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## Effects of Casing Materials and Ripening Period on Some Microbiological Properties of Tulum Cheese

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**Abstract:** Microbiological contents of Tulum cheese samples made from raw and pasteurized milk and ripened in goatskin, wooden box and plastic were compared. The casing materials affected yeast and mould counts significantly ( $P < 0.01$ ). The yeast and mould counts in wooden box ripened cheese (5.61 log fu/g) was significantly lower than those of tulum and plastic box ripened cheese. Pasteurization of milk resulted in a decrease in number of coliform bacteria. Casing material had significant effect on TAMB, yeast, moulds, coliform and *E. coli* counts. Ripening period had also influenced the microorganism in cheese. Mostly the count of all above mentioned microorganisms were decreased at longer ripening period (90th day).

**Key words:** Tulum cheese, microbiological quality, pasteurization

### Introduction

Tulum cheese is one of the traditional cheese types that is preferred by a large community in Turkey. It is made especially in East Regions of Turkey, where due to lack of modern facilities and difficult transportation the production of white and kasar cheese types are not economical. Recent years exceeding being a cheese type unique to Erincan, Elazığ, Tunceli, Bingöl cities it is gradually becoming popular all over Turkey. Its name, tulum, comes from casing material, goatskin. Tulum cheese is characterized with white, cream-like colour, lightly acidic, butter-like aroma, high fat content, easily digestible with semi-hard body. Although Karaibrahimoglu and Üçüncü (1988) proposed a model method, Tulum cheese still has no standard production method. Processing practice changes from city to city and factory to factory. On the other hand Tulum cheese is produced from unhomogenized milk. Strength and amount of rennet used, the size of casing material, ripening conditions and period is not standard, therefore it is not possible to obtain Tulum cheese with high standard quality in markets.

Tulum cheese is generally made from raw sheep milk. Sometimes goat milk and cow milk can be added to sheep milk. Raw milk is sieved through a piece of cloth and its temperature is adjusted to that of a fresh drawn milk by warmth of finger. Renneting temperature ranged between 27-41°C during cold and 28-39°C during hot weathers. Unheated raw milk cheese gives superior taste. Since raw milk is not pasteurized, it is probably a source of pathogen and some putrefactive microorganisms. Probably due to unique micro flora, Tulum cheese has superior taste and aroma to pasteurized milk cheese (Kurt *et al.*, 1984).

A spoonful of rennet is added very slowly to approximately 20 litter milk. The container is smothered with a cloth of some-fold to maintain the constant temperature. Controlled by finger, curd formation is completed when it does not stick to finger within approximately 57-160 min. It is filled into cloth bags of 1.5-kg with ladle. After removing whey curd is transferred to a large vat and kneaded with bare feet thoroughly, salt is added followed by filling into goatskin, tulum, tightly to remove air. The presence of air in cheese may result in spoilage. Some producers knead the cheese with some milk. It is believed that milk enables cheese to be soft, and gets it easy to press into tulum. Salt is sprinkled on cheese and edge of tulum is knitted tightly removing air. Cheese in tulum is ripened at 6-8°C and 75-80% RH for 90-

120 days (Akyuz, 1981).

Since production of sheep milk is insufficient, the objective of this research was to investigate the possibility of production of tulum cheese from cow milk which could be obtained throughout the year enabling tulum cheese supply at retailers with high quality and standard properties.

The effect of pasteurization of milk and different casing materials on some microbiological properties of Tulum cheese were also studied.

### Materials and Methods

Cow milk was obtained from Ataturk University Agriculture Management. Goatskins (tulum) of 8-10 kg capacity that were cleaned from blood and meat particles, washed thoroughly with warm water and dried, plastic boxes made from high-density polythene (Petkim 0464) and wooden boxes of 2.5kg capacity (15x17x12 cm<sup>3</sup>) made from dry, odorless cedar were used as casing materials.

Lyophilized cultures of *S. lactis* and *L. casei* were obtained from Christian Hansen's Laboratory.

**Cheese production:** Raw cow milk was divided into two equal parts. The first part was processed into tulum cheese directly, the second part was processed into tulum cheese following pasteurization (60°C, 30 min) and inoculation with a mixture of *S. lactis* 71580 and *L. casei* 71650 (1:1) at 1%.

Both types of tulum cheese were ripened at  $7 \pm 1^\circ\text{C}$  and 70 % RH in three kinds of casing materials, tulum (goatskin), wooden box and plastic box.

