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Chemical, Microbiological and Sensorial Properties of Tulum Cheese

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This study was carried out to determine the chemical, microbiological and sensorial properties of Tulum cheese, a raw milk cheese. 50 Randomly selected samples of Tulum cheese purchased from different retail markets in Erzurum and Konya regions, were analyzed. The production technique of Tulum cheese was investigated too. It was found that chemical, microbiological and sensorial properties of the samples varied in large range; especially microbiological quality was not very good. It is concluded that it is necessary to undertake hygienic measurements and use heat-treated milk in production and further investigations are required for standardizing the production technique of Tulum cheese.

Key Words: Tulum cheese, Coliform, Enterobacteriaceae, Fecal streptococci, *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella*.

INTRODUCTION

The variety of cheese depends upon cultural habits, natural conditions, milk variety and the production methods employed. There are more than 100 varieties of cheese in Turkey. Mostly produced one is white pickled cheese. Tulum cheese is third in production after white pickled and kasar cheeses. In recent years Tulum cheese production is having an increasing trend. Tulum cheese, a kind of local cheese special for Turkey, is not known worldwide. Tulum cheese is produced traditionally for own consumption of the nomadic people especially those living in high plateaus where milk transportation to the factories is very difficult. It is preferred for its characteristically natural mouldy taste and flavour. During the ripening period, naturally contaminated moulds grow and contribute to the ripening process. Traditionally sheep and goat's milk is used in Tulum cheese production but in recent

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years cow's milk is also in use because of increasing consumption preference. Tulum cheese quality in markets is variable because of difference in kind of milk used in its production. In traditional production procedure milk is renneted after milking with its own temperature and pasteurization and starter culture are not used in production.

Tulum cheese is produced almost in every region of Turkey but production procedures, ripening types and periods differ from each other. As such, Tulum cheese is called by different names, of which mostly known are Erzincan, Izmir, Divle and Çimi Tulum cheese varieties. Izmir Tulum cheese is very different from the others because of its production and ripening type. It's ripened in brine solution like white pickled cheese and packed in tin cans and is also called pickled Tulum cheese. Çimi Tulum cheese variety also differs from others as it is produced from goat's milk while the others are produced from sheep's milk. It's produced in Mediterranean region especially in Antalya region. Erzincan Tulum cheese is produced in East Anatolian Region especially in Erzincan and Elazig provinces. Erzincan and Divle Tulum cheese varieties are similar; there are some differences in production and ripening procedure. For example, there is curd washing procedure in Divle Tulum cheese production. Tulum cheese is placed into goat pelt case (Tulum). In general, Tulum cheese is stored and ripened in natural cold rooms[1-9]. Temperature in ripening rooms is 4-12 °C and relative humidity is about 65-85 %. Generally Tulum cheese is produced in spring and early summer and stored for 3-7 months and then consumed. In recent years cold storage is also in use in ripening. Some researchers[10,11] reported that plastic, semi-synthetic or polyethylene materials used in the packaging of Tulum cheese had positive effects on ripening time (shorter) and cheese quality. However, Guven and Konar[12] reported opposite stating that the Tulum cheese ripened in the goat pelt case had the best sensorial properties. Flow diagram of production process of Tulum cheese is shown in Fig. 1.

Investigations on Tulum cheese are limited in history. But in recent years studies on this cheese type have been an increasing trend. These studies are concentrated especially on microbiological properties of Tulum cheese[3,5,7,8,12-14], pasteurization application and starter culture addition in production of Tulum cheese[3,12,15,16].

In absence of a standard production technique, chemical composition of Tulum cheese is so varied. Chemical compositions of Tulum cheese according to several investigators is given in Table-1.

Table-1 shows that moisture content of Tulum cheese is very variable and it can be said that this type of cheese belongs to semi-hard cheese group. According to Turkish Standard Institution (TS 3001, Tulum Cheese Standard), the moisture content of Tulum cheese should be maximum 40 %.

