 0.06 <sup>ycb</sup>	4.85 $\pm$ 0.07 <sup>ab</sup>	4.98 $\pm$ 0.04 <sup>a</sup>	4.98 $\pm$ 0.04 <sup>a</sup>	4.98 $\pm$ 0.04 <sup>a</sup>	4.98 $\pm$ 0.04 <sup>a</sup>	4.98 $\pm$ 0.04 <sup>a</sup>	4.98 $\pm$ 0.04 <sup>a</sup>	
	E	4.04 $\pm$ 0.04 <sup>f</sup>	4.10 $\pm$ 0.06 <sup>f</sup>	4.28 $\pm$ 0.04 <sup>Ye</sup>	4.37 $\pm$ 0.03 <sup>Ze</sup>	4.73 $\pm$ 0.06 <sup>d</sup>	4.90 $\pm$ 0.03 <sup>c</sup>	5.13 $\pm$ 0.05 <sup>b</sup>	5.34 $\pm$ 0.08 <sup>a</sup>	5.40 $\pm$ 0.07 <sup>a</sup>	5.40 $\pm$ 0.07 <sup>a</sup>	5.40 $\pm$ 0.07 <sup>a</sup>	

Table 1: (Continued)

Microorganisms	Groups	Storage Days											
		0	30	60	90	120	150	180	210	240			
Yeast-Mold	A	3.98±0.02 <sup>Wc</sup>	4.10±0.06 <sup>Wc</sup>	4.70±0.11 <sup>Vb</sup>	5.11±0.06 <sup>Wa</sup>	NA	NA	NA	NA	NA	NA	NA	NA
	B	3.73±0.13 <sup>WKe</sup>	4.00±0.00 <sup>WXd</sup>	4.17±0.05 <sup>Wc</sup>	4.30±0.01 <sup>Xb</sup>	4.63±0.06 <sup>Wa</sup>	NA	NA	NA	NA	NA	NA	NA
	C	3.76±0.09 <sup>Kc</sup>	3.82±0.07 <sup>Xbc</sup>	3.98±0.02 <sup>Xb</sup>	4.14±0.10 <sup>Xa</sup>	NA	NA	NA	NA	NA	NA	NA	NA
	D	3.06±0.03 <sup>Vd</sup>	3.15±0.03 <sup>Vcd</sup>	3.22±0.05 <sup>Vc</sup>	3.50±0.03 <sup>Vb</sup>	3.62±0.06 <sup>Xb</sup>	3.83±0.04 <sup>Wa</sup>	NA	NA	NA	NA	NA	NA
	E	2.03±0.00 <sup>Zh</sup>	2.10±0.01 <sup>Zg</sup>	2.12±0.01 <sup>Zg</sup>	2.18±0.01 <sup>Zi</sup>	2.27±0.01 <sup>Ye</sup>	2.41±0.01 <sup>Xd</sup>	2.52±0.01 <sup>c</sup>	2.69±0.01 <sup>b</sup>	2.74±0.01 <sup>a</sup>	NA	NA	NA
<i>Staphylococcus aureus</i>	A	1.41±0.04 <sup>a</sup>	1.14±0.09 <sup>b</sup>	1.00±0.00 <sup>b</sup>	< 1.0	NA	NA	NA	NA	NA	NA	NA	NA
	B	1.39±0.13 <sup>a</sup>	1.00±0.00 <sup>b</sup>	1.00±0.00 <sup>b</sup>	< 1.0	< 1.0	NA	NA	NA	NA	NA	NA	NA
	C	1.70±0.09 <sup>a</sup>	1.00±0.00 <sup>b</sup>	< 1.0	< 1.0	NA	NA	NA	NA	NA	NA	NA	NA
	D	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	NA	NA	NA	NA	NA	NA
	E	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0

a-h: The mean values with different letters in the same line are significantly different (P < 0.05); VWXYZ: The mean values with different letters in the same column are significantly different (P < 0.05); NA: No analysis performed; < 1.0: Below the detectable level; A: Control group (normal atmosphere / dry air); B: % 100 CO<sub>2</sub>; C: % 100 N<sub>2</sub>; D: % 70 N<sub>2</sub> + % 30 CO<sub>2</sub>; E: % 75 N<sub>2</sub> + % 25 CO<sub>2</sub>.

Lactic acid bacteria also contribute to the biological preservation of foods by secreting antimicrobial substances (acetic acid, bacteriocin, and lactic acid) (Quttara et al., 2023). It was seen that CO<sub>2</sub> gas alone (the B group) and the D group had no effect on *LLP* when compared to the control. The reason for this result may be because *LLP* are facultative anaerobe microorganisms, and they are not affected by a high amount of CO<sub>2</sub>. No statistical difference was detected between the groups on day 0. (P>0.05). Some researchers reported findings analogous to the outcomes of this study (Papaioannou et al., 2007). It was determined that the *LLP* numbers in group C were lower than those in the control and B groups (P<0.05), but it was similar to group D (P>0.05). However, *LLP* counts were suppressed when the amount of CO<sub>2</sub> decreased, and the N<sub>2</sub> amount increased, as was seen in the E group.

