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Prevalence of *L. monocytogenes* and *Salmonella* spp. in Tulum cheese

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Abstract

Tulum cheese, which is produced from raw milk, is one of the most popular semi-hard cheeses in Turkey. The growth of some food pathogens such as *Listeria monocytogenes* and *Salmonella* spp. in cheese and other dairy products may cause serious health problems for consumers. The aim of this study was to assess the presence of *L. monocytogenes* and *Salmonella* spp. in Tulum cheese sold in Istanbul. During the period March 2004–March 2005, a total of 250 Tulum cheese samples were obtained from various markets located in Istanbul and the presence of *L. monocytogenes* and *Salmonella* spp. was analyzed according to "The US Food and Drug Administration" (FDA) methods. The results were positive for *L. monocytogenes* and *Salmonella* spp. in 12 (4.8%) and 6 (2.4%) samples respectively.

Keywords: *L. monocytogenes*; *Salmonella* spp.; Tulum cheese

1. Introduction

Tulum cheese is one of the most popular semi-hard cheeses in Turkey, and is produced from raw milk. Despite its popularity, unfortunately there are no standard techniques in Tulum cheese production. In many regions of Turkey, Tulum cheese is produced with traditional methods and marketed in public bazaars by village people. On the other hand, modern production procedures are carried out by a few dairies (Aygun, Aslantas, & Oner, 2005; Oksuztepe, Patir, & Calicioglu, 2005).

Traditionally, Tulum cheese is manufactured from skimmed sheep, goat or cow milk. After curd formation by the addition of rennet, the curd is salted and packed in goat skin (Tulum) for ripening at 6–10 °C, in 85% relative humidity for 3–6 months (Fig. 1). During the ripening, the goat skin gives the characteristic taste and sharp aroma to Tulum cheese. In dairies where the cheese is ripened in hygienic plastic containers have been used instead of goat skin, in order to prevent the contamination by some hazardous microorganisms (Erdogan, Gurses, & Sert, 2003; Yilmaz, Ayar, & Akin, 2005).

The microbiological characteristics of this cheese depend on the quality of raw milk, the procedures and the conditions of production, the personnel and the storage conditions. After the ripening period, some hazardous food pathogens such as *Listeria monocytogenes* and *Salmonella* spp. may still cause serious food safety problems for consumers, in spite of added salt, the antimicrobial metabolites, the presence of low pH and moisture levels (Oksuztepe et al., 2005).

Consumption of cheese contaminated with the mentioned pathogens can lead to serious health problems which can occasionally be fatal for consumers. In the last few years, several outbreaks of disease due to consumption of contaminated dairy products have been reported. Outbreaks implicating various cheese types in different countries can be seen in Table 1 (De Buyser, Dufour, Maire, & Lafarge, 2001).

Among dairy products, traditional production and consumption of Tulum cheese is widespread in Turkey. For this reason, this study was planned to detect the presence of *L. monocytogenes* and *Salmonella* spp. in Tulum cheese sold

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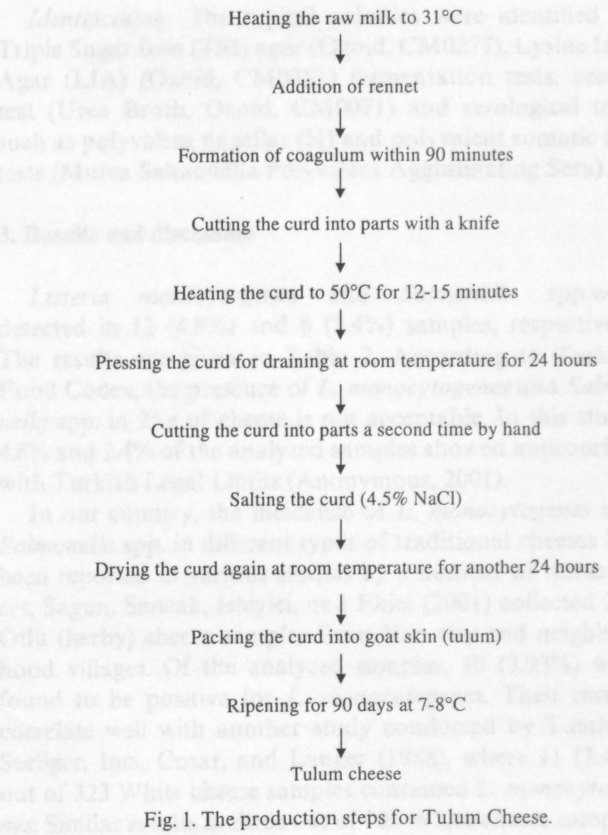