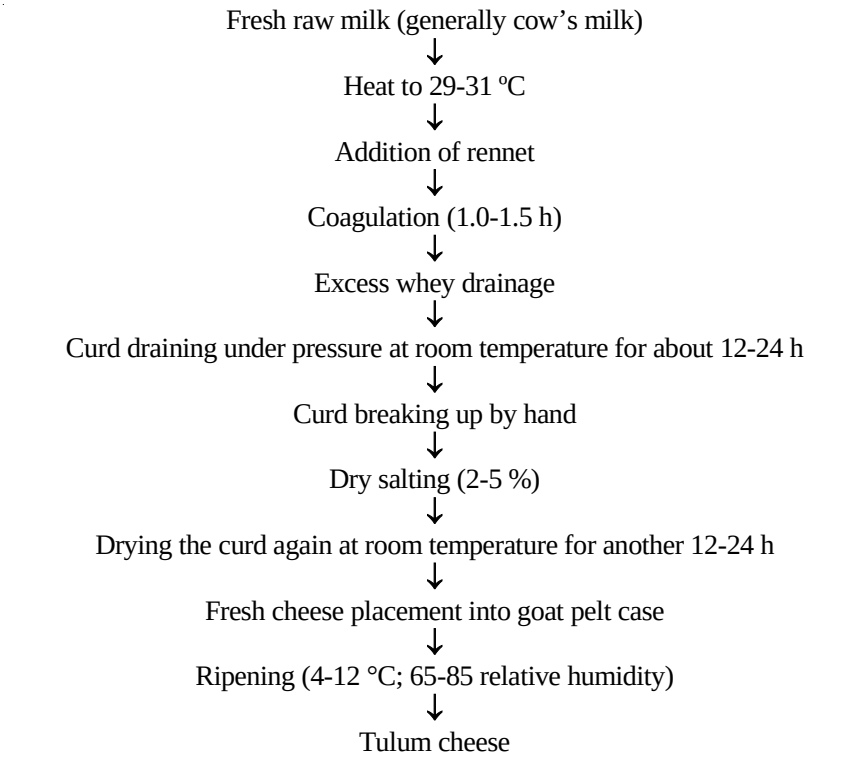

Fig. 1. Flow diagram of production of Tulum cheese

TABLE-1
CHEMICAL COMPOSITION OF TULUM CHEESE

Moisture (%)	Fat (%)	Protein (%)	Salt (%)	Ash (%)	Acidity (lactic acid) (%)	pH	Ref. no.
37.29	34.96	21.54	4.66	5.50	1.66	–	1
44.50	25.73	24.56	3.26	–	1.82	–	15
34.16	–	–	5.02	–	0.58	6.20	28
42.07	24.49	–	3.38	4.77	1.39	–	3
42.81	–	–	–	–	–	5.30	10
46.29	27.76	16.91	3.44	5.22	1.61	–	17
38.19	26.60	27.44	5.96	7.84	2.60	–	24
40.43	30.89	22.91	3.83	–	2.96	–	25
42.86	25.15	25.98	3.36	5.06	1.73	–	4
42.99	21.33	–	3.00	3.78	0.50	5.42	5
45.44	24.28	24.80	4.74	–	1.83	–	26
46.79	28.20	18.51	3.44	–	1.83	–	7
42.25	26.17	–	3.48	–	1.46	5. 38	32
42.87	28.70	21.27	5.81	7.22	1.54	–	27