Since lactic acid is a bacterial group (microaerophilic) it is not affected by the inhibition of CO<sub>2</sub> and N<sub>2</sub> gases used. Their numbers increased continuously in all the groups during the storage (Table 1). These increases during storage are in agreement with the reports of some researchers (Irkin, 2011; Pala et al., 2016). Similar to the findings observed in the numbers of *LLP*, the E group has a slowing down effect on the growth of *lactic streptococcus* in Tulum cheese.

Coliform bacteria showed a continuous decrease in all the groups during the storage (Table 1). They were below the detection limit (<1.0 log<sub>10</sub> CFU/g) in the B and D groups after day 90 and in the E group after day 60. The decrease of coliform bacteria during the storage period is similar to the findings of many researchers who noted that coliform bacteria count decreased in the cheese unpasteurized technique (Conte et al., 2011).

*Enterobacteriaceae* showed a continuous decrease in all the groups during the storage (Table 1). The C, D, and E groups had lower numbers of *Enterobacteriaceae* than the B and control groups (P<0.05). They were below the detection limit (<1.0 log<sub>10</sub> CFU/g) in the group E after day 60. The continuous decrease of this group of microorganisms during the storage period is compatible with the outcomes acquired in the studies using the MAP technique on different varieties of cheeses (Pintado and Malcata 2000; Caridi 2003; Tsiraki and Savvaidis, 2013).

The number of lipolytic microorganisms in the E group was 5.07 log<sub>10</sub> CFU/g, while the number of proteolytic microorganisms in the E group was 5.40 log<sub>10</sub> CFU/g at the end of the storage (on day 240) (Table 1).

In present study, yeast and mold counts increased slowly in all groups during the storage (P<0.05). This finding is

Table 2: The chemical analysis results of Tulum cheese samples (Arithmetic mean  $\pm$  Standard error)