The cheese put into tulum was divided into three equal parts. When 2.5 kg cheese was put, tulum was tied tightly on which two other cheese parts were added in the same way pressing to remove air. Some microbiological properties of all cheese samples (ripened in tulum, wooden box and plastic bag) were investigated on 30<sup>th</sup>, 60<sup>th</sup> and 90<sup>th</sup> days of ripening period. The contents of TAMB, yeast-mould, lactic bacteria, coliform, *S. aureus* and *S. aureus* C (+) were determined, and the effects of casing materials and ripening period on the microbiological properties of cheese were also evaluated.

Each part of cheese was analyzed at each period. Total aerobic mesophilic bacteria, yeast and mould, lactic bacteria, coliform bacteria and *S. aureus* counts were determined using PCA, PDA (with pH adjusted at 3.5), MRS Agar, VRBA and Staph. Medium-110 after incubation at 30°C for 48 hr, 22-25°C for 5-days, 30°C for 72 hr, 37°C for 24 hr and 37°C for

48 hr (Hausler *et al.*, 1974). In order to count and identify the *E. coli* of the micro-organisms grown on solid breed (agar), those which were considered coliform, were inoculated into liquid buyon in tubes and incubated at 45.5 °C for 24-48 hr. IMVIC tests were applied to identify those (+) isolates which formed gas in tubes. To determine the number of *S. aureus* Coagulase (+) all colonies were inoculated into Brain Heart Infusion bouyyon. After incubation at 35-37°C for 24 hr, 0.1 ml from each culture was transferred to little tubes and added with 0.3 ml plasma (human blood). During incubation of first 6 hr at 37°C, the tubes were controlled with an hour intervals. Those tubes in which coagulation occurred were considered as C (+) and those in which coagulation did not occur for 24 hr were considered as C (-) (Speck, 1976). This study was carried out according to Completely randomized blocks design with two replications.

### Results and Discussion

As is evident from Table 1, pasteurization of raw milk resulted in a decrease in number of coliform by approximately 5.26 log units ( $p < 0.01$ ) indicating the importance of studies to produce Tulum cheese from pasteurized milk.

The results of analysis of variance showed that the casing material had significant effect on TAMB, yeast-mould, coliform, *E. coli* counts and lactic acid bacteria counts. The results of Duncan's New multiple range test are given in Table 2.

**Coliform group bacteria:** The presence of coliform group bacteria in cheese is not desired because these cause structural defects in cheese and *E. coli* is considered as indicator of contamination. According to the Turkish Standards Institute (TS 3001) a maximum count of 100 cfu/g of coliform group bacteria and  $< 10$  cfu/g *E. coli* are allowable in Tulum cheese (Anon., 1989). Tulum cheese is produced in Turkey from raw milk that usually contains high number of coliform and *E. coli*. According to findings of other workers, there have been instances, that high counts of coliform bacteria were found in Tulum cheese. Bostan (1991) found coliform in 21 samples out of 38 and *E. coli* in 29 samples. He also reported that the average coliform group bacteria count in Tulum cheese samples was  $2.2 \times 10^3$  cfu/g. The coliform bacteria counts in 14 out of 28 samples in plastic casing and 7 out of 10 samples in goatskin were below detectable level ( $< 10$  cfu/g). Bostan (1991) also reported that 20 out of 28 samples in plastic and 9 out of 10 samples in goatskin contained  $< 10$  cfu/g *E. coli*, while average number of *E. coli* was  $2.9 \times 10^3$  cfu/g.

Kurt *et al.* (1991) determined that coliform group bacteria content of Tulum cheese samples ranged between  $3.75 \times 10^2$  and  $2.50 \times 10^7$  cfu/g, average count was  $3.20 \times 10^3$  cfu/g.

Arici and Simsek (1991) found coliform group bacteria in control (raw milk cheese) ranging from  $7.0 \times 10^7$  to  $4.0 \times 10^3$  cfu/g during ripening period. They also reported that only one of the fresh (just made) pasteurized milk cheese samples contained coliform but it disappeared at 4<sup>th</sup> week of ripening period.

Digrak *et al.* (1994) found that 70.5 % of 17 Tulum cheese samples obtained from retailers in Elazig Kapaliçarsi (Turkey) contained *E. coli*. They also reported that coliform group bacteria counts ranged from  $2.4 \times 10^2$  to  $2.4 \times 10^3$  cfu/g. Ceylan *et al.* (2000) found coliform counts 3.852 log cfu/g in spicy Tulum cheese.