Arici and Simsek[15] had reported that in initial stages of Tulum cheese samples produced from raw milk, coliform counts were 7.0×10^7 cfu/g and after 16 weeks of ripening it decreased to 4.0×10^3 cfu/g. Bostan *et al.*[10] determined coliforms in 38 Tulum cheese samples at an average rate of 7.3×10^3 cfu/g. 17 cheese samples obtained from Elazig markets had coliform counts between 2.4×10^2-3.0×10^4 cfu/g according to Digrak *et al.*[17] and the investigators found *E. coli* in 70.5 % of the samples. Kilic and Gonc[13] surveyed 35 Izmir Tulum cheese samples and reported coliforms between 1.2×10^2-2.7×10^7 cfu/g with average value at 1.15×10^5 cfu/g. Kurt *et al.*[6] reported that Erzincan Tulum cheese samples had coliform counts between 3.75×10^2-$2.5x10^7$ cfu/g at average value of 3.2×10^6 cfu/g. Keles and Atasever[5] reported the coliform counts between 0-1.05×10^6 cfu/g and an average of $1.64 \times 10^5 \pm 6.67 \times 10^4$ cfu/g in Divle Tulum cheese.

In a study Bostan and Ugur[3] produced Tulum cheese samples from raw milk experimentally and observed that fecal streptococci counts were in initial stages 2.5×10^5 cfu/g which decreased to 5.9×10^3 cfu/g at 60th and 2.6×10^3 cfu/g at 90th day of ripening. Same authors found fecal streptococci counts in samples from pasteurized and starter culture used Tulum cheese as 2.6×10^7 cfu/g in initial stages including *S. feacalis* decreasing to 3.7×10^6 cfu/g at 90th day of ripening. Other samples not having *S. feacalis* in starter combination, fecal streptococci counts were between 3.3×10^3-4.1×10^3 cfu/g and after 90 d of ripening it was not detected. Keles and Atasever[5] reported the fecal streptococci counts between 2.45×10^5-6.80×10^8 cfu/g at an average of $5.58 \times 10^7 \pm 3.51 \times 10^7$ cfu/g in Tulum cheese.

Bostan *et al.*[10] surveyed 38 Tulum cheese samples for yeast and moulds and reported the counts between 2.1×10^3-2.2×10^7 cfu/g and an average of 1.1×10^6 cfu/g. Digrak *et al.*[17] reported yeast and mould counts between 3.6×10^6-2.5×10^7 cfu/g with average of 3.6×10^6 cfu/g in 17 Erzincan Tulum cheese samples. Kilic and Gonc[13] surveyed 35 Izmir Tulum cheese samples and reported the counts between 8.2×10^3-5.73×10^6 cfu/g and an average of 7.5×10^5 cfu/g. In another study Kurt *et al.*[6] reported that these counts were found at an average of 1.87×10^6 cfu/g in Tulum cheese samples. Keles and Atasever[5] surveyed 20 Divle Tulum cheese samples and reported the counts between 0-3.0×10^7 cfu/g and an average of $3.5 \times 10^6 \pm 1.72 \times 10^6$ cfu/g.

In this study, it was aimed to determine some chemical, microbiological and sensorial properties of Tulum cheese sold in Erzurum and Konya regions.

EXPERIMENTAL

50 Tulum cheese samples (~ 200 g each) were collected randomly from different retail markets in Erzurum and Konya regions and transported in sterile jars to the laboratory. Moisture, fat, salt and ash contents, acidity values and pH degrees were determined according to Tekinsen *et al.*[18].

For microbiological analysis 10 g of samples were homogenized with 90 mL of a sterile 0.1 % peptone water solution by stomacher (Lab Blender 400) and then samples were diluted to 10^{-7} dilution by sterile peptone water.

Total counts of mesophilic aerobic bacteriae were determined using plate count agar (Oxoid CM 325) after incubation for 72 h at 30 ºC. Numbers of coliforms were detected by using Violet Red Bile Agar (Merck, 101406). Plates were incubated at 30 ± 1 °C for 24 ± 1 h.

The enumeration of *E. coli* was performed on TBX medium (Oxoid CM 945) after incubation for 4 h at 30 ºC and after that for 24 h at 45 ºC. At the end of the incubation *E. coli* isolates were biochemically characterized by IVMIC tests[19].

