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Research Article

Some Characteristics of Erzincan Tulum Cheese Produced Using Different Probiotic Cultures and Packaging Material

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Abstract

In this study, Erzincan tulum cheeses produced using pasteurized sheep milk and probiotic bacterial cultures were filled in different packaging materials (skin bag, intestine, and appendix) and stored for 90 days. Subsequently, the chemical, microbiological and quality criteria of the samples were examined on the 2nd, 30th, 60th and 90th days of the storage period. Although the acidity values of cheese samples increased during the storage period, the pH and water activity values decreased. Similarly, TAMB, TAPB, yeast/mold, lactic acid bacteria, lipolytic bacteria, proteolytic bacteria, *Lactococcus* spp. and *Pseudomonas* spp. counts increased; however, the total coliform and *Enterobacteriaceae* counts decreased. The probiotic culture counts added in cheese production also decreased during storage. Moreover, no *Escherichia coli, Staphylococcus aureus, Salmonella* spp., *Listeria* spp. and *Brucella* spp. development was determined in the samples.

Keywords: *Erzincan tulum cheese, Probiotic bacteria, Appendix, Small intestine, Quality criteria*

Farklı Probiyotik Kültür ve Ambalaj Malzemesiyle Üretilen Erzincan Tulum Peynirlerinin Bazı Özelliklerinin İncelenmesi

Öz

Bu çalışmada, pastörize koyun sütleri kullanılarak ve probiyotik bakteri kültürleri ilave edilerek üretilen Erzincan tulum peynirleri farklı ambalaj materyallerine doldurularak (tulum, ince bağırsak, kör bağırsak, 90 gün süre ile depolanmıştır. Depolamanın 2., 30., 60., ve 90. günlerinde peynirlerin bazı kimyasal ve mikrobiyolojik, kalite kriterleri incelenmiştir. Depolama süresi boyunca peynir örneklerinin % asitlik değerleri artış göstermesine karşın, pH ve aw değerlerinde azalma olduğu belirlenmiştir. Benzer şekilde depolama süresince TAMB, TAPB, maya/küf, laktik asit, lipolitik bakteri, proteolitik bakteri sayıları, *Lactococcus* spp. ve *Pseudomonas* spp. cinsi bakteri sayılarında artış tespit edilmesine karşın, Toplam koliform ve *Enterobacteriaceae* sayılarında ise azalma olduğu belirlenmiştir. Peynir üretiminde ilave edilen probiyotik kültür sayıları da yine depolama süresince azalmıştır. Ayrıca peynirlere yapılan *Escherichia coli, Staphylococcus aureus, Salmonella* spp, *Listeria* Spp. *Brucella* spp. analizleri sonucunda herhangi bir gelişme tespit edilememiştir.

Anahtar sözcükler: *Erzincan tulum peyniri, Probiyotik bakteri, kör bağırsak, İnce bağırsak, Kalite kriterleri*

INTRODUCTION

Cheese was first produced 8000 years ago, and approximately, 4000 different types of cheese are currently found in the world [1]. Turkey produces approximately 200 types of cheese [2]. The method of cheese production, the mixtures added to the cheese and fermentation process and other variables in this process differentiate the cheese types from each other. Cheese is an extensive microbial ecosystem, and the complex microbiota directly influences the formation of various kinds of cheese [3].

Tulum is the most consumed cheese after the white cheese and kashar cheese types in Turkey. Moreover, tulum cheese

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has the highest consumption rate among the traditionally produced cheese types [4]. Although tulum cheese is mostly produced in small family businesses in Turkey, in recent years its production has expanded to large factories [2,4].

Tulum cheese is produced in all regions in Turkey except Trakya region and is referred to by different names according to the areas where they are produced [5]. The most common tulum cheese types are Erzincan tulum cheese, Çimi tulum cheese (Antalya), Divle tulum cheese (Karaman), Afyon tulum cheese (Afyon), Kargı tulum cheese (Çankırı, Çorum), Isparta tulum cheese (Isparta), Selçuklu tulum cheese (Konya) and İzmir tulum cheese (ripened in brine). The production and storage conditions of tulum cheese are very different from other tulum cheese types [6].

Among these cheese types, Erzincan tulum cheese is the most produced and consumed tulum cheeses in Turkey and has a significantly higher economic value much higher than most cheese types [7]. So far, the Erzincan tulum cheese has no standard production method and is mostly produced in small dairy plants by traditional methods using fatty sheep milk, sometimes mixed with cow and goat milk [8]. Milk is processed with no fat standardization [9]. The milk used is renneted at its temperature following milking or heated to 30-32°C with no pasteurization application [10] and no starter culture addition [11,12]. Tulum cheese is marketed by traditionally filling in skin bags mostly obtained from the skins of small cattle [13]. However, in recent years, excessive microbial load in such packaging has had a negative impact on the consumer and economical aspects; therefore, various materials have been used in the production of cheeses such as plastic drums, artificial bags and intestines [14-16].

