



Microbiological Quality Evaluation of Various Types Of Cheese *

Pelin KOÇAK KIZANLIK, Ergün Ömer GÖKSOY

Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Aydın-TURKEY

Summary: This study was conducted to determine microbiological quality of various cheese samples. A total of 120 cheese samples (30 white brined cheese, 30 tulum cheese, 30 kashar cheese, 30 lor cheese) were collected from dairy processing plants' output shops. Cheese samples produced in various processing plants located in Western Aegean region of Turkey (İzmir, Aydın, Muğla) were obtained from the output shops in Aydın. Enumeration of total viable count (TVC), *Staphylococcus aureus* and coliforms were carried out, and the presence of *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes* were investigated. The results showed that the mean TVC in white brined cheese, Tulum cheese, kashar cheese and lor cheese were 9.43 log cfu/g, 9.87 log cfu/g, 7.71 log cfu/g and 9.80 log cfu/g, respectively. The mean numbers of *S. aureus* were found in these samples 4.87 log cfu/g, 5.61 log cfu/g, 4.14 log cfu/g and 5.09 log kob/g, respectively. When the levels of coliform bacteria considered, the least contaminated cheese was found to be kashar. The presences of *E. coli* and *L. monocytogenes* were determined in 32 samples (26.6%) and one kashar cheese sample (0.83%), respectively. No *Salmonella* spp. were encountered in any of the cheese samples investigated. It was concluded that microbiological quality of the cheese samples were generally poor due to inadequate hygienic conditions during production processes and marketing. High numbers of *S. aureus* contaminated cheese samples may cause food poisoning cases due to its enterotoxins. Contamination with *L. monocytogenes* and *L. ivanovii* may also cause public health risks.

Key words: Cheese, microbiological quality, public health

Çeşitli Peynir Türlerinde Mikrobiyolojik Kalitenin Değerlendirilmesi

Özet: Bu çalışma, Türkiye'nin Batı Ege Bölgesinde çeşitli mandıralarda üretilen Aydın ilinde bulunan mandıra satış noktalarından satışa sürülen 120 adet peynir örneğinin (30 beyaz peynir, 30 tulum peyniri, 30 kaşar peyniri ve 30 lor peyniri) mikrobiyolojik kalitelerini belirlemek amacıyla yapılmıştır. Alınan peynir örnekleri soğuk zincirde laboratuara getirilerek Toplam Mezofilik Aerob Canlı Bakteri (TMACB), *S. aureus*, koliform bakteri sayıları ile *E. coli*, *Salmonella* spp. ve *L. monocytogenes* varlığı yönünden incelenmiştir. Mikrobiyolojik analizler sonucunda ortalama TMACB sayıları beyaz, tulum, kaşar ve lor peynir örneklerinde sırasıyla 9.43 log kob/g, 9.87 log kob/g, 7.71 log kob/g ve 9.80 log kob/g olarak bulunurken, *S. aureus* sayıları ise sırasıyla 4.87 log kob/g, 5.61 log kob/g, 4.14 log kob/g ve 5.09 log kob/g olarak belirlenmiştir. Örnekler koliform grubu bakteriler yönünden incelendiğinde en az kontamine olan peynir grubunun kaşar peyniri olduğu tespit edilmiştir. İncelenen örneklerin 32 tanesinin (%26.6) *E. coli* ile bir adet kaşar peyniri örneğinin ise *L. monocytogenes* ile kontamine olduğu gözlenmiştir. Örneklerin hiçbirinde *Salmonella* spp. varlığına rastlanılmamıştır. Peynir örneklerinin, üretimi ve pazarlanması sırasında hijyenik koşullara yeterince dikkat edilmemesi nedeniyle, genel olarak mikrobiyolojik kalitelerinin düşük olduğu sonucuna varılmıştır. Yapılan analizler sonucunda örneklerde *S. aureus* yükünün yüksek olduğu belirlenmiştir. Bu durum stafilokokal enterotoksinlerin oluşturabileceği gıda zehirlenmeleri riskini arttırmaktadır. Peynirlerin *L. monocytogenes* ve *L. ivanovii* ile kontaminasyonu da halk sağlığı açısından risk teşkil etmektedir.

