# Psychrotrophic Bacteria and Yeast Populations in Turkish White and Tulum Cheeses

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#### Abstract

In this study, pH and microbiological (psychrotrophic bacteria and yeast) properties of White and Tulum cheeses were investigated. According to the results of our study, the lowest pH value identified was 4.42, and the highest pH was 5.84 in the cheese samples. Psychrotrophic bacteria counts ranged from 4.15 to 6.21 log CFU/g for White cheese and 4.02 to 6.15 log CFU/g for Tulum cheese. Psychrotrophic yeast counts ranged from 2.98 to 7.58 log CFU/g for White cheese and 2.99 to 6.77 log CFU/g for Tulum cheese. The highest values of psychrotrophic yeast were detected in the Tulum cheese samples. As a result, it was determined that the numbers of psychrotrophic bacteria and yeast were high in both Tulum and White cheese samples. These high numbers of psychrotrophic microorganisms during cheese storage in the refrigerator may increase even further and reduce shelf life. To extend the shelf life of these cheeses, it is recommended to ensure hygienic handling practices and explore the use of natural preservatives or modified atmosphere packaging.

Keywords: Bacteria, Yeast, Psychrotrophic, Tulum cheese, White cheese.

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# 1. Introduction

Cheese is a dairy product that is widely consumed around the world. It contains high quality proteins, fat, minerals and vitamins. It also contains essential amino acids, which are considered essential and must be taken from external sources. As cheese is a concentrated nutrient, it has more compounds such as protein, fat and vitamins than milk. It is also rich in calcium and phosphorus (Hayaloğlu & Özer, 2011; Üçüncü, 2008).

In case the proper hygienic and technological conditions are not followed during cheese production, the microorganisms and their metabolites that contaminate the cheese can make this very healthy product harmful. All the molds that were isolated from moldy cheese (blue cheese) samples were species of *Penicillium* and *Aspergillus*. Toxic molds are able to alter the majority of foodstuffs, and cheese is one of the most important of them. Under optimal fermentation conditions, molds derived primary metabolites are beneficial in the production of various fermented

products. Secondary metabolites known as mycotoxins, on the other hand, lead to economic damages and health problems (Özkalp & Durak, 1998).

Another classification of microorganisms based on their temperature requirements is psychrotrophic microorganisms. They are microorganisms that show the ability to flourish at temperatures of 7°C or lower and are commonly found in water, soil, plants, animal products in nature. Important psychrotrophic bacteria include bacteria such as *Pseudomonas, Acinetobacter, Alcaligenes, Flavobacterium* (Akan et al., 2014).

In order to prevent psychrotrophic bacteria from contaminating milk, hygiene should be paid attention. Since psychrotrophic bacteria lead to economic loss, complicate the processing of milk, have pathogenic effects on processed products and shorten the storage period, they are the microorganisms that need attention. In pasteurization (65-69 °C for 15 s), Gramnegative psychrotrophic bacteria are decreased by 77-97%, whereas Gram-positive bacteria may still be found in the environment. It has been noted that when the number of bacteria does not exceed 10<sup>6</sup> CFU/g, there is no problem (Akan et al., 2014). This study is aimed to identify the psychrotrophic bacteria and yeast populations in White and Tulum Cheeses, which are produced and consumed widely in Türkiye.

## 2. Material and Methods

#### 2.1. Material

White cheeses were collected from 10 different sellers in Kayseri market as material in this study. Tulum cheeses were collected from Tunceli, Erzincan and Elazığ provinces of Eastern Anatolia Region of Türkiye. Each sample was transported to the laboratory under refrigerated conditions (4°C).

#### 2.2. pH

After weighing 10 g of each cheese sample, 100 ml of distilled water was added. Afterwards, the samples were homogenized in Ultraturrax (IKA T18 Basic, Germany). Samples homogenized were measured with a calibrated pH meter (WTW, Inolab 720, Germany) (Ayar et al., 2023; Ozturk et al., 2021).