Values	Groups	Storage Days												
		0	30	60	90	120	150	180	210	240				
pH	A	5.48 $\pm$ 0.29	5.44 $\pm$ 0.31	5.35 $\pm$ 0.36	5.26 $\pm$ 0.39	NA	NA	NA	NA	NA	NA	NA	NA	NA
	B	5.47 $\pm$ 0.31	5.44 $\pm$ 0.32	5.38 $\pm$ 0.31	5.31 $\pm$ 0.31	5.20 $\pm$ 0.35	NA	NA	NA	NA	NA	NA	NA	NA
	C	5.38 $\pm$ 0.30	5.34 $\pm$ 0.31	5.30 $\pm$ 0.30	5.23 $\pm$ 0.32	NA	NA	NA	NA	NA	NA	NA	NA	NA
	D	5.47 $\pm$ 0.32	5.41 $\pm$ 0.30	5.39 $\pm$ 0.29	5.33 $\pm$ 0.31	5.29 $\pm$ 0.30	5.13 $\pm$ 0.26	NA	NA	NA	NA	NA	NA	NA
	E	5.59 $\pm$ 0.28	5.58 $\pm$ 0.27	5.53 $\pm$ 0.27	5.48 $\pm$ 0.28	5.46 $\pm$ 0.28	5.40 $\pm$ 0.27	5.36 $\pm$ 0.27	5.33 $\pm$ 0.26	5.33 $\pm$ 0.26	5.33 $\pm$ 0.26	5.28 $\pm$ 0.26	NA	NA
Acidity (% lactic acid)	A	0.70 $\pm$ 0.11	0.75 $\pm$ 0.11	0.78 $\pm$ 0.10	0.83 $\pm$ 0.11	NA	NA	NA	NA	NA	NA	NA	NA	NA
	B	0.63 $\pm$ 0.24	0.73 $\pm$ 0.19	0.83 $\pm$ 0.19	0.98 $\pm$ 0.25	1.10 $\pm$ 0.27	NA	NA	NA	NA	NA	NA	NA	NA
	C	0.71 $\pm$ 0.26	0.83 $\pm$ 0.27	0.88 $\pm$ 0.28	0.94 $\pm$ 0.29	NA	NA	NA	NA	NA	NA	NA	NA	NA
	D	0.70 $\pm$ 0.16	0.81 $\pm$ 0.16	0.90 $\pm$ 0.18	0.98 $\pm$ 0.19	1.08 $\pm$ 0.15	1.17 $\pm$ 0.15	NA	NA	NA	NA	NA	NA	NA
	E	0.62 $\pm$ 0.21	0.68 $\pm$ 0.21	0.79 $\pm$ 0.26	0.83 $\pm$ 0.26	0.86 $\pm$ 0.24	0.95 $\pm$ 0.28	0.99 $\pm$ 0.28	1.11 $\pm$ 0.33	1.23 $\pm$ 0.37	NA	NA	NA	NA
Dry matter (%)	A	62.24 $\pm$ 0.80	64.70 $\pm$ 0.96	65.31 $\pm$ 1.23	66.94 $\pm$ 0.86	NA	NA	NA	NA	NA	NA	NA	NA	NA
	B	54.04 $\pm$ 0.51 <sup>c</sup>	56.06 $\pm$ 1.02 <sup>bc</sup>	59.52 $\pm$ 1.78 <sup>ab</sup>	62.14 $\pm$ 1.19 <sup>a</sup>	65.07 $\pm$ 1.80 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA
	C	54.76 $\pm$ 0.76	57.58 $\pm$ 2.27	59.62 $\pm$ 2.28	61.54 $\pm$ 1.76	NA	NA	NA	NA	NA	NA	NA	NA	NA
	D	54.65 $\pm$ 1.19 <sup>d</sup>	57.35 $\pm$ 1.31 <sup>cd</sup>	59.59 $\pm$ 1.35 <sup>c</sup>	64.92 $\pm$ 1.22 <sup>b</sup>	67.30 $\pm$ 0.63 <sup>b</sup>	69.32 $\pm$ 0.48 <sup>w</sup>	NA	NA	NA	NA	NA	NA	NA
	E	53.35 $\pm$ 0.86	56.92 $\pm$ 3.07	57.70 $\pm$ 2.74	57.83 $\pm$ 2.71	58.07 $\pm$ 2.57	58.24 $\pm$ 2.63 <sup>x</sup>	58.35 $\pm$ 2.59	58.60 $\pm$ 2.64	59.78 $\pm$ 2.63	NA	NA	NA	NA
Salt (%)	A	3.90 $\pm$ 0.33	4.00 $\pm$ 0.31	4.16 $\pm$ 0.42	4.22 $\pm$ 0.41	NA	NA	NA	NA	NA	NA	NA	NA	NA
	B	3.86 $\pm$ 0.29	4.21 $\pm$ 0.18	4.36 $\pm$ 0.12	4.45 $\pm$ 0.15	4.82 $\pm$ 0.21	NA	NA	NA	NA	NA	NA	NA	NA
	C	3.84 $\pm$ 0.51	4.31 $\pm$ 0.31	4.43 $\pm$ 0.20	5.13 $\pm$ 0.28	NA	NA	NA	NA	NA	NA	NA	NA	NA