Anahtar kelimeler: Halk sağlığı, mikrobiyolojik kalite, peynir

Introduction

Cheese is one of the oldest human foods and is thought to have been developed approximately

8 000 years ago (16). The long tradition of consumption of fresh and fermented raw milk products was subject to an important change in the late 19th century, as the developed countries began wide scale pasteurization of milk to eliminate foodborne (zoonotic bacterial) pathogens (14). However, cheeses are ready-to-eat (RTE) food products that do not undergo any further treatment to ensure their safety before consumption (28).

Geliş Tarihi/Submission Date : 07.03.2017

Kabul Tarihi/Accepted Date : 22.08.2017

*This study is a short summary of the first author's MSc Thesis which was supported by Adnan Menderes University Scientific Research Project Coordination (Project Number: VTF-12044). This study was also presented in the 6th National Veterinary Food Hygiene Congress (October 7-11, 2015) in Van, Turkey.

In the previous years, there have been many outbreaks of infection associated with the consumption of various types of cheese (10). Four bacterial pathogens including *Staphylococcus aureus*, pathogenic *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes* are considered as the predominant organisms that are responsible to outbreaks associated cheese (32). Pathogenic bacteria contamination of cheese can be attributed to many sources such as raw milk used unpasteurized or inadequate pasteurization for the production of cheese, environmental sources (utensil and equipment during the processing), post processing, retail process and personnel (28,41).

Consumption of cheese contaminated with the mentioned pathogens can lead to serious health problems which can occasionally be fatal for consumers (11). *S. aureus* and *E. coli* in cheese are frequently used as indicators of hygienic quality and show lack of microbiological safety (40). Staphylococcal food poisoning is an intoxication that results from the consumption of foods containing sufficient amounts of one (or more) preformed enterotoxin (31).

Salmonella spp. infections have been linked to outbreaks associated with the consumption of various types of cheese (13). The typical symptoms of the illness include nausea, vomiting, diarrhea, abdominal cramps and fever while additional complications associated with the infection include septicemia or reactive arthritis (12). Although cheese have some features to inhibit the growth of *L. Monocytogenes* such as added salt, the antimicrobial metabolites, the presence of low pH and moisture levels, as well as low refrigeration temperature, it still may cause health hazards for consumers (11). Listeriosis is characterised with a very high fatality rate compared with those of other foodborne bacteria, preferentially affects individuals, whose immune system is perturbed, including pregnant women, newborns, old and immunocompromised people (28).

In Turkey, more than 40 varieties of cheese are produced. The other cheese varieties such as kashar, lor, tulum and soft cheese types are well known that occupy a large place in national consumption (25). This study was planned to examine various microbiological parameters such as total viable count, the numbers of *S. aureus* and coliforms, and to detect the presence of *E. coli*, *Salmonella* spp. and *L. mono-*

cytogenes in various types of cheese, in order to observe hygienic quality and possible health risks from these cheeses.

Materials and Methods

Sampling

A total of 120 cheese samples (30 white brined cheese, 30 tulum cheese, 30 kashar cheese, 30 lor cheese) were examined in order to evaluate their microbiological quality. Cheese samples produced in various processing plants located in Western Aegean region of Turkey (Izmir, Aydın, Mugla) were obtained from the output shops in Aydın. In this study cheese samples were collected from overall nine local Dairy Products Producing Premises (DPPP) located in various parts of Western Aegean Region. All samples were immediately transported to the laboratory for microbiological analysis under aseptic conditions and in insulated cold boxes at 4°C.

Microbiological analysis

The microbiological analysis procedures were carried out as stated in TSE (Turkish Standards Institute) standards (45,48,49). Ten gram cheese sample was homogenized in 90 mL of a sterile 0.1% pepton water solution (Merck 107228, Germany) using a Lab-Blender 400 Stomacher (Interscience, France) for at least two minutes in order to enumerate total viable count (TVC), *S. aureus*, coliform levels and to determine the presence of *E. coli* (21). Decimal serial dilutions were prepared up to 10⁻⁶. Serial dilutions were plated on to selective agar media [Plate Count Agar (oxid CM0325), Baird Parker Agar (Oxid CM0961), Lauryl Sulphate Broth (Oxid CM0451), Tryptone Bile X-glucuronide Agar (Oxid CM0961)] for each group bacteria.

