Microbiological Quality Evaluation of Various Types Of Cheese

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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 cfu/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 mandira 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 yapılmış. Alınan peynir örnekleri soğuk zincirde laboratuarda geceleyerek 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 TVC sayıları beyaz, tulum, kaşar ve lor peynir örneklerinde sırasıyla 9.43 log k/b/g, 9.87 log k/b/g, 7.71 log k/b/g ve 9.80 log k/b/g olarak bulunurken, S. aureus sayıları ise sırasıyla 4.87 log k/b/g, 5.61 log k/b/g, 4.14 log k/b/g ve 5.09 log k/b/g olarak belirlenmiştir. Örnekler koliform grubu bakteriler yönünden incelenildiğinde en az kontamine olan peynir grubunun kaşar peyniri olduğu tespit edilmiştir. İncelenmiş örneklerin 32 tanesinin (%26.6) ile bir adet kaşar peynir örneğinin ise L. monocytogenes ile kontamine olduğu gözlenmiştir. Örneklerin hiçbirinde Salmonella spp. varlığına rastlanmamıştır. Peynir örneklerinin, üretimi ve pazarlanmasının sırasında hygienik 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 oluşturulabileceği gazda zehirlenmeleri riskini artırmaktadır. 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).
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 characterized 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. monocytogenes* 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, Aydin, Mugla) were obtained from the output shops in Aydin. 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 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 (oxoid CM0325), Baird Parker Agar (Oxoid CM0961), Lauryl Sulphate Broth(Oxoid CM0451),Tryptone Bile X-glucoronide Agar (Oxoid 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 (Oxoid CM509, UK) and 225 mL Half Fraser Broth (Oxoid CM895, UK) with Half Fraser supplement (Oxoid 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 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 and 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 were not determined, due to the high determination limit of the plating out method used (Table 1).

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 (X^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 analysed were not determined.

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

<table>
<thead>
<tr>
<th>Types of cheese</th>
<th>TVC</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n/N</td>
<td>Mean±SEM</td>
</tr>
<tr>
<td>White brined cheese</td>
<td>30/30</td>
<td>9.43±0.18^a</td>
</tr>
<tr>
<td>Tulum cheese</td>
<td>30/30</td>
<td>9.87±0.10^a</td>
</tr>
<tr>
<td>Kashar cheese</td>
<td>26/30</td>
<td>7.71±0.12^b</td>
</tr>
<tr>
<td>Lor cheese</td>
<td>30/30</td>
<td>9.80±01^a</td>
</tr>
<tr>
<td><strong>F</strong></td>
<td>46.23***</td>
<td>3.99**</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Types of cheese</th>
<th>MPN/g(n/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;1100</td>
</tr>
<tr>
<td>White brined cheese</td>
<td>21/30 (70.0%)</td>
</tr>
<tr>
<td>Tulum cheese</td>
<td>14/30 (46.7%)</td>
</tr>
<tr>
<td>Kashar cheese</td>
<td>3/30 (10.0%)</td>
</tr>
<tr>
<td>Lor cheese</td>
<td>22/30 (73.3%)</td>
</tr>
</tbody>
</table>

n: positive samples N: number of total samples X^2: 39.533***, ***: P<0.001
found to be contaminated with *Listeria* spp. other than *L. monocytogenes* (Table 3).

**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 Koutsa et al. (28) regarding to the cheese borne outbreaks occurred in different countries. Especially in local areas, local DPPPs 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 Aygün 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 pathogens (32). However, for fermented foods high microbial load observed in the samples may not 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. Taka-hashi 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 log10 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

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

<table>
<thead>
<tr>
<th></th>
<th>White brined cheese</th>
<th>Tulum cheese</th>
<th>Kashar cheese</th>
<th>Lor cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. monocytogenes</em></td>
<td>-</td>
<td>-</td>
<td>1/30 (3.33%)</td>
<td>-</td>
</tr>
<tr>
<td><em>L. ivanovii</em></td>
<td>2/30 (6.66%)</td>
<td>3/30 (10%)</td>
<td>-</td>
<td>1/30 (3.33%)</td>
</tr>
<tr>
<td><em>L. seeligeri</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1/30 (3.33%)</td>
</tr>
<tr>
<td><em>L. welshimeri</em></td>
<td>-</td>
<td>1/30 (3.33%)</td>
<td>-</td>
<td>1/30 (3.33%)</td>
</tr>
<tr>
<td><em>Listeria</em> spp.</td>
<td>10/120 (8.33%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The prevalence of Listeria spp. in cheese samples was 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 CaCl₂, 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 kashar 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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