Fig. 1. The production steps for Tulum Cheese.

in Istanbul and to evaluate the results according to Turkish Food Codex.

2. Materials and methods

2.1. Samples

During the period March 2004–March 2005, a total of 250 Tulum cheese samples were randomly collected from various markets located in Istanbul. Samples were transported under cold conditions from their place of collection to the laboratory.

2.2. Microbiological analysis

The US Food and Drug Administration (FDA) methods were used for the detection of *L. monocytogenes* (Hitchins, 1995) and *Salmonella* spp. (Andrews, June, Sherrod, Hammack, & Amaguana, 1995).

Detection of *L. monocytogenes:*

Enrichment: 225 ml Listeria Enrichment Broth (LEB) (Oxoid, CM862) without selective supplement was added to 25 g Tulum cheese, and incubated at 30 °C for 4 h. Then, Listeria Enrichment Broth Selective Supplement (Oxoid, SR0141E) was added and incubated for another 44 h, for a total of 48 h, at 30 °C.

Isolation: At 24 and 48 h, the test culture (from LEB) was streaked onto both OXFORD (Oxoid, CM856) and PALCAM Agar (Oxoid, CM877), and incubated for 24–48 h at 35 °C. After incubation, five typical colonies from these media were transferred to Tryptic Soy Agar with Yeast Extract (TSAYE) (Oxoid, CM0131) and incubated for 24–48 h at 30 °C.

Identification: The purified isolates were identified by classic tests such as examination of TSAYE plates with oblique Henry illumination, Gram staining, examination of catalase activity, rotating or tumbling motility, haemolysis zone on blood agar, motility in Semisolid Indol Motility (SIM) medium (Oxoid, CM0435) for typical umbrella shape, and carbohydrate fermentation tests in Purple Carbohydrate Broth, and the CAMP test.

Detection of *Salmonella* spp.:

Enrichment: 225 ml Lactose Broth (LB) (Oxoid, CM0137) was added to 25 g Tulum cheese, and incubated at 35 °C for 24 h. Then, 0.1 ml of mixture transferred to 10 ml Rappaport Vassiliadis (RV) medium (Oxoid, CM0669) and another 0.1 ml mixture to 10 ml Tetrathionate (TT) Broth (Oxoid, CM0671). RV medium was incubated for 24 h, at 42 °C and TT broth for 24 h, at 43 °C.

Isolation: The test cultures from RV and TT separately were streaked onto Bismuth Sulfite (BS) Agar (Oxoid, CM0201), Xylose Lysine Desoxycholate (XLD) Agar (Oxoid, CM0469) and Hectoen Enteric (HE) Agar (Oxoid, CM0419), and incubated for 24 h at 35 °C.

