Study on the growth and survival of *Escherichia coli* O157:H7 during the manufacture and storage of Iranian white cheese in brine

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(Received 12 Apr 2009; revised version 2 Aug 2009; accepted 11 Aug 2009)

**Summary**

The behaviour of *Escherichia coli* O157:H7 was studied during the manufacture and storage of Iranian white cheese in brine. Cheese was manufactured using pasteurized cow milk and inoculated with *E. coli* O157:H7 with inoculum level of 10^3 cfu/ml. Four treatments were designed. Cheeses were made with or without starter culture and kept immersed in 6 or 8% salt brine during ripening and storage. Cheese samples were analysed for *E. coli* O157:H7 during manufacture and storage period. During cheese manufacture the number of *E. coli* O157:H7 increased by 10^6 cfu/g, but during ripening and cheese storage the number of organism decreased significantly in the cheese samples made with starter culture (P<0.05). The results showed an inhibitory effect of starter culture on *E. coli* O157:H7, but the organism can survive in this kind of cheese for up to 60 days of storage, respecting using starter culture, salt brine concentration and cheese storage time.

**Key words:** *Escherichia coli* O157:H7, Iranian white cheese, Starter culture, Brine salting

**Introduction**

Since the identification of *E. coli* O157:H7 as a human pathogen in 1982 (Fratamico and Smith, 2006), *E. coli* O157:H7 has become a pathogen of major concern for the food and dairy products because of it’s ability to cause severe illness, in particular, haemorrhagic colitis, haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura (Govaris *et al.*, 2001; Maher *et al.*, 2001). Water and other foodstuffs such as lettuce, alfalfa sprouts and apple juice have also been implicated in outbreaks (Buchanan and Doyle, 1997).

Most of the foodborne outbreaks of *E. coli* O157:H7 have been associated with the consumption of foods originated from cattle, especially foods contaminated with cattle faeces. Because *E. coli* O157 has been found regularly in healthy cattle faeces, this animal is known to be an asymptomatic carrier (Öksüz *et al.*, 2004). In 1999, over 11% of the total number of reported cases of infection caused by *E. coli* O157:H7 in England and Wales were due to dairy products (Vernozy-Rozand *et al.*, 2005). *Escherichia coli* O157 serotypes are identified as enterohaemorrhagic *E. coli* and categorized as verotoxin-producing *E. coli*. Verotoxin is also known as shiga-like toxin (Jamshidi *et al.*, 2008).

The organism is destroyed in pasteurization process, but insufficient heat-treatment of ground meat and raw milk forms a potential infection risk (Betts, 2000; Öksüz *et al.*, 2004). The processing
conditions for different milk products are very important from the standpoint of the organism’s infection risk. It can grow in trypticase soy broth (TSB) acidified with lactic acid at pH = 4.6 but not at pH = 4.5 (Glass et al., 1992).

Cheese made with unpasteurised milk is a potential vehicle for transmission of *E. coli* O157 to the consumer. In Iran, similar to other countries, a large amount of traditional cheeses are manufactured from raw milk and consumed freshly or after ripening in salt brine. The aim of the present study was to determine the effect of pH and different salt brine concentrations of cheese on survival of *E. coli* O157:H7 during manufacture and storage of Iranian white cheese in brine.

**Materials and Methods**

**Cow milk**

Pasteurized cow milk was obtained from Iranian Dairy Industries Co., and stored at 4°C. The quality of the milk was within the limits specified in the current Iranian standard for production of cheese (Fat = 2.5%, SNF = 8.9% and pH = 6.7) (Anon. 2002). It was evaluated for the lack of antibiotic residues (copan test).

**Bacterial strain and preparation of inocula**

Toxigenic strain of *E. coli* O157:H7 (ATCC 25922) was obtained from Faculty of Veterinary Medicine, University of Tehran. This strain was activated during two consecutive cultures in 50 ml brain-heart infusion (BHI) broth for 18-20 h until the optical density reached 0.8 to 0.9 at 600 nm, which corresponded to approximately $1 \times 10^8$ cfu/ml. The culture was diluted to obtain a concentration of $10^7$ cfu/ml. One ml of this culture was added to 10 L of milk to obtain a $10^5$ cfu/ml.