# 2.3. Microbiological Analysis of Cheese Samples

The samples were brought to the laboratory in sterile packages and 10 g samples were placed in a stomacher bag under sterile conditions. Then 90 ml of sterile Ringer's solution (Merck) was added and homogenized in Stomacher for 1 min. Subsequently, serial dilutions up to 106 were prepared (Sagdic et al., 2010).

#### 2.4. Enumeration of Psychrotrophic Bacteria

For determination of psychrotrophic bacteria counts, Plate Count Agar (PCA, Merck) was used. Suitable dilutions were inoculated on PCA medium by using the spread plate method and the petri dishes were incubated at 4°C for 10 days. After incubation, the colonies were counted and the results were given as log CFU/g (Munsch-Alatossava & Alatossava, 2006).

#### 2.5. Enumeration of Psychrotrophic Yeast

To determine the number of psychrotrophic yeasts in cheese samples, Dichloran Rose Bengal Chloramphenicol Agar (DRBC, Merck) was used. Suitable dilutions were inoculated on DRBC Agar medium using the spread plate method and the petri dishes were incubated at 4°C for 10 days. After incubation, colonies were counted and the results were given as log CFU/g (Kobatake et al., 1992).

#### 2.6. Statistical Analysis

The statistical analysis of the cheese samples was conducted using the SPSS 18 software package. A oneway analysis of variance (ANOVA) was performed to determine the differences between the samples. Subsequently, Duncan's multiple range test was applied to identify significant differences at the 0.05 significance level.

## 3. Results and Discussion

The pH values of Tulum and White cheese samples are given in Table 1. The pH values of Tulum cheese samples ranged between pH 4.82-5.84. It was determined that the pH values were pH<5 especially in TC4 and TC6 samples. In addition, pH values of other Tulum cheese samples were found to be pH>5 as shown in the related table.

The pH values of TC2 and TC9 samples were higher than the other samples. The pH values of WC1, WC2 and WC7 samples were pH 5.12, 5.10 and 5.10, respectively. In the other White cheese samples were observed to have pH<5 and WC8 sample had the smallest pH with pH 4.42.

Table 1. pH values of Cheese Samples

Tulum Cheese		White Cheese	
Sample	pH	Sample	pH
TC1	$5.19 \pm 0.08^{cd}$	WC1	$5.12 \pm 0.02^{f}$
TC2	$5.63 \pm 0.06^{e}$	WC2	$5.10\pm0.02^{\mathrm{f}}$
TC <sub>3</sub>	$5.26 \pm 0.05^{d}$	WC3	$4.55 \pm 0.05^{b}$
TC4	$4.96 \pm 0.04^{b}$	WC4	4.67±0.04 <sup>c</sup>
TC5	$5.17 \pm 0.05^{\circ}$	WC5	$4.88{\pm}0.18^{de}$
TC6	$4.82 \pm 0.01^{a}$	WC6	$4.78 \pm 0.04^{cd}$
TC7	$5.00\pm0.04^{\mathrm{b}}$	WC7	$5.10 \pm 0.11^{\mathrm{f}}$
TC8	$5.03 \pm 0.05^{b}$	WC8	$4.42 \pm 0.05^{a}$
TC9	$5.84 \pm 0.05^{f}$	WC9	$4.96 \pm 0.08^{e}$
TC10	5.16±0.08 <sup>c</sup>	WC10	4.72±0.10 <sup>c</sup>

TC: Tulum cheese, WC: White cheese, Values with the same letters in the columns are not significantly different at the 0.05 level.