The intestinal systems of humans and warm-blooded animals are complex ecosystems where 400 different microorganism species live together. The microorganisms found in this ecosystem are defined as "natural flora" and are divided into two groups as "beneficial" and "harmful" [17]. The functions of beneficial microorganisms in the intestinal system include assisting digestion of foods, protecting the host from pathogenic microorganisms, and promoting the defense mechanism of the host. In this context, microorganisms that have therapeutic effects on the health of the host by regulating the intestinal flora are described as "probiotics". The most important of these microorganisms include the genera *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* [18].

A substantial increase in studies on the addition of probiotic microorganisms into various foods has been noted in recent years. The food groups to which the probiotic microorganisms are added the most include milk and dairy products [19]. The proportion of probiotic dairy products in total dairy production worldwide increases every year and is expected to continue increasing in the following years [20].

This study aimed to investigate the physicochemical and microbiological properties of Erzincan tulum cheeses, produced using different probiotic bacteria and packaging materials, during the storage period.

MATERIAL and METHODS

Production of Tulum Cheeses

In the production of tulum cheese, the production method applied by Dikici [21] has been modified. Sheep milk needed to produce cheese used in the research was obtained from the sheep farmers in the Erzincan provincial highlands and brought to a milk factory also operating in Erzincan province borders under cold chain with the milk tanks. After passing through the separator, the milk was cleaned and heat treated at 75°C for 15 sec. Then, pasteurized milk was coagulated with calf rennet (Mayasan Inc., Istanbul, Turkey) having a coagulation strength of 1:16000 MCU mL^{-1} at 33°C for 90 min.

After the coagulation and the appropriate curding time, the curds were broken to the size of chickpea and filled in bags produced from cheesecloth-with an average weight of 5 kg and placed in a 20-22°C storage. Following the filling process, the cotton bags filled with curd were whirled around at regular intervals (1 h), and the whey was drained more easily and quickly.

After 24 h olmalı, the cotton bags were opened, and the curd was removed carefully without breaking it and was stuffed in tighter and roughly woven cotton cloths for 6-7 days of storage at 20-22°C. At the end of storage period, the curd was crushed to the size of a chickpea by hand and granulated Erzincan rock salt was added at 3.5% and stirred thoroughly until a homogenous form was obtained. Following this procedure activated probiotic bacterial cultures such as *Lactobacillus acidophilus* (Pro Lafti L-10) and *Bifidobacterium animalis* spp. *lactis* (Pro Lafti B-94) were added separately as 10^7 cfu/mL minimum and filled in special bags that could contain 45-50 kg cheese. The cheeses were then pre-ripened in storage at 22-23°C for three days. Then, the cheese was thoroughly crumbled and filled in small skin bags or appendix or intestines.

The cheese bags were stored for 90 days at -1°C at 75-80% relative humidity. On the 2nd, 30th, 60th, and 90th days of ripening, the physicochemical and microbiological analyses were conducted, and the results were evaluated comparatively.

Methods

Physiochemical Analysis

Titratable acidity was measured according to AOAC [22]. The pH was measured in a homogenate prepared by blending 10 g of a sample with 90 mL of distilled water for 30 s. pH values were obtained with glass electrode attached to a

Hanna pH meter (Model 2215, Hanna Instruments, USA). Water activity of the samples was recorded by Novasina TH-500 a_w Sprint (Novasina, Axair Ltd., Switzerland).

Microbial Analyses

Samples of 10 g were taken from the tulum cheese aseptically. A sterilized ringer solution at a dilution of 1:9 (w/v) was added, and the samples were homogenized for 3 min in a stomacher Lab-Blender 400 (London, UK). The serial decimal dilutions were sterilized and plated for bacterial counts [23,24].

Total aerobic mesophilic bacteria (TAMB), Total aerobic psychrophilic bacteria (TAPB) yeast/mold, lipolytic bacteria, proteolytic bacteria, Lactic Acid bacteria, *Lactoccocus* spp., *Listeria* spp., *Salmonella* spp., *Pseudomonas* spp., Total coliform, *Enterobacteriaceae*, *Staphylococcus aureus, Escherichia coli, Lactobacillus acidophilus* and *Bifidobacterium animalis* spp. *lactis* were counted using a spread plate technique *(Table 1, Table 2)*.

