Survival of *Escherichia coli* O157:H7 during processing and ripening period of Keş Cheese

Özen Kurşun Yurdakula, Seval Sevgi Kırdarb, Aylin Kasimoğlu Doğruc, Erhan Keyvana*

aDepartment of Food Hygiene and Technology, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, İstiklal Campus, 15030 Burdur, Turkey.
bDepartment of Food Processing, Milk and Dairy Products Technology Programme, Mehmet Akif Ersoy University, İstiklal Campus, 15030 Burdur, Turkey.
cDepartment of Food Hygiene and Technology, Faculty of Veterinary Medicine, Kırıkkale University, 71450 Kırıkkale, Turkey.

*Author for correspondence: Asst. prof. Erhan KEYVAN. Tel: +90 248 213 21 27. Fax: +90 248 213 20 01. e-mail: erhankeyvan@mehmetakif.edu.tr. Address: Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, İstiklal Campus, 15030 Burdur, Turkey

**ABSTRACT**

In this study the survival of *Escherichia coli* O157:H7 during the production process and ripening period of Keş cheese was investigated. Three vats containing equal amounts of raw milk were inoculated with $10^2$, $10^4$, $10^6$ cfu mL$^{-1}$ *E. coli* O157:H7, respectively and one vat remained uninoculated. Keş cheese was produced from both inoculated and uninoculated milks with the traditional method of the region. Cheese samples ripened at 6°C for 90 days and *E. coli* O157:H7 enumerations were done from uninoculated milk, inoculated milks, curds and different stages of ripening period. Most Probable Number technique was used for the analysis of *E. coli* O157:H7. Classical culture methods were used for the detection of aerobic mesophilic bacteria, lactococci and coliform bacteria. Also, control cheese was analysed for dry matter, salt content in dry matter and pH value. Elimination times were 45 days for $10^2$ cfu mL$^{-1}$, 75 days for $10^4$ cfu mL$^{-1}$, and 90 days for $10^6$ cfu mL$^{-1}$ of *E. coli* O157:H7. The final dry matter, salt content in dry matter and pH value were 82.52%, 3.16% and 4.58, respectively. Consequently, Keş cheese should be ripened at least 90 days in order to be considered as safe for public health.

Keywords: Elimination times, *Escherichia coli* O157:H7, Keş cheese, traditional cheese, dairy products


1. INTRODUCTION

*Escherichia coli* O157:H7 has been an important role as a foodborne pathogen since it was identified in 1982 for the first time (Riley et al. 1983). The main symptom of the disease of EHEC is hemorrhagic colitis (HC) referred as bloody diarrhea (Coia et al. 1998). It was reported that cattle, especially dairy cows, were the main reservoir, followed by sheep and pigs (Zhao et al. 1995). Also, outbreaks are though to be occurring due to faecal contamination originated from cattle (Wang et al. 1996). Dairy products made with raw milk and/or insufficiently heated milk can cause outbreaks (Honish et al. 2005; Oksuz et al. 2004). Cultured dairy products may be a high risk group for *E. coli* O157:H7 infections above human being. *E. coli* O157:H7 was reported to persist in white cheese for 120 days (Kuplulu et al. 2000), cheddar cheese for 158 days (Reitsma et al. 1996), smear-ripened cheese for 90 days (Maher et al. 2001) and camembert cheese for 75 days (Ramsaran et al. 1998). Keş cheese is a traditional cheese in Turkey and produced in different regions especially in the Mediterranean region. However there are differences in production this cheese even within the same region. In Burdur, Keş cheese is homemade cheese sold in bazaars. Commonly, Keş cheese’s production process contains boiling raw milk, cooling and boiling lactic coagulum steps, without adding any spices (Tarakçı et al. 2010). But in Burdur (in Mediterranean region of Turkey), Keş cheese is produced with self-coagulation of raw milk without any starter culture addition, than cutting coagulum, draining curd, kneading the curd with a mixture of spices such as nigella, cumin, red pepper, and black pepper. In this production process of Keş cheese, natural flora of raw milk
plays an important role in the formation of ripening. Considering that Keş cheese is commonly consumed in Burdur (Kirdar 2004) and this cheese has no pasteurisation and/or boiling stages in production process, it becomes very important to draw attention to potential risks in terms of public health. Also, due to handmade production human sourced contaminations are unavoidable.

Therefore, this study was conducted to determine the survival of E. coli O157:H7 in Keş cheese during production stages and ripening period.