Bostan and Ugur (1992) reported that coliform group bacteria disappeared on 60<sup>th</sup> day of ripening period in raw milk Tulum cheese samples and on 15<sup>th</sup> day in pasteurized milk cheese. In this study it was determined that the casing material

affected the coliform group bacteria counts ( $p < 0.01$ ) significantly. The least coliform bacteria count was determined in cheese ripened in wooden box while the highest number was in plastic bag ripened cheese. The coliform content of both wooden box and plastic bag ripened cheese were statistically similar to that of tulum ripened cheese. On the other hand the number of *E. coli* in Tulum ripened cheese was fewer than those of both wooden box and plastic bag ripened cheese samples probably due to contamination during processing (Table 2).

Fresh cheese from pasteurized milk contained it below detectable level ( $< 10$  cfu/g) (Table 1). Fresh raw milk cheese contained the highest number of coliform group bacteria. During first stage of ripening period it increased and the number of coliform determined at each period was statistically different ( $p < 0.01$ ) (Table 3). The highest number of coliform and *E. coli* were found on 30<sup>th</sup> day. And then it decreased again. The least count of *E. coli* was determined on 90<sup>th</sup> day of storage. The coliform group bacteria count in wooden box decreased during ripening to  $< 10$  cfu/g on 90<sup>th</sup> day. The coliform counts determined in raw milk cheese conformed only on 90<sup>th</sup> day to TS limits.

**Yeast and Mould:** According to Turkish Tulum Cheese Standard TS 3001, a maximum of 100 cfu/g yeast and mould is allowable (Anonymous, 1989). In this study it was determined that all the Tulum cheese samples contained yeast and mould starting from first stages of ripening period. The yeast and mould counts were  $1.10 \times 10^5$  cfu/g in raw milk fresh cheese. It was significant that fresh Tulum cheese made from pasteurized milk also contained 3.76 log cfu/g yeast and mould due to insufficiently aseptic conditions during production and packaging process (Table 1). The casing materials affected yeast and mould count significantly ( $p < 0.01$ ). The yeast and mould count in wooden box ripened cheese, average of 5.61 log cfu/g, was significantly lower than those of Tulum, (average 5.969 log cfu/g) and plastic ripened cheese, (average of 5.801 log cfu/g) (Table 2). The changes in yeast-mould during ripening period was significant ( $p < 0.01$ ). During ripening period, the yeast and mould counts of raw milk cheese samples in wooden box and plastic dropped constantly (Table 1). However, mould and yeast counts of pasteurized milk cheese packed in goatskin decreased on 60<sup>th</sup> day (4.90 log cfu/g) as compared to that of 30<sup>th</sup> day ( $9.80 \times 10^7$  cfu/g) and increased again on 90<sup>th</sup> day. But, yeast and mould counts of pasteurized milk cheese samples those ripened in wooden boxes and plastic increased constantly during storage period.

Bostan (1991) reported that the average yeast and mould content in 38 Tulum cheese samples was  $1.1 \times 10^5$  cfu/g. Bostan and Ugur (1992) found that there was no apparent difference between raw milk cheese and pasteurized milk cheese in the number of yeast and mould. They reported that small number of yeast and mould in curd increased at first and then decreased slightly. The least number of yeast and mould was  $8.2 \times 10^4$  cfu/g. Kurt *et al.* (1991) found the average yeast and mould count,  $1.99 \times 10^3$  cfu/g ranging from  $4.0 \times 10^3$  to  $6.8 \times 10^3$  cfu/g. They attributed the high yeast and mould content of Tulum cheese to contamination from air during ripening.

Pasteurization process affected total aerobic mesophilic bacteria (TAMB) counts in fresh cheese significantly ( $p < 0.01$ ). The TAMB counts were 9.11 log cfu/g in fresh Tulum cheese made from raw milk and 8.76 log cfu/g in fresh Tulum cheese made from pasteurized milk (Table 1). The casing material had significant effect ( $p < 0.01$ ) on TAMB count.