	D	3.97 $\pm$ 0.24	4.04 $\pm$ 0.24	4.25 $\pm$ 0.31	4.35 $\pm$ 0.31	4.56 $\pm$ 0.28	4.97 $\pm$ 0.38	NA	NA	NA	NA	NA	NA	NA
	E	3.61 $\pm$ 0.21 <sup>d</sup>	3.82 $\pm$ 0.04 <sup>cd</sup>	3.93 $\pm$ 0.03 <sup>bc</sup>	3.97 $\pm$ 0.03 <sup>b</sup>	4.20 $\pm$ 0.20 <sup>ab</sup>	4.39 $\pm$ 0.25 <sup>a</sup>	4.46 $\pm$ 0.23 <sup>a</sup>	4.62 $\pm$ 0.25 <sup>a</sup>	4.91 $\pm$ 0.38 <sup>a</sup>	NA	NA	NA	NA
Ash (%)	A	5.40 $\pm$ 0.22	5.42 $\pm$ 0.22	5.52 $\pm$ 0.19	5.69 $\pm$ 0.14	NA	NA	NA	NA	NA	NA	NA	NA	NA
	B	5.16 $\pm$ 0.46	5.38 $\pm$ 0.29	5.52 $\pm$ 0.27	5.67 $\pm$ 0.23	6.01 $\pm$ 0.10	NA	NA	NA	NA	NA	NA	NA	NA
	C	5.27 $\pm$ 0.34	5.45 $\pm$ 0.27	5.49 $\pm$ 0.26	5.58 $\pm$ 0.30	NA	NA	NA	NA	NA	NA	NA	NA	NA
	D	5.34 $\pm$ 0.27	5.36 $\pm$ 0.26	5.43 $\pm$ 0.25	5.53 $\pm$ 0.21	5.65 $\pm$ 0.26	5.78 $\pm$ 0.29	NA	NA	NA	NA	NA	NA	NA
	E	5.40 $\pm$ 0.21	5.45 $\pm$ 0.21	5.52 $\pm$ 0.25	5.57 $\pm$ 0.21	5.62 $\pm$ 0.21	5.67 $\pm$ 0.21	5.73 $\pm$ 0.22	6.17 $\pm$ 0.03	6.32 $\pm$ 0.07	NA	NA	NA	NA
Fat (%)	A	40.15 $\pm$ 2.59	39.70 $\pm$ 2.13	41.15 $\pm$ 2.59	41.37 $\pm$ 2.96	NA	NA	NA	NA	NA	NA	NA	NA	NA
	B	44.15 $\pm$ 3.37	45.49 $\pm$ 2.42	44.15 $\pm$ 1.85	42.63 $\pm$ 2.58	NA	NA	NA	NA	NA	NA	NA	NA	NA
	C	40.79 $\pm$ 3.28	43.00 $\pm$ 1.75	44.93 $\pm$ 1.71	48.32 $\pm$ 2.06	NA	NA	NA	NA	NA	NA	NA	NA	NA
	D	45.57 $\pm$ 3.70	43.98 $\pm$ 3.52	44.04 $\pm$ 3.33	40.77 $\pm$ 2.68	39.93 $\pm$ 2.81	39.21 $\pm$ 2.70	NA	NA	NA	NA	NA	NA	NA
	E	44.93 $\pm$ 2.18	44.17 $\pm$ 0.44	44.13 $\pm$ 0.72	44.60 $\pm$ 0.72	45.54 $\pm$ 1.06	45.82 $\pm$ 1.24	46.45 $\pm$ 1.27	46.77 $\pm$ 1.75	46.15 $\pm$ 1.58	NA	NA	NA	NA
Water activity (a <sub>w</sub> )	A	0.881 $\pm$ 0.01	0.889 $\pm$ 0.01	0.890 $\pm$ 0.02	0.893 $\pm$ 0.02	NA	NA	NA	NA	NA	NA	NA	NA	NA
	B	0.887 $\pm$ 0.02	0.913 $\pm$ 0.01	0.907 $\pm$ 0.02	0.897 $\pm$ 0.01	0.898 $\pm$ 0.01	NA	NA	NA	NA	NA	NA	NA	NA
	C	0.893 $\pm$ 0.02	0.900 $\pm$ 0.01	0.908 $\pm$ 0.01	0.918 $\pm$ 0.01	NA	NA	NA	NA	NA	NA	NA	NA	NA
	D	0.882 $\pm$ 0.01	0.884 $\pm$ 0.01	0.889 $\pm$ 0.01	0.891 $\pm$ 0.01	0.895 $\pm$ 0.01	0.904 $\pm$ 0.01	NA	NA	NA	NA	NA	NA	NA
	E	0.913 $\pm$ 0.00 <sup>a</sup>	0.908 $\pm$ 0.00 <sup>a</sup>	0.901 $\pm$ 0.00 <sup>ab</sup>	0.897 $\pm$ 0.00 <sup>b</sup>	0.893 $\pm$ 0.01 <sup>bc</sup>	0.890 $\pm$ 0.01 <sup>bc</sup>	0.887 $\pm$ 0.00 <sup>bc</sup>	0.885 $\pm$ 0.00 <sup>c</sup>	0.865 $\pm$ 0.01 <sup>d</sup>	NA	NA	NA	NA