2. MATERIALS AND METHODS

2.1. Test strain

As preliminary study, E. coli O157:H7 detection was done in 33 bulk tank bovine milks in order to isolate E. coli O157:H7, but none of the samples were positive. Considering human sourced contaminations, a human strain E. coli O157:H7 (ATCC 35150) was used in this study. The E. coli O157:H7 ATCC 35150 test strain was obtained from the Institute of Refik Saydam Hıfzıssıhha (Ankara, Turkey) and kept in cryogenic vials at -85 °C until use. Then at the start of the experiment, ATCC 35150 was activated in tryptic soya broth (TSB; BD Diagnostic Systems, Sparks, MD, USA) and kept in cryogenic vials at -85 °C until use. Then at the start of the experiment, ATCC 35150 was activated in tryptic soya broth (TSB; BD Diagnostic Systems, Sparks, MD, USA) at 37 °C for 24 h.

2.2. Cheese production

Sixty liter antibiotic-free bovine milk (pH 6.8) was taken from the milk producing farms in Burdur. Firstly, raw milk analysed for the E. coli O157:H7 presence. None of the raw milks were harboured E. coli O157:H7 that used for all three trials to produce Keş cheese. For Keş cheese production, milk was heated to 32 °C and transferred to stainlesssteel cheese vats, each containing 15 L of milk. Three vats were inoculated with 10², 10⁴ and 10⁶ cfu mL⁻¹ reference strain. One vat was not inoculated with E. coli O157:H7 and used as control. Chymosin was not added. Vats were kept under sunshine until lactic coagulum formed (about 8 hours). The coagulum was cut into 2 or 3 curds, then the curds were transferred to cheese cloth and drained in cool room (15 °C) for 24 hours. Drained curds were crumbled and solid salt (2%), nigella (1%), cumin (1%), and red pepper (1%) were added. The amount of spice mixture was decided according to the suggestion of the regional dairies that produce Keş cheese. Afterwards, the curd was kneaded manually for 5 minutes to distribute the salt and spices uniformly throughout the curd. The curd was shaped into cubic or colonial form and dried at room temperature (25°C) for 4 days. After the fifth day, cheeses ripened at 6 °C for 90 days. Experimental Keş cheese productions were replicated three times.

2.3. Sampling

Twenty five milliliter raw milk sample taken from uninoculated milk and the Most Probable Number (MPN) technique was used for E. coli O157:H7 enumeration. For the same purpose, 10 mL samples taken from 10², 10⁴, 10⁶ cfu mL⁻¹ E. coli O157:H7 inoculated milks, 10 g samples taken from curds (8th h of production), drained curds (24th h of production), dried cheeses in the fifth day of production, 1st, 7th, 15th, 30th days of ripening period of cheese and classical culture method was used. After 30 days of ripening period, to ensure recovery of levels of the pathogen, Most Probable Number technique was used for 60, 75, 90 day cheese samples. Also, classical culture methods were used for aerobic mesophilic bacteria, lactococci and coliform bacteria enumerations. For the compositional analysis and pH measurements, cheese samples were taken at 1, 30, 60, and 90 days of ripening.

2.4. Microbiological analysis

For the classical culture method enumeration of E. coli O157:H7, inoculated milk (10 mL), curd (10 g) and cheese (10 g) samples were diluted with 90 mL of 0.1% peptone water and homogenized for 2 minutes with a Labblender 400 stomacher (Seward Laboratory, London, UK). Serial dilutions were prepared with 9 mL sterile peptone water. Each dilutions were 0.1 mL spread onto a sorbitol MacConkey agar (Oxoid) plate, containing 0.1% 4-methylumbelliflore-b-d-glucuronide (SMA-MUG) in duplicate and incubated at 37 °C for 18 h. Suspicious colonies which were MUG and sorbitol negative were confirmed by E. coli O157:H7 latex agglutination assay (Oxoid). O157 and H7 antisera (BD Diagnostic Systems) were used to identify presumptive E. coli O157:H7 isolates for further accurate isolation. To determine the levels of E. coli O157:H7 that under the detection limit (<1.0 x 10¹ cfu mL⁻¹ MPN technique was used. For this purpose, cheese samples (1 g) were transferred into three tubes each containing 9 mL mEC (E. coli) broth (BD Diagnostic Systems) supplemented with novobiocin (Sigma, St Louis, MO, USA). Similarly, decimal dilutions of the samples were added to three tubes of mEC broth (9 mL). All tubes were incubated at 37 °C for 24 h. After incubation, enriched cultures (0.1 mL) were inoculated on SMA-MUG plates in duplicate and incubated at 37 °C for 18 h. Typical E. coli O157:H7 colonies were selected and confirmed
Survival of Escherichia coli O157:H7 during processing and ripening period of Keş Cheese

The changes in E. coli O157:H7 populations in Keş cheese are shown in Fig. 1. In all inoculated groups, while E. coli O157:H7 levels remained stable during the production process (5 days), E. coli O157:H7 counts were increased about 1 log from the initial numbers of 10^2, 10^4 and 10^6 cfu mL^-1 during formation lactic coagulum (8 h). Then E. coli O157:H7 bacterial cells began to decrease in all groups at the first day of ripening. The average pH levels were determined as pH 6.80, 5.24, 4.87 for uninoculated milk, lactic coagulum and curd, and first day of ripening, respectively. The increase and decrease of E. coli O157:H7 counts might be both explained by pH levels.