For the isolation of *Salmonella* spp. and *L. monocytogenes*, 25 g cheese samples were put into stomacher bags containing 225 mL buffered pepton water (Oxid CM509, UK) and 225 mL Half Fraser Broth (Oxid CM895, UK) with Half Fraser supplement (Oxid SR166E, UK), respectively then homogenized least two minutes. The inoculation, incubation and identification procedures were carried out as stated in TSE standards (46,47). Suspected colonies for *Salmonella* spp. and *L. monocytogenes* were subjected to serological and biochemical test kits (Salmonella Latex Test, Oxoid FT 0203A and Microbact 12L, Listeria Identification System, Oxoid MB1128, UK) according to the manufacturer's instructions.

Statistical analysis

One-way ANOVA were carried out to determine the statistical difference between the groups for TVC and the numbers of *S. aureus*. When an

analysed were not determined, due to the high determination limit of the plating out method used (Table 1).

Table 1. The level of TVC and *S. aureus* (log cfu/g) obtained from cheese samples

	TVC		<i>S. aureus</i>	
	n/N	Mean±SEM	n/N	Mean±SEM
White brined cheese	30/30	9.43±0.18 ^a	24/30	4.87±0.30 ^{ab}
Tulum cheese	30/30	9.87±0.10 ^a	28/30	5.61±0.22 ^a
Kashar cheese	26/30	7.71±0.12 ^b	14/30	4.14±0.36 ^b
Lor cheese	30/30	9.80±0.1 ^a	26/30	5.09±0.25 ^a
F	46.23 ^{***}		3.99 ^{**}	

n: positive samples N: number of total samples *: P<0.05, ***: P<0.001.
a,b: Different superscripts in the same row were significantly different (P<0.05).

overall significance was observed, Post Hoc tests were performed using Duncan test. For most probable number (MPN) method used for coliform count, samples were divided into 3 groups; 1) group with a coliform load of >1100, 2) group with a coliform load of between 1100 ve 3, and 3) group with a coliform load of <3. Significant differences between groups were tested using Pearson chi-square test and Fisher's exact chi-square test was performed if there was a low value (<5) in the expected cell counts.

Results

The lowest mean TVC was observed in kashar cheese samples. Although there was not any significant difference between tulum, white brined and lor cheese samples, a significant difference (P<0.001) was found between kashar cheese and other cheese samples analysed. The TVC levels of four kashar cheese samples

The lowest mean of *S. aureus* was also determined in kashar cheese samples. There was a significant difference (P<0.05) in the numbers of *S. aureus* between kashar cheese, and lor and tulum cheese samples (Table 1).

The lowest level of coliform was observed in kashar cheese samples (Table 2). A significant difference was found between the observed and statistically expected levels of coliforms in white brined cheese, tulum cheese and lor cheese (χ^2 : 39.533^{***}, ***: P<0.001). Tulum cheese was found to be the most contaminated cheese by *E. coli* with a prevalence of 36.60%. The prevalence of *E. coli* in white brined cheese, kashar cheese and lor cheese were 33.3%, 13.3%, and 23.3%, respectively.

Salmonella spp. was not determined in any of the 120 cheese samples examined. *L. monocytogenes* was found in one kashar cheese sample, and nine other cheese samples were also

Table 2. The levels (MPN/g) of coliform in various cheese samples

Types of cheese	MPN/g(n/N)		
	>1100	1100-3	<3
White brined cheese	21/30 (70.0%)	5/30 (16.7%)	4/30 (13.3%)
Tulum cheese	14/30 (46.7%)	13/30 (43.3%)	3/30 (10.0%)
Kashar cheese	3/30 (10.0%)	14/30 (46.7%)	13/30 (43.3%)
Lor cheese	22/30 (73.3%)	8 (26.7%)	0

n: positive samples N: number of total samples χ^2 : 39.533^{***}, ***: P<0.001

found to be contaminated with *Listeria* spp. other than *L. monocytogenes* (Table 3).

gens (32). However, for fermented foods high microbial load observed in the samples may not

Table 3. The distribution of *Listeria* spp. in various cheese types

	White brined cheese	Tulum cheese	Kashar cheese	Lor cheese
<i>L. monocytogenes</i>	-	-	1/30(3.33%)	-
<i>L. ivanovii</i>	2/30 (6.66%)	3/30 (10%)	-	1/30(3.33%)
<i>L. seeligeri</i>	-	-	-	1/30(3.33%)
<i>L.welshimeri</i>	-	1/30(3.33%)	-	1/30(3.33%)
<i>Listeria</i> spp.		10/120 (%8.33)		