Statistical Analysis

The research design was completely randomized having a factorial structure (3 x 3 x 4). The factors were probiotic *(Lactobacillus acidophilus*, *Bifidobacterium animalis* spp. *lactis*, and *Lactobacillus acidophilus* + *Bifidobacterium animalis* spp. *lactis)*, packaging (Skin bag, appendix, and intestine), and storage time (2, 30, 60 and 90 day). Three-way ANOVA was applied to data using procedure of the SPSS statistical package program (SPSS Inc., Chicago, IL) to do this analysis. Lsmeans values were generated and corresponding Duncan multiple comparison test. The treatment structure was completely randomized with 2 replications.

Table 1. *Pre-enrichment broths used in microbiological analysis, incubation conditions and methods used*

Microorganisms	Broth	Supplement	Incubation Conditions	Method Used
Listeria spp.	Fraser (Merck 1. 10398)	FLSS (Merck 1.0092) FLAIS (Merck 1.0093)	30°C - 24 h - aerobic	ISO 11290-1:2017 [25] ISO 11290-2:2017 [26]
Salmonella spp.	NB (Merck 1.05443) RVS (Merck 1.07700)	--	37°C - 24 h - aerobic 42°C - 24 h - aerobic	[27] ISO 6579-1:2017 [28]

***FLSS:** Fraser Listeria Selective Supplement; **FLAIS:** Fraser Listeria Ammonium Iron (III) Supplement; **NB:** Nutrient Broth; **RVS:** Rappaport Vassiliadis Salmonella Enrichment Broth*

Table 2. *Analysis of the microorganism groups and reproduction condition*

Microorganisms	Medium	Incubation Conditions	Method Used
TAMB	Plate Count Agar (Merck 1.05463)	30°C - 48/72 h - aerobic	ISO 4833-2:2013 [29] ISO 4833-2:2013 [30]
TAPB	Plate Count Agar (Merck 1.05463)	4°C - 5/7 day - aerobic	[24]
Yeast/Mold	Potato Dextose Agar (Merck 1.10130)	22°C - 4/5 day - aerobic	[24]
Salmonella spp.	Brilliant Green Phenol Red Lactose Sucrose Agar (Merck 1.10747.0500)	37°C - 24/48 h - aerobic	ISO 6579-1:2017 [27,31]
Pseudomonas spp.	*Pseudomonas* Selective Agar Base (PSA) (Merck 1.07620)	37°C - 24/48 h - aerobic	ISO 13720:2010 [32]
Esherichia coli	Chromocult TBX Agar (Merck 1.16122)	44°C - 24/48 h - aerobic	ISO 16649-1:2001 [33] ISO 16649-2:2001 [34] ISO 16649-3:2015 [35]
Enterobacteriaceae	Eosin Methylene-Blue Lactose Sucrose Agar (Merck 1.01347)	30°C - 24/48 h - aerobic	ISO 21528-2:2004 [36]
Total Coliform Group	Violet Red Bile Agar (Merck 1.01406)	30°C - 24/48 h - aerobic	ISO 4832 [37]
Staphylococcus aureus	Baird Parker Agar (Merck 1.05406)	37°C - 24/48 h - aerobic	ISO 6888-1 [38]
Lipolytic Bacteria	Tributyrin Agar (Merck 1.01957)	30°C - 48/72 h - aerobic	[24]
Proteolytic Bacteria	Plate Count Skim Milk Agar (Merck 1.15338)	21°C - 72 h - aerobic	[24]
Lactobacillus acidophilus	MRS-sorbitol Agar (Merck 1.10660)	30°C - 24/48 h - anaerobic	[24]
Bifidobacterium animalis spp. lactis	Propionate agar (Merck 1.00043)	30°C - 24/48 h - anaerobic	[24]
Lactic Acit Bacteria	MRS (Man Rogasa) Agar (Merck 1.10661)	30°C - 24/48 h - anaerobic	[24]
Lactococcus spp.	M17 Agar (Merck 1.15108)	30°C - 24/48 h - aerobic	[24]
Listeria spp.	Oxford (Merck 1.07004)	37°C - 24/48 h - aerobic	ISO 11290-1:2017 [25] ISO 11290-2:2017 [26]
Brucella spp.	Farrell's Agar (Oxoid CM 169) Brucella Sellective Supplement (Oxoid SR 83)	37°C - 21 day 6% CO_2	[24]

***TAMB:** Total Aerobic Mesophilic Bacteria, **TAPB:** Total Aerobic Psychrophilic Bacteria*

RESULTS

Probability values of physiochemical and microbiological analyses (P-values) for all main effects and interactions source of variation are presented in *Table 3* and *Table 4*. Chemical analysis results of Erzincan tulum cheese produced with the addition of probiotic bacteria packed using different packaging materials are shown in *Table 5* while microbiological analysis results are shown in *Table 6* and *Table 7*. During the three-month-storage period, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp.*, *Listeria* spp. and *Brucella* spp. were not detected. *Enterobacteriaceae* counts were <2 log cfu/g during the storage period.