Growth of E. coli O157:H7 during cheese production was reported by many researchers previously. Reitsma and Henning (1996) stated that the pathogen grew during the production of cheddar cheese. Arocha et al. (1992) stated that E. coli O157:H7 grew from the inoculation level of 5 log cfu mL^-1 to 7 log cfu g^-1 during the production process of fresh cottage cheese. Ramsaran et al. (1998) expressed that a significant increase in the number of E. coli O157:H7 (10^4 cfu mL^-1) during the production of Camembert cheese.

### 3. RESULTS AND DISCUSSION

Microbiological changes of the control Keş cheese flora are shown in Table I. The number of aerobic mesophilic bacteria, lactococci and Lactobacillus spp. increased until 30th day of ripening, then they slowly decreased until at the end of ripening. Likewise, the number of coliform bacteria increased during production process, then they showed a strong decreasing. The increment of bacterial flora might be due to the fermentation of lactose while the reduction may be attributed to the decrease of pH level. On the other hand, microbiological results of the present study for total bacterial count, lactic acid bacteria, and coliform bacteria were differ from other studies (Akyüz and Gülümser 1987, Tarakçı et al. 2001). These differences may be caused by differences between production process such as boiling or not boiling milk.

#### Table I. Microbiological flora of Keş cheese during manufacturing and ripening (90 d).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Production (hour)</th>
<th>Ripening (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Milk (mL^-1)</td>
<td>Curd (8 h)</td>
</tr>
<tr>
<td>AMGC</td>
<td>6.7±0.1</td>
<td>8.6±0.3</td>
</tr>
<tr>
<td>LC</td>
<td>5.3±0.6</td>
<td>7.3±0.8</td>
</tr>
<tr>
<td>LB</td>
<td>5.1±0.3</td>
<td>7.4±0.7</td>
</tr>
<tr>
<td>CB</td>
<td>2.9±0.5</td>
<td>3.9±0.8</td>
</tr>
</tbody>
</table>

*AMGC, Aerobic mesophilic bacteria. **LC, Lactococci. *LB, Lactobacillus spp. **CB, Coliform bacteria *=<1.0, Under detection limit
Table II. Composition of Keş cheese during ripening (90 d).

<table>
<thead>
<tr>
<th>Compositional parameters</th>
<th>Time (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>pH</td>
<td>4.87±0.13</td>
</tr>
<tr>
<td>DM (%)</td>
<td>64.46±0.72</td>
</tr>
<tr>
<td>SDM (%)</td>
<td>1.90±0.09</td>
</tr>
</tbody>
</table>

Figure 1: The survival of *E. coli* O157:H7 (10^2, 10^4 or 10^6 cfu mL^-1) during manufacturing and ripening stages of Keş cheese.

The increasing, stabilizing and remaining at about 10^4 cfu g^-1 for 75 days of *E. coli* O157:H7 connected with the low pH level of the cheese (pH 5.0) by the researchers. Also, Kuplulu et al. (2000) reported that the number of *E. coli* O157:H7 increased 1 log cycle in the curd for all three inoculation levels (10^2, 10^4, and 10^6 cfu mL^-1). *E. coli* O157:H7 is most resistant to stress and lactic acid in late log phase (Benjamın & Datta, 1995) and stationary phase (Buchanan and Edelson 1999). This knowledge may explain the number of *E. coli* O157:H7 remain constant at the begining of ripening stage in the present study.