(Contd...)

Table 2: (Continued)

Values	Storage Days											
	0	30	60	90	120	150	180	210	240			
Total protein and nitrogen (%)	A	27.83±2.43	28.07±2.41	28.12±2.37	28.16±2.46	NA	NA	NA	NA	NA	NA	NA
	B	26.73±2.40	27.76±2.45	28.27±2.47	28.40±2.77	28.65±2.54	NA	NA	NA	NA	NA	NA
	C	27.87±2.39	28.97±2.17	30.72±2.05	33.86±2.50	NA	NA	NA	NA	NA	NA	NA
	D	26.65±0.10 <sup>e</sup>	27.35±0.37 <sup>d</sup>	29.79±1.44 <sup>cd</sup>	32.49±1.67 <sup>bc</sup>	35.39±1.26 <sup>ab</sup>	37.89±0.90 <sup>Wa</sup>	NA	NA	NA	NA	NA
	E	24.15±1.22	25.99±0.86	26.62±1.05	27.04±1.22	28.03±2.11	28.23±2.12 <sup>X</sup>	28.52±2.00	29.75±1.66	31.90±2.07		
Water-soluble nitrogen (SC-N) (%)	A	0.24±0.01 <sup>c</sup>	0.32±0.02 <sup>Wb</sup>	0.36±0.01 <sup>Wab</sup>	0.39±0.01 <sup>a</sup>	0.40±0.01 <sup>a</sup>	NA	NA	NA	NA	NA	NA
	B	0.24±0.02 <sup>d</sup>	0.30±0.01 <sup>Wc</sup>	0.34±0.01 <sup>Wbc</sup>	0.37±0.01 <sup>ab</sup>	0.40±0.01 <sup>a</sup>	NA	NA	NA	NA	NA	NA
	C	0.23±0.01 <sup>c</sup>	0.26±0.01 <sup>WXbc</sup>	0.31±0.02 <sup>Wab</sup>	0.35±0.02 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA
	D	0.24±0.01 <sup>e</sup>	0.30±0.02 <sup>Wide</sup>	0.33±0.01 <sup>Wcd</sup>	0.37±0.01 <sup>bc</sup>	0.39±0.01 <sup>ab</sup>	0.41±0.01 <sup>Wa</sup>	NA	NA	NA	NA	NA
	E	0.22±0.00 <sup>g</sup>	0.23±0.01 <sup>Xig</sup>	0.25±0.01 <sup>Xef</sup>	0.27±0.01 <sup>de</sup>	0.29±0.00 <sup>d</sup>	0.31±0.00 <sup>Xc</sup>	0.33±0.00 <sup>b</sup>	0.35±0.00 <sup>sa</sup>	0.36±0.00 <sup>sa</sup>		
Degree of ripening in Water-soluble nitrogen (%)	A	5.47±0.38 <sup>b</sup>	7.26±0.58 <sup>a</sup>	8.36±0.53 <sup>a</sup>	8.94±0.61 <sup>Wa</sup>	9.02±0.67 <sup>Wa</sup>	NA	NA	NA	NA	NA	NA
	B	5.76±0.28 <sup>c</sup>	6.88±0.38 <sup>bc</sup>	7.68±0.54 <sup>ab</sup>	8.37±0.68 <sup>WXab</sup>	9.02±0.67 <sup>Wa</sup>	NA	NA	NA	NA	NA	NA
	C	5.41±0.48	5.85±0.45	6.43±0.72	6.61±0.67 <sup>XY</sup>	NA	NA	NA	NA	NA	NA	NA
	D	5.83±0.36	7.00±0.54	7.14±0.57	7.24±0.48 <sup>WX</sup>	7.35±0.34 <sup>X</sup>	7.91±0.20	7.36±0.00 <sup>c</sup>	7.50±0.00 <sup>b</sup>	7.70±0.01 <sup>a</sup>		
	E	5.81±0.01 <sup>i</sup>	5.85±0.00 <sup>h</sup>	5.99±0.00 <sup>g</sup>	6.38±0.01 <sup>Yf</sup>	6.60±0.00 <sup>Ye</sup>	7.01±0.01 <sup>d</sup>	7.36±0.00 <sup>c</sup>	7.50±0.00 <sup>b</sup>	7.70±0.01 <sup>a</sup>		
% 12'lik Trichloroacetic acid soluble nitrogen ratio (TCA-N) (%)	A	0.38±0.01 <sup>Wc</sup>	0.42±0.01 <sup>Wb</sup>	0.45±0.01 <sup>Wab</sup>	0.48±0.01 <sup>Wa</sup>	0.47±0.02 <sup>Wa</sup>	NA	NA	NA	NA	NA	NA
	B	0.37±0.01 <sup>WXc</sup>	0.39±0.01 <sup>WXbc</sup>	0.43±0.01 <sup>Wab</sup>	0.45±0.02 <sup>Wa</sup>	0.47±0.02 <sup>Wa</sup>	NA	NA	NA	NA	NA	NA
	C	0.32±0.01 <sup>XYb</sup>	0.36±0.02 <sup>Yb</sup>	0.43±0.01 <sup>Wa</sup>	0.45±0.02 <sup>Wa</sup>	NA	NA	NA	NA	NA	NA	NA
	D	0.32±0.01 <sup>Ye</sup>	0.35±0.01 <sup>Yde</sup>	0.37±0.01 <sup>Xcd</sup>	0.40±0.01 <sup>Xbc</sup>	0.42±0.01 <sup>Wab</sup>	0.45±0.01 <sup>Wa</sup>	NA	NA	NA	NA	NA
	E	0.20±0.00 <sup>Zg</sup>	0.21±0.01 <sup>Zg</sup>	0.23±0.00 <sup>Yf</sup>	0.25±0.00 <sup>Ye</sup>	0.27±0.01 <sup>Xde</sup>	0.29±0.00 <sup>Xcd</sup>	0.31±0.01 <sup>bc</sup>	0.32±0.01 <sup>b</sup>	0.34±0.00 <sup>sa</sup>		
% 5'lik Phosphotungstic acid soluble nitrogen ratio (PTA-N) (%)	A	0.42±0.01 <sup>W</sup>	0.43±0.02 <sup>W</sup>	0.45±0.02 <sup>W</sup>	0.48±0.01 <sup>W</sup>	0.48±0.01 <sup>W</sup>	NA	NA	NA	NA	NA	NA
	B	0.35±0.01 <sup>Xd</sup>	0.40±0.01 <sup>WXc</sup>	0.43±0.01 <sup>WXbc</sup>	0.47±0.01 <sup>Wab</sup>	0.48±0.01 <sup>Wa</sup>	NA	NA	NA	NA	NA	NA
	C	0.37±0.01 <sup>WX</sup>	0.41±0.01 <sup>WX</sup>	0.42±0.01 <sup>WX</sup>	0.44±0.02 <sup>W</sup>	NA	NA	NA	NA	NA	NA	NA
	D	0.34±0.02 <sup>Xc</sup>	0.35±0.02 <sup>Xc</sup>	0.37±0.02 <sup>Xbc</sup>	0.41±0.02 <sup>Wabc</sup>	0.44±0.02 <sup>Wab</sup>	0.47±0.02 <sup>Wa</sup>	NA	NA	NA	NA	NA
	E	0.19±0.00 <sup>Yg</sup>	0.19±0.00 <sup>Yg</sup>	0.21±0.01 <sup>Yg</sup>	0.23±0.00 <sup>Xf</sup>	0.25±0.00 <sup>Xe</sup>	0.27±0.00 <sup>Xd</sup>	0.29±0.00 <sup>c</sup>	0.31±0.00 <sup>b</sup>	0.33±0.00 <sup>sa</sup>		

a-i: The mean values with different letters in the same line are significantly different (P < 0.05); WXY: The mean values with different letters in the same column are significantly different (P < 0.05); NA: No analysis performed; A: Control group (normal atmosphere / dry air); B: % 100CO<sub>2</sub>; C: % 100N<sub>2</sub>; D: % 70 N<sub>2</sub> + % 30 CO<sub>2</sub>; E: % 75 N<sub>2</sub> + % 25 CO<sub>2</sub>.