The psychrotrophic bacteria counts of Tulum cheeses and White cheeses are given in Table 2. The psychrotrophic bacteria counts ranged between 4.59 and 6.21 log CFU/g in the Tulum cheese and between 4.02 and 7.40 log CFU/g in the White cheese. The TC3 sample contained the least psychrotrophic bacteria with 4.59 log CFU/g. The highest level of psychrotrophic bacteria was observed in the TC2 sample as 6.21 log CFU/g. The lowest number of psychrotrophic bacteria among the White cheese samples was found in the WC8 sample, while the highest was 7.40 log CFU/g in the WC4 sample.

Tulum Cheese		White Cheese	
Bacteria Count (log CFU/g)	Sample	Bacteria Count (log CFU/g)	
4.89±0.27 <sup>ab</sup>	WC1	$4.09 \pm 0.20^{a}$	
$6.21 \pm 0.09^{d}$	WC2	$6.09 \pm 0.05^{\circ}$	
$4.59 \pm 0.16^{a}$	WC <sub>3</sub>	$5.51 \pm 0.15^{b}$	
5.40±0.20 <sup>c</sup>	WC4	$7.40 \pm 0.08^{d}$	
$4.65 \pm 0.07^{a}$	WC5	$4.11 \pm 0.07^{a}$	
$5.13\pm0.25^{\mathrm{bc}}$	WC6	4.11±0.10 <sup>a</sup>	
$4.85\pm0.21^{ab}$	WC7	6.15±0.04 <sup>c</sup>	
$5.17 \pm 0.08$ bc	WC8	$4.02 \pm 0.05^{a}$	
$5.31 \pm 0.11^{\circ}$	WC9	6.11±0.05 <sup>c</sup>	
5.44±0.07 <sup>c</sup>	WC10	$5.73 \pm 0.15^{b}$	
	m Cheese Bacteria Count (log CFU/g) 4.89±0.27 <sup>ab</sup> 6.21±0.09 <sup>d</sup> 4.59±0.16 <sup>a</sup> 5.40±0.20 <sup>c</sup> 4.65±0.07 <sup>a</sup> 5.13±0.25 <sup>bc</sup> 4.85±0.21 <sup>ab</sup> 5.17±0.08 <sup>bc</sup> 5.31±0.11 <sup>c</sup> 5.44±0.07 <sup>c</sup>	Im Cheese       Wf         Bacteria Count       Sample $(log CFU/g)$ 4.89±0.27 <sup>ab</sup> WC1 $6.21\pm0.09^d$ WC2 $4.59\pm0.16^a$ WC3 $5.40\pm0.20^c$ WC4 $4.65\pm0.07^a$ WC5 $5.13\pm0.25^{bc}$ WC6 $4.85\pm0.21^{ab}$ WC7 $5.17\pm0.08^{bc}$ WC8 $5.31\pm0.11^c$ WC9 $5.44\pm0.07^c$ WC10	

Table 2. Psychrotrophic Bacteria Count of Cheese Samples (log CFU/g)

TC: Tulum cheese, WC: White cheese, Values with the same letters in the columns are not significantly different at the 0.05 level.

The psychrotrophic yeast results of White and Tulum cheeses are presented in Table 3. Psychrotrophic yeasts counts of Tulum and White cheeses were in the range of 2.98-7.58 log CFU/g and 2.99-6.77 log CFU/g, respectively. The psychrotrophic yeasts were 2.98 log CFU/g in TC3 sample and 7.58 log CFU/g in TC9 sample. The lowest number of psychrotrophic yeasts was determined in WC1 sample with 2.99 log CFU/g, while the highest number of yeasts was determined in WC2 sample with 6.77 log CFU/g. In general, it was identified that the counts of psychrotrophic yeast in the samples had high values. It is observed that psychrotrophic yeast counts of Tulum cheeses had more than White cheeses. The WC1 and TC3 samples were found to have psychrotrophic yeast <3 log CFU/g.