Table 1
Salmonella spp. and *L. monocytogenes* outbreaks implicating various cheese types in different countries

Country	Year	Microorganism	Cheese type	Cases/deaths
Italy	1981	*Salmonella* spp.	Mozzarella	>100
USA	1981	*Salmonella* spp.	Mozzarella	321/2
Switzerland	1983–87	*L. monocytogenes*	Vacherin Mont D'or	122/33
Canada	1984	*Salmonella* spp.	Cheddar	>1700
Finland	1985	*Salmonella* spp.	Farm cheese	35
USA	1985	*L. monocytogenes*	Mexican soft cheese	142/48
England	1989	*Salmonella* spp.	Soft Cheese	42
Luxembourg	1989	*L. monocytogenes*	Camembert	2
Denmark	1989–1990	*L. monocytogenes*	Hard & blue cheese	26/6
France	1993	*Salmonella* spp.	Goat milk cheese	273/1
Canada	1994	*Salmonella* spp.	Farm soft cheese	35
France	1995	*Salmonella* spp.	Mont D'or cheese	25/5
France	1995	*L. monocytogenes*	Brie de Meaux cheese	36/11
France	1996	*Salmonella* spp.	Mont D'or cheese	14/1

Identification: The typical colonies were identified by Triple Sugar Iron (TSI) agar (Oxoid, CM0277), Lysine Iron Agar (LIA) (Oxoid, CM0381) fermentation tests, urease test (Urea Broth, Oxoid, CM0071) and serological tests such as polyvalent flagellar (H) and polyvalent somatic (O) tests (Murex Salmonella Polyvalent Agglutinating Sera).

3. Results and discussion

Listeria monocytogenes and *Salmonella* spp.were detected in 12 (4.8%) and 6 (2.4%) samples, respectively. The results are given in Table 2. According to Turkish Food Codex, the presence of *L. monocytogenes* and *Salmonella* spp. in 25 g of cheese is not acceptable. In this study, 4.8% and 2.4% of the analyzed samples showed impropriety with Turkish Legal Limits (Anonymous, 2001).

In our country, the incidence of *L. monocytogenes* and *Salmonella* spp. in different types of traditional cheeses has been reported in various studies by a number of researchers. Sagun, Sancak, Isleyici, and Ekici (2001) collected 254 Otlu (herby) cheese samples from Van city and neighborhood villages. Of the analyzed samples, 10 (3.93%) were found to be positive for *L. monocytogenes*. Their results correlate well with another study conducted by Tumbay, Seeliger, Inci, Cosar, and Langer (1988), where 11 (3.4%) out of 323 White cheese samples contained *L. monocytogenes*. Similar results in three out of 105 White cheese samples and in one out of 40 Cecil cheese samples were reported by Ciftcioglu and Ugur (1991) and Gulmez and Guven (2001), respectively. Our findings showed similarity with these results. On the other hand, in a study performed by Cetinkaya, Ertas, and Muz (1999), it was reported that *L. monocytogenes* could not be detected in 52 Tulum cheese samples.

Salmonella spp., were detected by Tekinsen and Ozdemir (2006) in 50 unripened Van otlu (herby) cheese samples obtained in Van and Hakkari markets. Three (6%) of the 50 samples were found to be positive for *Salmonella* spp. These results are higher than ours. Contrary to this, it was reported that *Salmonella* spp. were not found in 26 Tulum cheese samples by Ozalp, Kaymaz, and Aksehirli (1978), in 20 Tulum cheese samples by Kivanc (1989), in 38 White cheese samples by Turantas, Unluturk, and Goktan (1989), in 80 White cheese and 40 Cecil cheese samples by Gulmez and Guven (2001) and in 50 Carra cheese samples by Aygun et al. (2005).

In countries other than Turkey, various results were established on the presence of *L. monocytogenes* and *Salmonella* spp. in different types of traditional cheeses. In Silva, Almeida, Alves, and Almeida (2003) found *L. monocytogenes* in 16.7% of analyzed Minas Frescal Cheese samples. Rudolf and Scherer (2001) reported that 6.4% of European red-smear cheese samples were contaminated with *L. monocytogenes*. In a study that was conducted by Makino et al. (2005) in Japan, *L. monocytogenes* was detected in 15 out of 123 traditional cheese samples. On the other, Ashenafi (1990), stated that *L. monocytogenes* was not isolated from 100 Ayib cheese samples in Ethiopia. Menéndez, Godínez, Centeno, and Rodríguez-Otero (2001), studied Tetilla, which is produced from raw cow's-