**Starter culture**

Lyophilized direct vat type thermophilic yoghurt culture containing *Streptococcus thermophilus* and *Lactobacillus delbruekii* subsp. *bulgaricus* (Chr. Hansen’s laboratory, FD-DVS CH-1, Denmark) was used to make the Iranian traditional white brined cheese.

**Procedure of making Iranian white cheese in brine**

To evaluate the effect of starter on *E. coli* O157:H7, two batches of cheese were prepared, one of them was treated with 0.2 U/L starter (at 35°C) while the other sample was left intact. To speed up the clotting or reducing the amount of rennet needed, CaCl$_2$ (0.02% w/v) was added. Rennet (Chr. Hansen’s Laboratory, HANILASE L 3500) was then added to achieve the final concentration of 0.002% (w/v). Cheese was maintained at 35°C for 1 h to curdle. The curd was cut into $2 \times 2 \times 2$ cm$^3$ and allowed to drain. The mixture was agitated and drained for 1 h. Following drainage, the curd was placed in stainless steel press for 6 h, to fuse the curd grains into a continuous mass (7 h). The moulded cheese was cut into $7 \times 7 \times 7$ cm$^3$ pieces and kept immersed in 20% solution of pasteurized salt brine for 8 h at 18°C (15 h). After salting, cheese pieces were aseptically packed in 6 and 8% salt brine and held at 14°C to ripen. The specimens were then kept at 4°C (Hanifian and Karim, 2006). During ripening and storage period, the samples were analysed on dogs 15, 30, 45 and 60.

**Enumeration and detection of *Escherichia coli* O157:H7**

MacConkey agar containing sorbitol instead of lactose (SMAC) was used for isolation of *E. coli* O157:H7. Due to the fact that these bacteria are unable to ferment sorbitol, non-sorbitol-fermenting (NSF) colonies were potentially considered as *E. coli* O157:H7 (McDonough et al., 2000; Meng et al., 2001; Jamshidi et al., 2008).

At each sampling period, 10 g of cheese was added to a bottle containing 90 ml of 0.1% peptone water and homogenized using a stomacher lab blender for 3 min. Serial 10-fold dilutions of test material were prepared in sterile peptone water, surface spread plated in duplicate on sorbitol MacConkey agar (HIMEDIA M298, India) surfaces containing cefixime (0.05 mg/l) and potassium tellurite (2.5 mg/l) (CT-SMAC), then incubated at 35°C for 24 h. Non-sorbitol-fermenting colonies on CT-SMAC were counted and 5-10 colonies were chosen to confirm by latex-agglutination with the *E.
coli O157 latex kit (Bahar afshan). Latex agglutinating isolates were further confirmed biochemically in SIM, MR-VP broth, Simon’s citrate agar and TS1 agar. E. coli O157:H7 are glucose, indole and methyl red positive, but negative for lactose, sucrose, Voges-Proskauer, citrate, CO₂ and SH₂ (Meng et al., 2001). Then biochemically confirmed E. coli O157:H7 colonies were counted.

Physicochemical analysis of cheese

Physicochemical analysis of the samples were made at each sampling time for enumeration of E. coli O157:H7. Salt content (Carpenter and Hendricks, 2003), total solid (Bradley, 2003) and pH (Sadler and Murphy, 2003) were determined. The pH of cheese samples was determined using a pH meter (Testo 230, pH-und temperature-meßgerät, EN 50081-1 + EN 50082-1, Gmbh, Germany).

Statistical analysis

A split plot experiment based on completely randomized design (CRD) with three replications was conducted. Factor A included starter (with and without starter), factor B included salt brine concentration (6 and 8%) and factor C was time (15, 30, 45 and 60 days). Data were analysed using the general linear model procedure (SAS, 1992). Analysis of the variance followed by Duncan’s multiple range test was employed to find significant differences (P<0.05) between the treatments.