Elimination times of *E. coli* O157:H7 were determined as 45 day for 10^2 cfu mL^-1, 75 day for 10^4 cfu mL^-1, and 90 day for 10^6 cfu mL^-1 in this study. According to the physico-chemical properties of cheese, pH level began to slow down after the first day, and reached to pH 4.58 after 90 days (Table II). In addition, dry matter (DM) content was quite high during the ripening period. Salt content in dry matter (SDM) increased from 1.90% to 3.16% at the end of the ripening period (Table II). There was a positive correlation between the numbers of 10^2 cfu mL^-1 *E. coli* O157:H7 sample group and pH (r: 0.628; P=0.029), DM (r: -0.829; P<0.001), and SDM (r: -0.758; P=0.004, Spearman). Similarly, there were a positive correlation between high inoculated groups (10^4 and 10^6 cfu mL^-1 *E. coli* O157:H7) and each physico-chemical properties of cheese. The statistical analysis results for 10^2 cfu mL^-1 *E. coli* O157:H7 sample group were r: 0.975, P < 0.05 for pH; r: -1.000, P < 0.01 for dry matter; r: -0.998, P<0.01 for SDM (Pearson). Also, the results of 10^6 cfu mL^-1 *E. coli* O157:H7 sample group were for pH, DM, and SDM were r: 0.711, P= 0.010; r: -0.774, P= 0.003; and r: -0.734, P= 0.007 (Pearson), respectively.

Guraya et al. (1998) notified that salt stimulates the pathogen inactivation. Also, there is a correlation between SDM and *E. coli* O157:H7 inactivation in this study. Conversely, it was reported that *E. coli* O157:H7 could tolerate NaCl concentrations as high as 8.5% by Glass et al. (1992). Salt concentrations of the present study were lower than previous studies (Arocha et al. 1992, Reıtsma and Henning 1996, Kuplu et al. 2000) but inactivation times of all *E. coli* O157:H7 doses were less than the previous studies. This can be an indication that also other factors play a role except for the amount of salt.
Survival of Escherichia coli O157:H7 during processing and ripening period of Keş Cheese

(3%) to inactivate the pathogen in Keş cheese. Kuplulu et al. (2000) found the elimination times for $10^2$, $10^4$, and $10^6$ cfu mL$^{-1}$ doses of E. coli O157:H7 as less than 120 days, 120 days and more than 120 days, respectively. The pathogen survived for 158 days when it is added 3 log mL$^{-1}$ dose to Cheddar cheese milk (Reitsma and Henning, 1996). On the other hand it was reported that E. coli O157:H7 remained for 30–40 days at pH 4.0–4.5 (Mcingvale et al. 2000). In the present study, pH values changed from 4.87 at the beginning of ripening to 4.58 at the end of 90 day ripening in Keş cheese. In the present study, pH reduction was longer than the previous studies. However, inactivation time of the pathogen was shorter. According to our results, the correlations between the number of all E. coli O157:H7 doses and pH values, DM, and SDM counts confirmed the effect of physicochemical properties on the pathogen inactivation. However, the effect of pH, DM, and SDM counts may be combined with other factors. Addition of the spices (Nigella sativa and cumin) to curd, during the production process of Keş cheese, may be considered as another inactivation factor as they possess antimicrobial agents.

There are numerous studies on antimicrobial effects of Nigella sativa and cumin (Sagdic et al. 2002, Nazma and Choudhury 2005, Agaoglu et al. 2007; Enany et al. 2009). Burits and Bucar (2000) analysed Nigella sativa for its essential oils. The researchers reported that according to the GC-MS results, essential oil content is taken from large quantities of thymoquinone, cymene, carvacrol, t-anethole, 4-terpineol and longifoline. Carvone, dihydro carvone, limonene and carvacrol are volatil oils of cumin. Also, different studies revealed that Nigella sativa seeds (Toama et al. 1974, Nazma and Choudhury 2005) and cumin have an inhibitory effect on E. coli (Agaoglu et al. 2007). According to another study, Nigella sativa oil extract has significant inhibition on E. coli on both human and animal strains as much as most of the standard antibiotics (Enany et al. 2009). Similarly, Sagdic et al. (Sagdic et al. 2002) reported that cumin extract (2%) prevented the growth of E. coli O157:H7. On the contrary, red pepper was reported as ineffective on E. coli growth (Agaoglu et al. 2007).

The results of the present study demonstrate that survival of E. coli O157:H7 in the lactic cheese production process is possible. Taking into account that the lack of pasteurisation and/or boiling stage in Keş cheese production, regional differences and cheese making process should be standardized as it may cause outbreaks from raw milk originated pathogens and human sourced contaminations. Considering the survival of even low numbers of E. coli O157:H7 in lactic environment can constitute a big threat to the consumers, it is recommended that Burdur Keş cheese should be ripened at least 90 days for the public health.

4. ACKNOWLEDGEMENTS

This study was supported by the Scientific Research Projects Unit of Mehmet Akif Ersoy University (Project no: NAP-0006-07).

5. REFERENCES


Association of Official Analytical Chemists Inc, Arlington, V.A.


SPSS. 1999. SPSS 10 for windows. Chicago, IL: SPSS Inc.