Discussion and Conclusion

Foodborne infections/intoxications are common public health problem in developed, developing and undeveloped countries in variable extends (18). Ninety outbreaks associated with cheese from 1998 to 2011 were reported by The Centers for Disease Control and Prevention (CDC). These outbreaks resulted in 1882 illnesses including 230 hospitalizations, and 6 deaths. A median of 6.5 outbreaks (range, 3-10) was reported each year, with no discernible pattern in the number of outbreaks overall or by pasteurization status. The pasteurization status of the milk used to make the cheese was reported for 82 (91%) outbreaks; 38 (46%) were caused by cheese made from unpasteurized milk and 44 (54%) by cheese made from pasteurized milk (17). A detailed review was presented by Koustta et al. (28) regarding to the cheese borne outbreaks occurred in different countries.

Especially in local areas, local DPPP's have their own consumers who trust the firms, their products are believed to be safe and consumers buy their product without any hesitation.

The mean TVC of cheese samples showed that the lowest microbial load was on kashar cheese samples with a level of 7.71 log cfu/g and this level was statistical significant than those observed from white brined cheese, Tulum cheese and Lor cheese. Studies conducted in Turkey by Aygun et al. (5), Simsek and Sagdic (38), Kursun et al. (30) were in agreement with the results reported here and showed TVC level between 10^6 and 10^9 cfu/g. For foods, in general the level of TVC indicates the presence of low hygienic practices and the possibility of other groups of microorganisms including patho-

give such a sophisticated result due to the difficulties in distinguishing contaminated microorganisms and routine microflora of fermented food (42).

Similar to TVC, the lowest *S. aureus* level was found in kashar cheese and there was a statistical significance between kashar cheese, and lor and tulum cheese. While 93.3% of tulum cheese, 86.6% of lor cheese and 80% of white brined cheese samples were contaminated with *S. aureus*, 46.6% of kashar cheese samples were found to be contaminated with this agent. Takahashi and Johns (39) showed the prevalence of *S. aureus* in cheddar cheese samples as 12% in Canada. Jorgensen et al. (24) reported that 75% of cheese samples investigated were contaminated with *S. aureus*. Tekinşen and Özdemir (40) reported the mean level of *S. aureus* as 6.1 log cfu/g in 50 cheese samples collected from the markets of Van and Hakkari provinces of Turkey. Contamination degree of *S. aureus* in milk and dairy products is mainly associated with clinical and subclinical mastitis and workers in the farm, and in the hygienic conditions of processing plant (9,35,50). The manufacturing process has also been demonstrated to be the source of high *S. aureus* numbers (28). Insufficient pasteurization and post pasteurization contaminations could also lead cheese to be contaminated with *S. aureus*. In raw milk cheeses the fermentation or ripening process prevent growth of *S. aureus* (1). Due to high number of *S. aureus* is needed to produce SE's ($>5 \log_{10}$ cfu/g) (23), any condition that stimulate growth of *S. aureus* to high levels may cause food poisoning cases. Therefore, although viable *S. aureus* cells are eliminated by processing

(pasteurization, fermentation or ripening etc), SE's once produced are present in the cheese. According to Turkish Food Codex (TFC) (44) for coagulase positive *S. aureus* m and M values are 10^2 and 10^3 cfu/g, respectively. Our mean values for this pathogen were ranging 4.14 log cfu/g and 5.61 log cfu/g for different cheese samples, far above the maximum limits stated by TFC (44), which might cause food poisoning cases with improper handling and temperature abuse conditions.

Kashar cheese was found to be with the lowest MPN for coliforms and the highest MPN was found in Lor cheese. The levels of coliforms in cheese samples other than kashar cheese were found to be statistically higher than which was expected. This group of bacteria cause some technological defects (swelling) in some sort of soft cheese samples due to heterofermentative degradation of lactose causing gaseous (CO_2), lactic acid, acetic acid and alcohol (26). The prevalence of *E. coli* in white brined cheese, Tulum cheese, kashar cheese and Lor cheese were 33.3%, 36.6%, 13.3%, and 23.3%, respectively. As a sum 26.6% of cheese samples were found to be contaminated with *E. coli*. The prevalence of *E. coli* in various types of cheese samples was reported between 4% and 74.3% by several researchers (7,20,34,43).