Table 3. *Probability values of physiochemical and microbiological analyses (p-values) for all main effects and interactions source of variation*

Source of Variation	Acidity	pH	a_w	TAMB	TAPB	Mold-Yeast	LAB
Probiotic	0.049	0.422	<.0001	<.0001	<.0001	<.0001	<.0001
Packaging	0.427	0.193	<.0001	<.0001	<.0001	0.037	<.0001
Storage time	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Probiotic x Packaging	0.186	0.012	0.271	<.0001	<.0001	<.0001	<.0001
Probiotic x Storage Time	0.031	0.047	<.0001	0.057	0.199	<.0001	0.075
Packaging x Storage Time	0.703	0.905	<.0001	0.112	0.129	0.007	0.007
Probiotic x Packaging x Storage Time	0.208	0.233	0.005	0.024	0.022	0.010	<.0001

TAMB: *Total Aerobic Mesophilic Bacteria,* ***TAPB:*** *Total Aerobic Psychrophilic Bacteria,* ***LAB:*** *lactic Acid Bacteria*

Table 4. *Probability values of microbiological analyses (p-values) for all main effects and interactions source of variation*

Source of Variation	*Lactococcus* spp.	*Pseudomonas* spp.	Lipolytic Bacteria	Proteolytic Bacteria	Total Coliform	*Bifidobacterium animalis* spp. *lactis*	*Lactobacillus acidophilus*
Probiotic	<.0001	<.0001	<.0001	<.0001	--	<.0001	<.0001
Packaging	<.0001	0.022	0.138	0.107	--	<.0001	<.0001
Storage Time	<.0001	<.0001	<.0001	<.0001	--	<.0001	<.0001
Probiotic x Packaging	<.0001	0.017	<.0001	0.663	--	<.0001	<.0001
Probiotic x Storage time	0.070	<.0001	<.0001	0.001	--	0.006	0.005
Packaging x Storage Time	0.009	0.817	0.061	0.480	--	0.042	0.704
Probiotic x Packaging x Storage Time	<.0001	0.799	0.344	0.798	--	0.618	0.447

Table 5. *Changes in the physicochemical and microbiological analysis results of tulum cheese samples during storage*

Samples	% Acidity				pH				a_w				TAMB				TAPB			
	Storage Time (Day)				Storage Time (Day)				Storage Time (Day)				Storage Time (Day)				Storage Time (Day)			
	2	30	60	90	2	30	60	90	2	30	60	90	2	30	60	90	2	30	60	90
TCBS	0.84^{d}	0.92^{c}	0.98^{b}	1.19^{a}	5.08^{a}	4.91^{b}	4.80^{c}	4.64^{d}	0.934^{a}	0.926^{b}	0.911^{c}	0.897^{d}	5.14^{d}	5.34^{c}	5.55^{b}	5.63^{a}	3.18^{d}	3.38^{c}	3.47^{b}	3.67^{a}
TCBC	0.85^{d}	0.90^{c}	0.98^{b}	1.33^{a}	5.06^{a}	4.92^{b}	4.80^{c}	4.54^{d}	0.929^{a}	0.910^{b}	0.906^{c}	0.898^{d}	4.12^{d}	4.70^{c}	5.03^{b}	5.32^{a}	2.16^{d}	2.74^{c}	3.07^{b}	3.36^{a}
TCBİ	0.86^{d}	0.92^{c}	0.98^{b}	1.33^{a}	5.03^{a}	4.92^{b}	4.77^{c}	4.54^{d}	0.931^{a}	0.912^{b}	0.899^{c}	0.886^{c}	5.19^{d}	5.41^{c}	5.61^{b}	5.68^{a}	3.23^{d}	3.36^{c}	3.65^{b}	3.72^{a}
TCLS	0.85^{d}	0.91^{c}	0.98^{b}	1.21^{a}	5.07^{a}	4.89^{b}	4.78^{c}	4.63^{d}	0.933^{a}	0.914^{b}	0.899^{c}	0.883^{d}	5.25^{d}	5.43^{c}	5.58^{b}	5.69^{a}	3.22^{d}	3.41^{c}	3.58^{b}	3.75^{a}
TCLC	0.85^{d}	0.90^{c}	0.97^{b}	1.04^{a}	5.04^{a}	4.97^{b}	4.82^{c}	4.74^{d}	0.930^{a}	0.906^{b}	0.894^{c}	0.881^{d}	5.21^{d}	5.41^{c}	5.57^{b}	5.71^{a}	3.25^{d}	3.45^{c}	3.61^{b}	3.75^{a}
TCLİ	0.86^{d}	0.91^{c}	0.99^{b}	1.17^{a}	5.00^{a}	4.85^{b}	4.76^{c}	4.65^{d}	0.930^{a}	0.904^{b}	0.887^{c}	0.849^{d}	5.21^{d}	5.40^{c}	5.56^{b}	5.66^{a}	3.25^{d}	3.45^{c}	3.60^{b}	3.69^{a}
TCMS	0.86^{d}	0.90^{c}	0.97^{b}	1.27^{a}	4.96^{a}	4.88^{b}	4.78^{c}	4.60^{d}	0.933^{a}	0.916^{b}	0.904^{c}	0.893^{d}	4.83^{d}	5.10^{c}	5.32^{b}	5.58^{a}	2.87^{d}	3.14^{c}	3.36^{b}	3.62^{a}
TCMC	0.85^{d}	0.91^{c}	0.98^{b}	1.06^{a}	5.07^{a}	4.83^{b}	4.80^{c}	4.76^{d}	0.925^{a}	0.908^{b}	0.899^{c}	0.893^{d}	5.25^{d}	5.42^{c}	5.63^{b}	5.77^{a}	3.35^{d}	3.52^{c}	3.72^{b}	3.86^{a}
TCMİ	0.85^{d}	0.90^{c}	0.97^{b}	1.09^{a}	5.08^{a}	4.97^{b}	4.79^{c}	4.71^{d}	0.940^{a}	0.921^{b}	0.885^{c}	0.858^{d}	5.06^{d}	5.24^{c}	5.40^{b}	5.56^{a}	3.21^{d}	3.39^{c}	3.54^{b}	3.71^{a}