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Table 1: Some microbiological properties of Tulum cheese samples (log cfu/g)

Casing material	Ripening	Coliform	<i>E. coli</i>	Yeast-mould	LAB	TAMB	<i>S. aureus</i>	<i>S. aureus</i>
Raw milk	Fresh	5.26	4.5	5.04	8.36	9.11	2.32	-
Tulum (*)	30.	2.58	-	6.58	7.00	7.36	-	-
	60.	1.48	-	7.52	8.20	8.34	-	-
	90.	1.88	-	6.38	7.65	7.60	-	-
Wooden box (*)	30.	2.38	2.2	7.23	6.18	7.26	-	-
	60.	1.48	1.3	6.18	8.15	8.26	-	-
	90.	-	-	5.48	8.30	7.23	-	-
Plastic bag (*)	30.	2.65	2	7.15	6.81	8.26	-	-
	60.	2.48	2.4	5.93	7.91	7.96	-	-
	90.	1.85	-	5.9	7.04	7.58	-	-
Pasteurized	Fresh	3.76	8.8	3.76	8.75	8.76	-	-
Tulum (**)	30.	4.98	-	7.99	9.48	9.04	-	-
	60.	3.7	2.8	4.9	9.94	8.97	-	-
	90.	2.78	-	5.57	8.90	8.93	-	-
Wooden box (**)	30.	4.98	4.9	4.53	9.56	9.30	-	-
	60.	4.3	4	5.81	8.98	8.93	-	-
	90.	3.6	-	6.2	8.57	8.58	-	-
Plastic bag (**)	30.	5.04	4.3	5.75	8.23	7.88	-	-
	60.	3.88	3.3	6.08	8.95	8.96	-	-
	90.	2.3	-	6.85	8.77	8.59	--	--

(\*) Cheese made from raw milk, (\*\*) Cheese made from pasteurized milk (-) below detectable level

Table 2: Average counts of some microorganisms found in Tulum cheese samples stored in different casing materials and the results of Duncan test (log cfu/g)

Casing material	Coliform	<i>E. coli</i>	TAMB	LAB	Yeast- mould
Tulum	2.854 ab	1.154 b	8.522 a	8.373 a	5.969 a
Wood	2.749 b	2.124 a	8.437 b	8.217 ab	5.606 b
Plastic	2.936 a	2.108 a	8.393 b	8.083 b	5.801 a

Averages (n = 4) followed by different letters are statistically different (p < 0.05)

Table 3: Average counts of some microorganisms found during storage period and the results of Duncan test

Ripening Period (d)	Coliform	<i>E. coli</i>	TAMB	LAB	Yeast- mould
Fresh	2.529 c	2.272 b	8.958 a	8.442 a	4.413 c
30	3.799 a	2.595 a	8.173c	7.901 b	6.522 a
60	2.940 b	2.315 b	8.581b	8.515 a	6.156 b
90	2.118 d	0.000 c	8.089 c	8.039 b	6.077 c

Averages (n = 4) followed by different letters are statistically different (p < 0.01)

The least TAMB count was found in plastic bag ripened cheese and it was statistically the same as that of the wooden box ripened cheese, while the highest TAMB count was determined in Tulum ripened cheese (Table 2). The ripening period had significant effect (p < 0.01) on TAMB content of cheese samples. Table 3 shows that fresh cheese samples had highest TAMB count. The fluctuation in number of TAMB was considered significant but generally it decreased during ripening period to the least number on 90<sup>th</sup> day.

During ripening period the TAMB counts in raw milk cheese samples stored in all three kinds of casing materials were lower than those of fresh cheese samples. On 90<sup>th</sup> day of ripening period, the least TAMB counts were determined in cheese samples packed in wooden box, plastic and Tulum respectively (Table 1). During ripening period, all cheese samples made from pasteurized milk contained higher level of TAMB than those of fresh cheese except packed in plastic and wooden box on 90<sup>th</sup> day than raw milk cheese (Table 1).

Bostan (1991) reported that no remarkable difference in micro-organism content of cheese samples ripened in goatskin and plastic bag. He concluded that cheese ripened in plastic were superior in sensory properties to that ripened in tulum on the other hand both had the same risk for health.

Bostan (1991) found that the cheese samples contained a wide range of microorganisms varying from 4.0x10<sup>7</sup> cfu/g to 5.7x10<sup>8</sup> cfu/g, with an average 3.2x10<sup>8</sup> cfu/g TAMB and the cheese samples in plastic casing and goatskin did not show a apparent difference in microbiological content. Digrak *et al.* (1994) found TAMB count in 17 Tulum cheese samples

between 3.2x10<sup>7</sup> and 9.5x10<sup>8</sup> cfu/g averaging 1.8x10<sup>8</sup> cfu/g. They concluded that the high TAMB content of Tulum cheese samples were due to raw milk used. Bostan and Ugur (1992) carried out a study to produce Tulum cheese from pasteurized milk with a combination of different culture microorganisms. They found the count of TAMB ranged from 5.2x10<sup>7</sup> to 9.1x10<sup>10</sup> cfu/g and reported that there was no apparent difference between pasteurized and raw milk cheese samples in TAMB count. Kurt *et al.* (1991) reported that the minimum, maximum and average numbers of TAMB in 26 Tulum cheese samples collected from Erzurum and Erzincan provinces of Turkey were 2.10x10<sup>7</sup>, 1.55x10<sup>10</sup> and 2.13x10<sup>9</sup> cfu/g. They stated that the high number of TAMB was due to raw sheep milk and long processing procedure (approximately 10 days) of tulum cheese. It was more difficult to obtain clean milk from sheep than cow manually. They also stated that the TAMB content of Tulum cheese decreased during ripening.