No *Salmonella* spp. was found in cheese samples investigated, our finding for *Salmonella* spp. is in agreement with the results of Keskin et al (27), Aygun et al. (5) and Gülmez and Güven (19). In contrary, Akkaya and Alişarlı (2), Tekinşen and Özdemir (40) found *Salmonella* spp. in the cheese samples investigated. While Kahraman et al. (25) showed that the prevalence of *Salmonella* spp. was 1.7% in 105 white brined cheese samples, Almeida et al. (3), Erkan et al. (15), Hayaloglu and Kirbag (22), Miranda et al. (33), reported the prevalence of *Salmonella* spp. as 10.0%, 23.3%, 4.3%, 9.1%, respectively.

The genus *Listeria* comprises six species: *Listeria monocytogenes*, *Listeria innocua*, *Listeria ivanovii*, *Listeria welshimeri*, *Listeria seeligeri* and *Listeria grayi*. Among the genus of *Listeria*, which cause the infection of listeriosis in both animals and man, *L. monocytogenes* is a major pathogenic microorganism, and bacterium *L. ivanovii* is rarely pathogenic for humans (4). According to TFC no *L. monocytogenes* must be found in cheese samp-

les (44). The prevalence of *Listeria* spp. in cheese samples were found to be as 8.33% (ten cheese samples) and 1 kashar cheese (3.33%) was found to be contaminated with *L. monocytogenes*. Several researchers reported the prevalence of *L. monocytogenes* in various types of cheese samples between 1.2% and 4.8% (6,8,11,19,25,30,37).

Differences between the results above may be related with the use of raw or pasteurized milk in cheese production, cheese production techniques, and hygienic conditions during the production, storage and sales process, and some characteristics of cheese such as pH and moisture content. For example, *L. monocytogenes* can be found more frequently in high-moisture cheeses, soft and semi-soft cheeses which have water activity higher than hard cheeses (4,36). Turkish white cheese contains high levels of moisture, and pH, particularly at the surface, increases during ripening; so that they are more prone to microbial growth than hard or semi-hard cheeses (25). Producing white cheese is different than kashar, tulum and lor cheeses. Producers sometimes do not heat milk up to pasteurization temperatures and/or add raw milk into pasteurized milk. The reason for this is to avoid adding CaCl_2 , which means extra cost and to save energy and time (29). The other reason for the high contamination rates for white brined cheese might be the brine. Pathogens may be transferred to cheese via contaminated brine (25). Lor cheese is produced from the whey leftover from cheese production. The more manipulation especially in unhygienic conditions may generally cause the highest levels of coliforms in lor cheese. In addition three lor cheese samples were also found to be contaminated with *Listeria* spp. Although a second heat treatment was also applied to kashar cheese processing, one kashar cheese sample was found to be contaminated with *L. monocytogenes*. It has been stated as rarely pathogenic for humans, *L. ivanovii* was found in all types of cheese samples except kashar cheese, which might also cause serious public health problems. *L. seeligeri* and *L. welshimeri* were also found in tulum and lor cheese samples analysed.

As a result, cheese processing contains integrated procedures from milk supply to retail market. Therefore, any hygienic problems associated with milk and processing stages cause cheese to be contaminated with pathogens.

Our results showed that cheese samples analysed contaminated with *S. aureus* higher than those stated in TFC. High level of *S. aureus* may cause food poisoning cases due to its toxins. *L. monocytogenes* is a major pathogenic microorganism, causing foodborne infections and deaths, and bacterium *L. ivanovii* is rarely pathogenic for humans. According to TFC (44), no *L. monocytogenes* must be found in food samples. Our findings showed that 10 cheese samples were contaminated with *Listeria* spp., and 7 of which were contaminated with *L. monocytogenes* (1 sample) and *L. ivanovii* (6 samples) that might also cause public health risks.

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Corresponding Author:

Pelin KOÇAK KIZANLIK
Adnan Menderes University
Faculty of Veterinary Medicine
Department of Food Hygiene and Technology
Aydın-TURKEY
Tel: +90 256 247 07 20
E-mail: pelin.kocak@adu.edu.tr