In the different studies, it was determined that the pH values of White cheeses were pH 4.84 (Sağun et al., 2001), pH 4.50 (Güler & Uraz, 2004), pH 4.88-4.96 (Öner et al., 2006), pH 4.6-5.3 (Cinbaş & Kılıç, 2005). These results are in line with the pH values found in our study. It was found that the numbers of psychrophilic bacteria in fresh White cheese samples sold in Elazığ were  $1.5 \times 10^2$ -8.3×10<sup>4</sup> CFU/g (Dığrak et al., 1996). Another study on home-made cheese samples produced in Muğla showed that the numbers of psychrophilic bacteria were between  $3.2 \times 10^3$  and  $2.5 \times 10^5$  CFU/g (Uğur 2001). The counts of psychrophile bacteria in Afyon Tulum cheeses were determined as 3.22-4.57 log CFU/g (Kara & Akkaya, 2015).

Table 3.	Psychrotrophic Yeast Count of Cheese Sam-
ples (log	CFU/g)

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Tulum Cheese		White Cheese	
Sample	Yeast Count (log CFU/g)	Sample	Yeast Count (log CFU/g)
TC1	$6.09 \pm 0.03^{de}$	WC1	$2.99 \pm 0.12^{a}$
TC2	$6.16 \pm 0.04^{\text{ef}}$	WC2	$6.77 \pm 0.06^{f}$
TC3	$2.98 \pm 0.03^{a}$	WC <sub>3</sub>	$4.39 \pm 0.12^{\circ}$
TC4	$5.90 \pm 0.02^{d}$	WC4	$6.27 \pm 0.01^{g}$
TC5	4.17±0.24 <sup>c</sup>	WC5	$6.01 \pm 0.03^{\text{ef}}$
TC6	$3.39 \pm 0.12^{b}$	WC6	$4.02 \pm 0.09^{b}$
TC7	$4.22 \pm 0.02^{\circ}$	WC7	$5.78 \pm 0.04^{d}$
TC8	$6.38 \pm 0.03^{f}$	WC8	$6.23 \pm 0.02^{g}$
TC9	$7.58 \pm 0.14^{g}$	WC9	$5.87 \pm 0.10^{de}$
TC10	$7.54 \pm 0.01^{g}$	WC10	$6.11 \pm 0.04^{fg}$
ma m 1	1	** 1	1.1

TC: Tulum cheese, WC: White cheese, Values with the same letters in the columns are not significantly different at the 0.05 level.

In the TS 591 Standard for White cheese, the number of yeasts and molds permitted was set as 10<sup>3</sup> CFU/g (TSE, 1995). If the number of yeasts is above the relevant TSE standard, it may indicate that hygienic conditions are not maintained in the period from milking to marketing of cheese. Yeast and mold that can be present in cheese can be a minimum of 102 CFU/g and a maximum of 10<sup>3</sup> CFU/g. There is no limit defined in the standard for psychrotrophic bacteria.

#### 4. Conclusions

A significant population of psychrotrophic bacteria and yeast has been observed in Tulum and White cheeses consumed in the Türkiye. The poor hygiene conditions of production affect the microbial load in cheeses. A high number of psychrotrophic microorganisms will adversely affect the cheese shelf life. It is important to take into account that the number of psychrotrophic bacteria and yeasts may increase due to the equipment, water, personnel, air and other contamination sources used during the cheese production process.

To mitigate these effects and extend the shelf life of these cheeses, it is recommended to ensure strict hygienic handling practices and explore the use of natural preservatives or modified atmosphere packaging to control psychrotrophic microorganism growth. Implementing these measures can significantly reduce microbial contamination and enhance the safety and quality of cheese products.

#### **Declaration of Competing Interest**

The authors declare that they have no financial or nonfinancial competing interests.

#### **Author's Contributions**

S.B. Tunaydin, S. Güvercin, A. Ervan: *Definition, Data Collection, Investigation, Methodology*.

İ. Öztürk (🕩 0000-0003-1434-4763): Definition,

Investigation Conceptualization, Writing, Editing, Methodology, Supervision.

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