Enterobacteriaceae counts were enumerated via surface plating in violet red bile glucose agar (Merck) and incubating at 37 °C for 24-48 h.

In detecting fecal streptococci, Barnes' thallus acetate tetrazolium glucose agar was used and plates were incubated at 45 ± 1 °C for 48 ± 1 h[20]. Yeast and moulds were detected by using Potato Dextrose Agar acidified with 10 mL/L of 10 % tartaric acid solution. Plates were incubated at 21 ± 1 °C for 5 d.

S. aureus was enumerated on Baird-Parker Medium (Oxoid CM 275) with Egg-Yolk Tellurite Selective (Oxoid SR 0054 C) and incubated for 24 h at 37 ºC. Colonies were examined by catalase test, staphylase test kit (Oxoid DR 0595 A), anaerobic utilization of glucose and mannitol, coagulase test[21].

For enumeration of number of yeasts and moulds were used Rose-Bengal Chloramphenicol Agar Base (RO) (Oxoid CM 549) with chloramplenicol selective supplement (Oxoid SR 0078 E) and OGYE agar (Merck, 1.05978) with OGYE selective supplement (Merck 1.09877). They are incubated for 5 d at 25 ºC.

Salmonella spp. was tested using a modification of the standard method suggested by BAM[21]. 25 g of the sample was aseptically added into 225 mL buffered peptone water (Oxoid CM 509) and stomached for 3 min. The pre-enrichment cultures were incubated for 18-20 h at 37 ºC. After that it was inoculated in the tube including 10 mL Salmonella Enrichment Broth acc to Rappaport-Vassiliadis (Merck 1.07700) and the tubes incubated for 48 h at 42 ºC. After this incubation, it was inoculated on surface drawing method in SS agar (Oxoid CM 99) and BPLS Agar (Modified) (Merck 1.10747) and incubated for 24 h at 35 ºC. After the end of this time, the salmonella colonies pink-coloured inoculated VRB-agar with drawing method for 24 h at 37 ºC. After incubation, presumptive colony of *Salmonella*

was chosen and identified using Gram staining, appropriate biochemical tests Triple Sugar Iron Agar (TSIA, Oxoid CM 277), Lysine Decarboxylase Broth (LDB, Oxoid CM 0308), Areas Test (Oxoid CM 53), Voges proskuer and tryptone water (TW, Merck, 1.10859) and then tested using *Salmonella typhi O, Salmonella typhi H* and *Salmonella antiserum* as received from Refik Saydam Hifsisaha Unit, Ankara, Turkey.

For the *Listeria monocytogenes* 25 g of the sample was aseptically added into 225 mL Palcam broth. To assess the maximum recuperation of *L. monoctogenes*, cultures were kept at 35 ± 1 ºC for 4 h[22] after this step selective agents (polymyxin, B-sulphate, lithium chloride, acriflavin, ceftazidime) were added to obtain selective enrichment for 7 d at 35 ºC under aerobic conditions. Isolations were examined on Palcam agar incubated under microaerophilic conditions (anaerocult C, Merck) in a jar of anaerobiosis for 48 h at 35 ºC. Presumptive isolates were evaluated by gram stain catalase reaction and motility test.

In sensorial analysis, flavour, texture, appearance and colour properties were detected. 5 panelists were experienced before and samples were examined by the panelists by using a 100 points chart[23].

The numerical results are given as mean and standard deviations of cheese samples using SPSS software (SPSS 10.0 for Windows program).

RESULTS AND DISCUSSION

Chemical compositions of Tulum cheese samples were varied. This seems to indicate that the producers use different production practices; there is no standard production method. Some of the chemical results are similar to those Tulum cheese chemical contents reported by investigators. There were some differences in chemical contents of Tulum cheese with some other researchers[3,4,7,15,17,24-27]. Differences resulted from different production methods (for example, quantity of added salt, washing application of the curd). Chemical composition and pH values of the cheese samples are shown in Table-2.