TCBS: *Tulum Cheese Bifidobacterium animalis spp. lactic Skin Bag,* ***TCBC:*** *Tulum Cheese Bifidobacterium animalis spp. lactic appendix,* ***TCBİ:*** *Tulum Cheese Bifidobacterium animalis spp. lactic intestine,* ***TCLS:*** *Tulum Cheese Lactobacillus acidophilus Skin Bag,* ***TCLC:*** *Tulum Cheese Lactobacillus acidophilus appendix,* ***TCLİ:*** *Tulum Cheese Lactobacillus acidophilus intestine,* ***TCMS:*** *Tulum Cheese Mix Skin Bag,* ***TCMC:*** *Tulum Cheese Mix appendix,* ***TCMİ:*** *Tulum Cheese Mix intestine,* ***a - d (→):*** *Values with the same capital letters in the same rows for each analysis differ significantly (P<0.05)*

Table 6. *Changes in the microbiological analysis results of tulum cheese samples during storage*

Samples	Mold-Yeast				Lactic Acid Bacteria				*Lactococcus* spp.				*Pseudomonas* spp.				Lipolytic Bacteria			
	Storage Time (Day)				Storage Time (Day)				Storage Time (Day)				Storage Time (Day)				Storage Time (Day)			
	2	30	60	90	2	30	60	90	2	30	60	90	2	30	60	90	2	30	60	90
TCBS	3.70^{d}	4.29^{c}	4.76^{b}	5.01^{a}	4.98^{d}	5.12^{c}	5.29^{b}	5.43^{a}	3.95^{d}	4.10^{c}	4.26^{b}	4.41^{a}	2.76^{d}	3.35^{c}	3.82^{b}	4.06^{a}	4.03^{d}	4.60^{c}	4.90^{b}	5.22^{a}
TCBC	3.55^{d}	4.23^{c}	4.66^{b}	4.89^{a}	3.50^{d}	4.14^{c}	4.61^{b}	4.94^{a}	2.47^{d}	3.11^{c}	3.58^{b}	3.91^{a}	2.61^{d}	3.28^{c}	3.71^{b}	3.95^{a}	4.11^{d}	4.42^{c}	4.74^{b}	5.06^{a}
TCBİ	3.48^{d}	4.11^{c}	4.58^{b}	4.72^{a}	4.99^{d}	5.27^{c}	5.52^{b}	5.66^{a}	3.96^{d}	4.24^{c}	4.49^{b}	4.63^{a}	2.53^{d}	3.17^{c}	3.63^{b}	3.77^{a}	4.09^{d}	4.42^{c}	4.63^{b}	5.03^{a}
TCLS	2.48^{d}	3.64^{c}	4.29^{b}	4.81^{a}	4.88^{d}	5.17^{c}	5.42^{b}	5.62^{a}	3.85^{d}	4.15^{c}	4.39^{b}	4.59^{a}	<2	2.70^{c}	3.34^{b}	3.86^{a}	3.86^{d}	4.52^{c}	4.90^{b}	5.11^{a}
TCLC	1.89^{d}	3.79^{c}	4.31^{b}	4.74^{a}	5.06^{d}	5.29^{c}	5.50^{b}	5.62^{a}	4.04^{d}	4.26^{c}	4.47^{b}	4.60^{a}	<2	2.85^{c}	3.37^{b}	3.80^{a}	3.91^{d}	4.50^{c}	4.86^{b}	5.28^{a}
TCLİ	2.05^{d}	3.87^{c}	4.40^{b}	4.73^{a}	5.09^{d}	5.33^{c}	5.50^{b}	5.61^{a}	4.06^{d}	4.31^{c}	4.47^{b}	4.58^{a}	<2	2.93^{c}	3.46^{b}	3.79^{a}	4.00^{d}	4.79^{c}	5.02^{b}	5.21^{a}
TCMS	3.41^{d}	3.85^{c}	4.54^{b}	4.88^{a}	4.52^{d}	4.77^{c}	5.05^{b}	5.41^{a}	3.49^{d}	3.74^{c}	4.02^{b}	4.38^{a}	3.77^{d}	4.22^{c}	4.24^{b}	4.64^{a}	4.14^{d}	4.95^{c}	5.12^{b}	5.27^{a}
TCMC	3.99^{d}	4.48^{c}	4.88^{b}	5.16^{a}	5.01^{d}	5.33^{c}	5.52^{b}	5.62^{a}	3.99^{d}	4.30^{c}	4.49^{b}	4.59^{a}	3.66^{d}	4.07^{c}	4.44^{b}	4.62^{a}	4.23^{d}	4.88^{c}	5.24^{b}	5.31^{a}
TCMİ	3.97^{d}	4.61^{c}	4.83^{b}	5.07^{a}	4.98^{d}	5.16^{c}	5.31^{b}	5.40^{a}	3.95^{d}	4.13^{c}	4.28^{b}	4.37^{a}	3.59^{d}	3.95^{c}	4.22^{b}	4.54^{a}	4.38^{d}	5.02^{c}	5.19^{b}	5.32^{a}