**Table 3: The sensory analysis results of Tulum cheese samples (Arithmetic mean  $\pm$  Standard error)**

Property	Groups	Storage Days							
		30	60	90	120	150	180	210	240
Packaging	A	8.00 $\pm$ 0.00 <sup>a</sup>	7.66 $\pm$ 0.07 <sup>Wb</sup>	7.42 $\pm$ 0.06 <sup>Wc</sup>	NA	NA	NA	NA	NA
	B	6.11 $\pm$ 0.22	6.00 $\pm$ 0.00 <sup>X</sup>	5.83 $\pm$ 0.11 <sup>X</sup>	5.78 $\pm$ 0.17	NA	NA	NA	NA
	C	7.78 $\pm$ 0.22 <sup>a</sup>	6.55 $\pm$ 0.53 <sup>Wb</sup>	6.00 $\pm$ 0.23 <sup>Yc</sup>	NA	NA	NA	NA	NA
	D	7.66 $\pm$ 0.19	7.40 $\pm$ 0.20 <sup>W</sup>	6.89 $\pm$ 0.11 <sup>W</sup>	6.67 $\pm$ 0.67	6.00 $\pm$ 0.00	NA	NA	NA
	E	7.78 $\pm$ 0.22 <sup>a</sup>	7.78 $\pm$ 0.22 <sup>Wa</sup>	7.60 $\pm$ 0.23 <sup>Wa</sup>	6.67 $\pm$ 0.67 <sup>ab</sup>	6.00 $\pm$ 0.00 <sup>b</sup>	6.00 $\pm$ 0.00 <sup>b</sup>	6.00 $\pm$ 0.00 <sup>b</sup>	6.00 $\pm$ 0.00 <sup>b</sup>
Appearance	A	23.78 $\pm$ 0.22 <sup>a</sup>	22.89 $\pm$ 0.44 <sup>a</sup>	20.78 $\pm$ 0.40 <sup>b</sup>	NA	NA	NA	NA	NA
	B	23.11 $\pm$ 0.89	23.78 $\pm$ 0.22	23.78 $\pm$ 0.22	23.55 $\pm$ 0.45	NA	NA	NA	NA
	C	23.78 $\pm$ 0.22	22.67 $\pm$ 1.33	23.11 $\pm$ 0.89	NA	NA	NA	NA	NA
	D	23.11 $\pm$ 0.89	23.78 $\pm$ 0.22	24.00 $\pm$ 0.00	23.78 $\pm$ 0.22	23.78 $\pm$ 0.22	NA	NA	NA
	E	24.00 $\pm$ 0.00	23.78 $\pm$ 0.22	23.78 $\pm$ 0.22	23.55 $\pm$ 0.45	23.11 $\pm$ 0.89	22.22 $\pm$ 1.18	20.89 $\pm$ 0.89	19.78 $\pm$ 2.51
Structure	A	8.00 $\pm$ 0.00	7.78 $\pm$ 0.22	7.55 $\pm$ 0.45	NA	NA	NA	NA	NA
	B	7.78 $\pm$ 0.22	7.78 $\pm$ 0.22	7.55 $\pm$ 0.45	7.17 $\pm$ 0.33	NA	NA	NA	NA
	C	8.00 $\pm$ 0.00	7.78 $\pm$ 0.22	7.55 $\pm$ 0.45	NA	NA	NA	NA	NA
	D	8.00 $\pm$ 0.00	8.00 $\pm$ 0.00	8.00 $\pm$ 0.00	8.00 $\pm$ 0.00	8.00 $\pm$ 0.00	NA	NA	NA
	E	8.00 $\pm$ 0.00	8.00 $\pm$ 0.00	8.00 $\pm$ 0.00	8.00 $\pm$ 0.00	8.00 $\pm$ 0.00	8.00 $\pm$ 0.00	8.00 $\pm$ 0.00	8.00 $\pm$ 0.00
Odour	A	19.78 $\pm$ 0.22	19.55 $\pm$ 0.45	19.00 $\pm$ 0.58	NA	NA	NA	NA	NA
	B	20.00 $\pm$ 0.00 <sup>a</sup>	20.00 $\pm$ 0.00 <sup>a</sup>	19.33 $\pm$ 0.67 <sup>ab</sup>	16.55 $\pm$ 0.87 <sup>b</sup>	NA	NA	NA	NA
	C	19.78 $\pm$ 0.22	19.33 $\pm$ 0.33	18.22 $\pm$ 0.73	NA	NA	NA	NA	NA
	D	20.00 $\pm$ 0.00	20.00 $\pm$ 0.00	19.78 $\pm$ 0.22	19.55 $\pm$ 0.45	19.00 $\pm$ 0.58	NA	NA	NA
	E	19.78 $\pm$ 0.22	19.33 $\pm$ 0.67	19.11 $\pm$ 0.11	18.44 $\pm$ 0.80	17.78 $\pm$ 0.22	16.67 $\pm$ 2.40	16.44 $\pm$ 2.62	16.00 $\pm$ 3.06
Taste	A	31.22 $\pm$ 0.40	29.89 $\pm$ 0.68	28.22 $\pm$ 0.97	NA	NA	NA	NA	NA
	B	30.70 $\pm$ 1.68	30.11 $\pm$ 0.89	29.72 $\pm$ 0.69	28.44 $\pm$ 0.44	NA	NA	NA	NA
	C	29.78 $\pm$ 1.18	29.64 $\pm$ 0.60	28.67 $\pm$ 0.33	NA	NA	NA	NA	NA
	D	31.15 $\pm$ 0.27	30.89 $\pm$ 0.80	30.43 $\pm$ 0.57	29.22 $\pm$ 0.49	28.78 $\pm$ 0.22	NA	NA	NA
	E	31.15 $\pm$ 0.27	30.67 $\pm$ 1.33	29.55 $\pm$ 0.80	29.11 $\pm$ 1.98	29.00 $\pm$ 0.58	28.89 $\pm$ 0.11	26.22 $\pm$ 1.18	24.44 $\pm$ 2.35
Total	A	90.77 $\pm$ 0.78	87.77 $\pm$ 1.23	83.08 $\pm$ 2.28	NA	NA	NA	NA	NA
	B	88.67 $\pm$ 1.33	87.44 $\pm$ 2.62	87.35 $\pm$ 1.85	82.17 $\pm$ 1.36	NA	NA	NA	NA
	C	89.11 $\pm$ 1.56	85.97 $\pm$ 1.40	83.55 $\pm$ 1.26	NA	NA	NA	NA	NA
	D	90.06 $\pm$ 0.90	89.93 $\pm$ 1.32	89.10 $\pm$ 0.90	87.22 $\pm$ 1.64	85.55 $\pm$ 0.99	NA	NA	NA
	E	90.71 $\pm$ 0.70 <sup>a</sup>	89.55 $\pm$ 2.45 <sup>abc</sup>	88.04 $\pm$ 1.09 <sup>b</sup>	85.94 $\pm$ 3.11 <sup>abc</sup>	83.89 $\pm$ 1.64 <sup>bc</sup>	81.77 $\pm$ 2.82 <sup>bc</sup>	77.55 $\pm$ 3.74 <sup>c</sup>	74.22 $\pm$ 7.51 <sup>c</sup>