TABLE- 2
CHEMICAL COMPOSITION (%) AND pH VALUES OF TULUM CHEESE SAMPLES

Properties	Mean	SD	Minimum	Maximum
Moisture (%)	38.45	2.11	32.73	46.82
Protein (%)	22.72	1.83	15.78	28.84
Fat (%)	25.09	1.94	12.03	35.20
Salt (%)	3.51	0.21	1.52	6.32
Ash (%)	4.65	0.45	1.90	7.92
Acidity*	1.07	0.04	0.37	1.93
pH	5.18	0.06	4.78	5.66

*Acidity in lactic acid percentage; SD = Standard deviation.

Coliform counts were found 5.27 ± 4.65 cfu/g. Results are found lower than some reports[6,13,28] while higher than some others[10,15,17]. The reason for high number of microorganisms (for example, coliform, *S. aureus*, *E. coli*) may be due to the use of raw milk and non-hygienic production processes and storage conditions. Fecal streptococci counts were found in high numbers as 6.58 ± 6.35 cfu/g. Results are similar to those reported by some investigators[3,6,13,17]. Yeast and mould counts were found as high as 6.65 ± 6.18 cfu/g. It probably resulted from the microflora of the ripening rooms (for example, cave).

Salmonella spp and *L. monocytogenes* were not detected in the Tulum cheese. Similar results are reported by some other researchers[29,30]. Colak *et al.*[31] determined *Salmonella* spp and *L. monocytogenes* 2.4 and 4.8 %, respectively in Tulum cheese samples. Microbiological properties of the cheese samples are shown in Table-3.

TABLE-3
MICROBIAL COUNTS OF TULUM CHEESE SAMPLES (log_{10} cfu/g)

Microorganisms	Mean	SD	Minimum	Maximum
Total aerobic mesophilic bacteriae	8.68	8.37	5.02	9.83
Coliforms	5.27	4.65	< 2	6.16
Enterobacteriaceae	5.38	5.88	< 2	7.27
Fecal *streptococci*	6.58	6.35	4.25	7.74
Yeast and mould	6.65	6.18	< 2	7.82
Escherichia coli	3.24	4.71	< 2	5.13
Staphylococcus aureus	3.12	4.02	< 2	4.93
Salmonella spp	n.d.			
Listeria monocytogenes	n.d.			

n.d. = not detectable; SD = standard deviation.

In sensorial evaluation; total sample points were detected between 55-94 and an average value of 70.62 ± 2.07. Keles and Atasever[5] have found total sensorial points in Divle Tulum cheese between 67-97 and an average of 79.30 ± 1.77. This difference is because of speciality of Divle Tulum cheese. It is ripened in a special cave. Ates and Patir[28] reported that total sensorial points in experimental Tulum cheese were found between 62.40-91.25. Sensorial properties of the cheese samples are shown in Table-4.

Conclusion

In this study, 50 Tulum cheese samples were examined for chemical, microbiological and sensorial properties. It was found that there were wide variations among the samples. Microbiological quality of Tulum cheese samples was found low. The number of microorganisms in analyzed Tulum cheese samples seems to be related with the use of raw milk and non-

TABLE-4
SENSORIAL EVALUATION OF TULUM CHEESE SAMPLES

Properties	Mean	SD	Minimum	Maximum
Flavour (45)	33.72	0.96	25	45
Texture (30)	20.91	0.73	15	30
Appearance (15)	9.26	0.52	5	15
Colour (10)	6.73	0.39	3	10
Total (100)	70.62	2.07	55	94

( ) = Numbers in the brackets show maximum points of properties.
SD = standard deviation.

hygienic production processes and storage conditions. It was concluded that Tulum cheese production should be standardized. Pasteurized milk should be used in Tulum cheese production and the processing, ripening and storage of the cheese should be carried out under good hygienic conditions. Further investigations should be carried out for improvement and standardization of cheese.

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