TCBS: *Tulum Cheese Bifidobacterium animalis spp. lactic Skin Bag,* ***TCBC:*** *Tulum Cheese Bifidobacterium animalis spp. lactic appendix,* ***TCBİ:*** *Tulum Cheese Bifidobacterium animalis spp. lactic intestine,* ***TCLS:*** *Tulum Cheese Lactobacillus acidophilus Skin Bag,* ***TCLC:*** *Tulum Cheese Lactobacillus acidophilus appendix,* ***TCLİ:*** *Tulum Cheese Lactobacillus acidophilus intestine,* ***TCMS:*** *Tulum Cheese Mix Skin Bag,* ***TCMC:*** *Tulum Cheese Mix appendix,* ***TCMİ:*** *Tulum Cheese Mix intestine,* ***a - d (→):*** *Values with the same capital letters in the same rows for each analysis differ significantly (P<0.05)*

Table 7. *Changes in the microbiological analysis results of tulum cheese samples during storage*

Samples	Proteolytic Bacteria				Total Coliform				*Bifidobacterium animalis* spp. *lactis*				*Lactobacillus acidophilus*				Total *Enterobacteriaceae*			
	Storage Time (Day)				Storage Time (Day)				Storage Time (Day)				Storage Time (Day)				Storage Time (Day)			
	2	30	60	90	2	30	60	90	2	30	60	90	2	30	60	90	2	30	60	90
TCBS	4.02^{d}	4.79^{c}	5.20^{b}	5.42^{a}	3.10	2.46	<2	<2	5.39^{a}	5.34^{b}	5.16^{c}	4.99^{d}	3.27^{a}	3.18^{b}	3.05^{c}	3.02^{c}	<2	<2	<2	<2
TCBC	4.00^{d}	4.75^{c}	5.01^{b}	5.41^{a}	2.45	<2	<2	<2	6.19^{a}	6.09^{b}	6.03^{c}	5.94^{d}	3.34^{a}	3.25^{b}	3.18^{c}	3.01^{d}	<2	<2	<2	<2
TCBİ	3.96^{d}	4.73^{c}	5.06^{b}	5.40^{a}	<2	<2	<2	<2	5.72^{a}	5.65^{b}	5.42^{c}	5.06^{d}	3.23^{a}	3.07^{b}	2.95^{c}	2.88^{d}	<2	<2	<2	<2
TCLS	3.94^{d}	4.80^{c}	5.19^{b}	5.31^{a}	<2	<2	<2	<2	3.55^{a}	3.31^{b}	3.07^{c}	2.91^{d}	5.42^{a}	5.25^{b}	5.11^{c}	4.94^{d}	<2	<2	<2	<2
TCLC	4.02^{d}	4.78^{c}	5.13^{b}	5.27^{a}	2.85	<2	<2	<2	3.43^{a}	3.21^{b}	3.03^{c}	2.75^{d}	5.39^{a}	5.26^{b}	5.17^{c}	5.03^{d}	<2	<2	<2	<2
TCLİ	3.44^{d}	4.82^{c}	5.03^{b}	5.23^{a}	<2	<2	<2	<2	3.41^{a}	3.19^{b}	2.75^{c}	2.55^{d}	5.42^{a}	5.26^{b}	5.14^{c}	4.94^{d}	<2	<2	<2	<2
TCMS	4.71^{d}	4.66^{c}	5.40^{b}	5.58^{a}	2.57	<2	<2	<2	5.36^{a}	5.15^{b}	5.02^{c}	4.87^{d}	5.25^{a}	5.18^{b}	5.13^{c}	4.86^{d}	<2	<2	<2	<2
TCMC	4.61^{d}	5.01^{c}	5.38^{b}	5.59^{a}	<2	<2	<2	<2	5.36^{a}	5.14^{b}	5.02^{c}	4.85^{d}	5.36^{a}	5.22^{b}	5.13^{c}	4.90^{d}	<2	<2	<2	<2
TCMİ	4.53^{d}	4.89^{c}	5.16^{b}	5.49^{a}	<2	<2	<2	<2	5.25^{a}	5.18^{b}	5.05^{c}	4.73^{d}	5.34^{a}	5.25^{b}	5.12^{c}	4.90^{d}	<2	<2	<2	<2