a-c: The mean values with different letters in the same line are significantly different ( $P < 0.05$ ); wxy: The mean values with different letters in the same column are significantly different ( $P < 0.05$ ); NA: No analysis performed; A: Control group (normal atmosphere / dry air); B: % 100 CO<sub>2</sub>; C: % 100 N<sub>2</sub>; D: % 70 N<sub>2</sub> + % 30 CO<sub>2</sub>; E: % 75 N<sub>2</sub> + % 25 CO<sub>2</sub>.

similar to those of some researchers (Oyugi and Buys 2007; Temiz, 2010; Gammariello et al., 2011; Olivares et al., 2012). The E group has a slowing down effect on the growth of yeast and molds in Tulum cheese. However, in a study conducted by Valle et al. (2023), they stated that the high percentage of CO<sub>2</sub> in MAP reduced yeast-mold growth.

*Staphylococcus aureus* bacteria showed a continuous decrease in the A, B, and C groups during the storage (Table 1), and there were no statistical differences between the groups on day 0 ( $P > 0.05$ ). *Staphylococcus aureus* was below the detection limit ( $< 1.0 \log_{10}$  CFU/g) in the D and E groups during the storage period. These findings are in line with ISO 6888-1:1999 concerning the microbiological criteria both for food safety and process hygiene criteria. The decrease of *Staphylococcus aureus* bacteria during the storage period is similar to the findings of many researchers who noted that the number of *Staphylococcus aureus* bacteria decreased in various dairy products with unpasteurized technique (Pintado and Malcata 2000; Mancuso et al., 2014). The

results found by Zulewska et al. (2023) in their study are the same as the findings in our study.

*E. coli* and sulfate-reducing anaerobic bacteria were not detected ( $< 1.0 \log_{10}$  CFU/g) in any of the cheese groups during the storage period. *E. coli* results are in accordance with the Turkish Standards Institute (Anonymous, 2016) and ISO 6888-1:1999 concerning the microbiological criteria. The findings of our research are similar to the findings in their study of Zulewska et al. (2023)

### Physicochemical results

The pH values of Tulum cheese samples showed a continuous decrease during the storage period. However, no statistically significant difference was determined between groups and within groups ( $P > 0.05$ ) (Table 2). The decrease in pH may be due to the inhibition of the growth of bacteria by CO<sub>2</sub> gas in all groups. It is seen that these results are similar to the findings of some researchers (Olarde et al., 2002; Thippeswamy and Venkateshaiah,



2011; Olivares et al., 2012; Piscopo et al., 2015; Zulewska et al., 2023).

The acidity (% lactic acid) value increased continuously during the storage period in all groups. No statistically significant dissimilarity was determined between groups and within groups ( $P>0.05$ ) (Table 2). The observed increase in acidity was most likely due to carbonic acid formation, as observed in previous studies (Olarde et al., 2002).

The dry matter value in Tulum cheese samples showed an increase continuously during the storage period in all groups. There were statistical dissimilarities between the groups on days 0, 30, 60, and 90 ( $P>0.05$ ). However, a significant difference was found between the D and E groups on day 150. There were statistical differences within the B and D groups. According to TS 3001 (Anonymous, 2015), the maximum moisture content in Tulum cheese is 45%. The dry matter values of our study are in accordance with TS 3001 standards. It is seen that the increase in the amount of dry matter during the storage period is similar to the findings of Pluta et al. (2005).

The amount of salt showed a continuous increase during the storage period (Table 2). There were statistical dissimilarities within group in the E group. However, no significant dissimilarity was found between the groups ( $P>0.05$ ).