**Lactic acid bacteria (LAB):** Lactic acid bacteria (LAB) count plays a fundamental role in ripening of cheese due to lactic acid fermentation. Otherwise it is impossible to prevent growth of coliform and especially proteolytic microorganisms in cheese. Bostan (1991) found the average lactic Streptococci content of 38 Tulum cheese samples 2.2x10<sup>8</sup> cfu/g. Digrak *et al.* (1994) found the number of LAB between 1.3x10<sup>9</sup> and 7.6x10<sup>7</sup> cfu/g, with an average of 1.15x10<sup>7</sup> cfu/g. Kurt *et al.* (1991) found the average number of LAB, 8.56x10<sup>8</sup> cfu/g ranging between 1.80x10<sup>5</sup> and 3.80x10<sup>7</sup> cfu/g. Bostan *et al.* (1992) isolated 684 LAB strains from

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experimental Tulum cheese samples and 488 LAB strains were isolated from good organoleptic quality Tulum cheese samples collected from retailers. At first stages of ripening period *S. lactis* and *S. faecium*; ongoing stages *L. casei*, *L. plantarum*, *S. faecalis* were dominant in experimental cheese samples. Whereas *S. faecium*, *S. faecalis*, *S. lactis*, *L. casei* and *L. plantarum* were dominant.

The lactic acid bacteria counts were 8.36 log cfu/g in fresh cheese made from raw milk and 8.75 log cfu/g in fresh cheese made from pasteurized milk cheese (Table 1).

Casing material had significant effect on LAB count. According to Duncan's multiple range test (Table 2) the LAB content of cheese samples ripened in tulum was significantly higher than that of plastic bag but similar to that of cheese ripened in wooden box.

The fluctuate change in LAB during ripening period was significant ( $p < 0.01$ ). It decreased on 30<sup>th</sup> day and increased on 60<sup>th</sup> day but decreased again on 90<sup>th</sup> day to level on 30<sup>th</sup> day (Table 3).

The highest number of *S. aureus* count was (2.32 log cfu/g) found in raw milk cheese but it was below detectable level ( $< 10$  cfu/g) (Table 1). It can be concluded that *S. aureus* was destructed during ripening of cheese as well as during pasteurization. Bostan (1991) found average Coagulase (+) *Staphylococcus* number as  $8.4 \times 10^3$  cfu/g in 38 Tulum cheese samples. Digrak *et al.* (1994) found that 8 out of 17 Tulum cheese samples contained the number of *S. aureus* below detectable level, while other samples contained ranging between  $4.8 \times 10^2$  and  $1.9 \times 10^5$  cfu/g, with an average of  $3.5 \times 10^4$  cfu/g. They reported that Coagulase (+) *S. aureus* was found in three samples. Arici and Simsek (1991) isolated Coagulate (+) *S. aureus* from raw milk ranging from  $1.2 \times 10^8$  to  $9.5 \times 10^4$  cfu/g during 16 weeks of storage period. The *S. aureus* count of pasteurized milk cheese samples were below detectable level. Bostan and Ugur (1992) reported that all the Tulum cheese samples contained *S. aureus*. The number of *Staphylococcus* in Raw milk Tulum cheese reduced to  $1.0 \times 10^4$  cfu/g on 90<sup>th</sup> day, while it disappeared on 60<sup>th</sup> day, in two other pasteurized milk cheese samples. *S. aureus* disappeared on 90<sup>th</sup> day in raw milk cheese and on 30<sup>th</sup> day in pasteurized milk cheese samples. Since exceeding  $1 \times 10^8$  cfu/g may cause food-borne poisoning so the presence of *Staphylococcus* is undesirable (Frazier *et al.*, 1978).

It can be concluded that Tulum cheese may be a potential source of health risk due to contamination during production,

casing and marketing stages in addition to production from raw milk. Therefore, the milk should be obtained clean and pasteurized. Production process should be standardized and suitable small casing materials should be selected and Tulum cheese should be ripened properly.

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