TCBS: *Tulum Cheese Bifidobacterium animalis spp. lactic Skin Bag,* ***TCBC:*** *Tulum Cheese Bifidobacterium animalis spp. lactic appendix,* ***TCBİ:*** *Tulum Cheese Bifidobacterium animalis spp. lactic intestine,* ***TCLS:*** *Tulum Cheese Lactobacillus acidophilus Skin Bag,* ***TCLC:*** *Tulum Cheese Lactobacillus acidophilus appendix,* ***TCLİ:*** *Tulum Cheese Lactobacillus acidophilus intestine,* ***TCMS:*** *Tulum Cheese Mix Skin Bag,* ***TCMC:*** *Tulum Cheese Mix appendix,* ***TCMİ:*** *Tulum Cheese Mix intestine,* ***a - d (→):*** *Values with the same capital letters in the same rows for each analysis differ significantly (P<0.05)*

DISCUSSION

Probiotic addition (P<0.05), storage time (P<0.0001), and probiotic x storage period interactions (P<0.05) were statistically significant on the acidity values of Erzincan tulum cheeses produced with probiotic bacteria and stored in different packages (P<0.05) *(Table 3)*. Acidity values of the samples were found to increase during storage (P<0.0001), and the highest acidity value was determined in TCBI samples with 1.36% *(Table 5)* at the end of storage, which could be attributed to the probiotic bacteria added during the production stage. Andiç et al.[39] and Çakır [40] have reported that the acidity values of tulum cheeses increase with an increase in the storage period.

Storage period (P<0.0001), probiotic bacteria x packaging (P<0.05) and probiotic x storage time (P<0.05) interactions influenced the pH values of the samples *(Table 3)*. During the storage period, the pH values of all cheese samples decreased (P<0.05) *(Table 5)*. Because of the 90-day-storage period, the lowest pH value was determined in TCBI with 4.54, and the addition of probiotic bacteria was associated with a decrease in pH values. Keles and Atasever [4] and Morul and İşleyici [41] have similarly reported that the pH values of tulum cheeses decreased during the storage period.

Our results indicate that the increase regarding milk acidity and the decrease in pH values in all the samples during storage period was due to the lactic acid produced by the activities of starter and non-starter bacteria found in the cheese which could ferment lactose.

Probiotic bacteria x Packaging x Storage Period *(Table 3)* had a significant effect on the water activity of the tulum cheeses. During the storage period, the water activity (a_w) of all tulum cheese samples decreased (P<0.05) *(Table 5)*. At the beginning of storage, the highest a_w value was determined in TCMI sample with 0.940, whereas the lowest aw value at the end of the 90-day-storage period was determined in TCLI sample with 0.849. Erdem and Patır [42] have reported that the average a_w values in tulum cheeses range between 0.910 and 0.930. However, and Morul and İşleyici [41] determined these values to range between 0.870 and 0.980. The differences between the studies were associated with the differences in the packing materials and storage period.

The effect of Probiotic bacteria x Packaging x Storage Period interactions had a significant effect on TAMB, TAPB, and yeast/mold counts *(Table 3)* (P<0.05). TAMB counts increased in all tulum cheese samples during the storage period (P<0.05) *(Table 5)*. The highest increase was found to be in TCBC with 1.2 log cfu/g. The results obtained were lower than those reported by Çağlar [43] thus, corroborating the results of Güven and Konar [44], Hayaloglu et al.[45], and Çakır [40].