Table 2
The distribution of *L. monocytogenes* and *Salmonella* spp. in Tulum cheeses

Sample number	*L. monocytogenes* positive samples	*L. monocytogenes* positive (%)	*Salmonella* spp. positive samples	*Salmonella* spp. positive (%)
250	12	4.8	6	2.4

Table 3
Incidence of *L. monocytogenes* and *Salmonella* spp. in various traditional cheese types in Turkey and other countries

Cheese type	No. of samples	Microorganism	Incidence	Country	Reference
Otlu cheese	254	*L. monocytogenes*	10 (3.93%)	Turkey	Sagun et al. (2001)
White cheese	323	*L. monocytogenes*	11 (3.4%)	Turkey	Tumbay et al. (1988)
White cheese	105	*L. monocytogenes*	3 (2.8%)	Turkey	Ciftcioglu and Ugur (1991)
Cecil cheese	40	*L. monocytogenes*	1 (2.5%)	Turkey	Gulmez and Guven (2001)
Tulum cheese	52	*L. monocytogenes*	0	Turkey	Cetinkaya et al. (1999)
Otlu cheese	50	*Salmonella* spp.	3 (6%)	Turkey	Tekinsen and Ozdemir (2006)
Tulum cheese	20	*Salmonella* spp.	0	Turkey	Kivanc (1989)
White cheese	38	*Salmonella* spp.	0	Turkey	Turantas et al. (1989)
Carra cheese	50	*Salmonella* spp.	0	Turkey	Aygun et al. (2005)
White cheese	80	*Salmonella* spp.	0	Turkey	Gulmez and Guven (2001)
Cecil cheese	40	*Salmonella* spp.	0		
Traditional cheese	14	*L. monocytogenes*	0	Greece	Angelidis et al. (2006)
		Salmonella spp.	0		
Red smear cheese	329	*L. monocytogenes*	21 (6.4%)	Germany	Rudolf and Scherer (2001)
Domestic cheese	123	*L. monocytogenes*	15 (12.1%)	Japan	Makino et al. (2005)
Ayib cheese	100	*L.monocytogenes*	0	Ethiopia	Ashenafi (1990)
Tetilla cheese	24	*L. monocytogenes*	2 (8.3%)	Spain	Menéndez et al. (2001)
		Salmonella spp.	0		
Cameros cheese	18	*L. monocytogenes*	1 (5.6%)	Spain	Olarte et al. (1999)
		Salmonella spp.	0		

milk cheese in Galicia, Spain. In 24 cheese samples, *L. monocytogenes* was detected in 2 (8.3%) whereas *Salmonella* spp. were not isolated in any samples. These findings are similar to the results of Olarte, Sanz, Fandos, and Torre (1999) who found *L. monocytogenes* in 1 out of 18 (5.6%) Spain Cameros cheese and could not isolate *Salmonella* spp. in any samples. In another study conducted by Angelidis et al. (2006) *L. monocytogenes* and *Salmonella* spp. were not detected in 14 semi hard traditional cheeses in Greece. Total survey results which are stated in this study can be seen in Table 3.

In this study, the presence of *Listeria* and *Salmonella* species in analysed Tulum cheeses seems to be related with the use of raw milk and non-hygienic production processes and storage conditions. Furthermore, it was reported by various researchers that Tulum cheese had a low microbiological quality, because of the poor hygienic conditions in small primitive production establishments which are widespread in our country (Bostan, Ugur, & Aksu, 1992; Kurt & Oztek, 1984; Tekinsen, Patir, & Alkan, 1993).

In conclusion, since cheese is a ready-to-eat product, even a low incidence of contamination may pose a risk to consumer health. Therefore, it is essential to ensure high safety standards such as raw milk quality, effective pasteurization process, hygienic production and storage conditions, proper cleaning and sanitation processes in production places.

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