It was observed that the amount of ash increased continuously during the storage period. No statistically significant dissimilarity was determined between groups and within groups ( $P>0.05$ ) (Table 2).

There was a continuous fluctuation in the A, B, D, and E groups in terms of the amount of fat during storage. There was a continuous increase in the C group (Table 2). No statistical was found dissimilarities between groups and within groups ( $P>0.05$ ) (Table 2).

Water activity ( $a_w$ ) values in Tulum cheese samples were observed to increase continuously in the A, C, and D groups, fluctuations in the B group, and continuous decreases in the E group during storage (Table 2). There were statistical differences within the group in the E group. However, no significant difference was found between the groups ( $P>0.05$ ). It was found that the MAP findings in this study were similar to the findings of some studies (Thippeswamy and Venkateshaiah, 2011; Mancuso et al., 2014) in which the effects of the MAP method on the shelf life of various cheese types were investigated.

The total protein amounts in the analyzed Tulum cheese samples were observed to continuously increase in whole groups during the storage period. Generally, the lowest

value was determined to be 24.15 on day 0 of the E group and the highest value was 37.89 on day 150 of the D group (Table 2). There were statistical differences within the group in the D group ( $P<0.05$ ). There were no statistical differences between groups ( $P>0.05$ ). However, between the D and E groups was found significant difference on day 150 ( $P<0.05$ ). It was determined that the findings in this study were compatible with the findings of some researchers (Pluta et al., 2005; Olivares et al., 2012).

Water-soluble nitrogen (SC-N) values showed an increase continuously during the storage period. During the storage period, it was observed that the values determined in the E group were consistently less than the values determined in the other groups. The statistical differences within the group in the A, B, C, D, and E groups were significant ( $P<0.05$ ). There were statistical differences between groups on days 30, 60, and 150 ( $P<0.05$ ) (Table 2). Our findings were compatible with Alam and Goyal's (2011) Mozzarella cheeses packaged with the MAP technique. The degree of ripening in water-soluble nitrogen showed an increase incessantly during the storage period. The statistical differences were significant between groups on days 90 and 120 of the storage ( $P<0.05$ ). However, the statistical differences between the other days were not significant ( $P>0.05$ ). The statistical differences within the group in the A, B, C, D, and E groups were significant ( $P<0.05$ ).

The 12% TCA-N value showed an increase continuously during the storage period. There were statistical differences between groups on days 0, 30, 60, 90, 120, and 150 ( $P<0.05$ ). The statistical differences within the group were significant in all groups ( $P<0.05$ ) (Table 2). It is seen that the findings found in the study are similar to the findings of some researchers (Ozer et al., 2002).

The 5% PTA-N soluble nitrogen ratio is also known as a measure of ripening and proteolysis in cheeses (Thippeswamy and Venkateshaiah, 2011). This value showed an increase continuously in all groups during the storage period. There were statistical differences between groups on days 0, 30, 60, 90, 120, and 150 ( $P<0.05$ ). The statistical differences within the group were significant in the B, D, and E groups ( $P<0.05$ ) (Table 2). The reason for the increase of the 5% PTA-N ratio during the ripening of cheeses is derived from the shown solubility feature in the 5% PTA-N of the small molecule peptides and amino acids formed during ripening (Hayaloglu, 2003). The findings obtained in this study are similar to the findings of some researchers (Ozer et al., 2002; Hayaloglu, 2003) who stated that the 5% PTA-N ratio increased during storage of cheeses.

## Sensory results

The packaging scores in Tulum cheese samples decreased continuously during the storage period. There were statistical differences between the groups in the packaging scores on days 60 and 90 ( $P < 0.05$ ). The statistical differences within the group were significant in the A, C, and E groups ( $P < 0.05$ ) (Table 3). However, when the scores of the E group were evaluated within themselves, significant differences were obtained ( $P < 0.05$ ). The findings obtained in this study are similar to the findings of some researchers (Marcuzzo et al., 2012; Acerbi et al., 2018; Darnay et al., 2019, Atallah et al., 2021).

## CONCLUSION

Consequently, it was observed that the Tulum cheese samples packaged in the MAP with 75% N<sub>2</sub>+25% CO<sub>2</sub> showed important changes in its quality, and the shelf life of this group was extended up to 240 days. Since the rate of CO<sub>2</sub> gas used in higher doses may cause sensory disorders in cheese, it can be recommended to use this gas mixture (E) for semi-hard cheeses such as Tulum cheese. Since traditional cheeses are produced in small family businesses or small-scale enterprises, they do not have a standard production. These cheeses are of great importance and consumed by the local people. For this reason, standardizing the production of traditional cheeses and developing technologies such as packaging will increase the quality standards of these cheeses, bring the products to the industrial sector, and contribute to both the region's and the country's economy.

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## CONFLICTS OF INTEREST

The authors declare that they have not any conflict of interest.

## Authors' contributions

Pelin DEMİR: Conceptualization, methodology, formal analysis, investigation, writing - original draft, writing- review & editing, visualization; Osman İrfan İLHAK: Writing- review & editing; Gülsüm ÖKSÜZTEPE: Conceptualization, methodology, formal analysis, writing - original draft, writing- review & editing, visualization.

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