Similarly, TAPB counts increased in all tulum cheese samples during the storage period (P<0.05) *(Table 5)*. At the beginning of the storage period, the lowest TAPB count was in TCBC with 2.16 log CFU/g whereas the highest value was in TCMC sample with 3.86 log cfu/g. Morul and İşleyici [41] have reported the TAPB counts in Divle tulum cheeses were between 2.70 and 8.48 log cfu/g while Kara and Akkaya [46] have reported the TAPB counts in Afyon tulum cheeses to range between 3.07 and 5.83 log cfu/g.

The total yeast/mold counts in cheese samples increased during the storage period (P<0.05) *(Table 6)*. The highest increase was in the TCLC sample with 2.85 log cfu/g, and the lowest increase was in the TCLS with 1.29 log cfu/g. These results are lower than those reported by Çağlar [43], Çetin et al.[47], Hayaloğlu et al.[45], and similar to those reported by Öksüztepe et al.[48]. The differences regarding TAMB, TAPB, and yeast/mold counts from similar previous studies was associated with the microbial quality of the milk used and the differences in hygiene and sanitation conditions during production.

The effects of adding probiotic bacteria, packaging, storage period, probiotic bacteria x packaging and probiotic bacteria x packaging interaction, probiotic bacteria x packaging x storage period interactions on lactic acid bacteria counts and *Lactococci* counts were statistically significant at P<0.01 level while probiotic bacteria x storage period was statistically significant at P<0.05 level *(Table 4)*.

Lactic acid bacteria count increased by an average of 0.5 log cfu/g during the entire storage period, The highest increase was determined in TCLS with 0.75 log cfu/g (P<0.05) *(Table 6)*. The results obtained in this study are higher those reported by Güven and Konar [44], lower than those of Morul and İşleyici [41] and Kara and Akkaya [46] and similar to those of Çetin et al.[47]. The difference between the results can be associated with the presence of probiotic bacteria used in production, packaging materials, and storage conditions.

There was an increase in the *Lactococci* count during the storage period (P<0.05) *(Table 4)*. The highest increase was determined in the TCMS sample *(Table 6)*. Kara and Akkaya [46], in their study on the determination of microbiological and physicochemical properties and lactic acid bacteria distribution of Afyon tulum cheeses, reported that the average *Lactococci* counts similar to those obtained in the present study.

There was an increase in the *Pseudomonas* count during storage in tulum cheese, and the highest increase was found in TCMS (P<0.05) *(Table 6)*. Morul and İşleyici [41] reported the average *Pseudomonas* counts to be 3.60 log cfu/g, which is similar to the counts reported in the present study.

The lipolytic bacterial counts of the samples increased during storage (P<0.05) *(Table 6)*. At the end of the 90-day-storage period, the highest numbers of lipolytic bacteria were detected in TCMC with 5.30 log cfu/g. Kara and Akkaya [46] have reported that the average lipolytic bacteria counts in Afyon tulum cheeses ranged between 2.55 and 4.60 log cfu/g. Similarly, the proteolytic bacteria count increased by an average of 1 log cfu/g during storage *(Table 6)*. The highest increase was found to be at TCLS with 1.32 log cfu/g. Kara and Akkaya [46] determined the average proteolytic bacterial counts to be 2.55 log cfu/g. The difference between the studies was attributed to the packaging material used and the duration of the storage period.

Bacterial counts of *Bifidobacterium animalis* spp. *lactis* species decreased throughout the entire storage period (P<0.05) *(Table 7)*. The highest bacterial count was determined in the TCBC sample with 5.93 log cfu/g.

Similar to the decrease in the Bifidobacterium bacteria counts, the number of *Lactobacillus acidophilus* were determined to decrease by an average of 0.5 log cfu/g during 90 days of storage (P<0.05) *(Table 7)*. The highest decrease was in the TCLS sample with 0.5 log cfu/g, and the lowest decrease was in TCBS with 0.25 log cfu/g.

The decrease in *Bifidobacterium animalis* spp. *lactis* and *Lactobacillus acidophilus* bacterial counts are associated with a decrease in a_w value and the increase in lactic acid bacteria and *Lactococci* counts and acidity percentage during the storage period.

Erzincan tulum cheese is a local cheese produced traditionally in Turkey. Pasteurization is not commonly used in traditional cheese production methods. This study aimed to produce a pasteurized novel dairy product with a good taste and rich nutritional value, which does not pose a health risk.

Moreover, the addition of probiotic bacteria to the tulum cheese increases the functional properties of the cheese that has beneficial effects on human health, particularly the digestive system.

Some adverse effects such as odor originating from the skin bag used in traditional Erzincan tulum cheese production were avoided by using different packaging materials. The taste and aroma of the product were enhanced to produce a more appealing product with aesthetic and health benefits for the food industry in both Turkey and the world.

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