Results

The physicochemical properties and counts of E. coli O157:H7 in milk and cheeses made with starter and without starter culture, during manufacture, ripening and storage are given in Table 1. Escherichia coli O157:H7 was not isolated from the samples of pasteurised milk, starter culture, rennet, CaCl₂ or salt brine.

The counts of E. coli O157:H7 in all of the cheeses increased continuously from the initial inoculum level by about 3 logs in 7 h during manufacture.

During brine salting (20% solution of NaCl) for 8 h at 18°C, the population of the pathogen remained relatively stable. At the end of 15 h, the NaCl concentration in the cheese was 4%. During ripening, in the cheeses made with starter culture, the pathogen population decreased significantly (P<0.05) to 4 log cfu/g, whereas they remained relatively stable (about 10⁶ cfu/g) in the cheeses made without starter culture. At those storage times, the pH was dropped to 5.1 and 6.2 in the cheese samples with and without starter, respectively. The pH of the cheeses made with starter dropped gradually to 4.5 on day 60.

At the end of the storage time, survival of E. coli was significantly lower (P<0.05) in cheese with starter (Fig. 1) compared to that without starter (Fig. 2). However, at 4°C, a rapid decline in E. coli O157:H7 population was observed in cheese samples made with starter, but it survived at approximately 80 and 630 cfu/g in 8 and 6% salt brine throughout storage.

Discussion

According to the USA mandates, acidic food processors should guarantee a 5-log reduction of E. coli O157:H7 during processing of fermented sausages and fruit juices (because of the survival of E. coli O157:H7 in acidic foods) (Getty et al., 2000; Samelis and Sofos, 2003). There is no published data on the 5D reduction of the organism for fermented milk products, yet. Validation studies on survival of E. coli O157:H7 and other pathogens are also necessary to indicate potential risks and preventive actions to be taken (Leuschner and Boughtflower, 2002; Lekkas et al., 2006). Such studies are particularly needed for traditional Iranian white brined cheese, as few data on growth and survival of E. coli O157:H7 in this product are currently available.

The results of this study indicated that E. coli O157:H7 may have a great potential for survival in Iranian white brined cheese. The lactic acid bacteria in the cheese made with starter culture had an inhibitory effect on E. coli O157:H7, since the population of E. coli O157:H7 was significantly (P<0.05) lower compared to the cheese made without starter culture.
Table 1: Changes in mean of total solid, NaCl content, pH and *E. coli* O157:H7 counts in cheese samples made with starter and without starter

<table>
<thead>
<tr>
<th>Cheese type</th>
<th>Brine concentration (%)</th>
<th>Time (d or h)</th>
<th>Total solid (%) ± SEM</th>
<th>NaCl content (%) ± SEM</th>
<th>pH ± SEM</th>
<th>Log <em>E. coli</em> O157:H7 ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>With starter</td>
<td>6</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>6.7 ± 0.02</td>
<td>3 ± 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 h</td>
<td>37.0 ± 0.60</td>
<td>ND</td>
<td>6.1 ± 0.05</td>
<td>5.8 ± 0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 h</td>
<td>43.3 ± 0.89</td>
<td>3.9 ± 0.03</td>
<td>5.6 ± 0.03</td>
<td>5.8 ± 0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 d</td>
<td>31.6 ± 0.25</td>
<td>4.0 ± 0.21</td>
<td>5.3 ± 0.07</td>
<td>6.2 ± 0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 d</td>
<td>35.3 ± 0.60</td>
<td>3.8 ± 0.26</td>
<td>5.0 ± 0.09</td>
<td>4.1 ± 0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45 d</td>
<td>37.0 ± 0.31</td>
<td>4.6 ± 0.17</td>
<td>5.4 ± 0.09</td>
<td>5.8 ± 0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 d</td>
<td>43.3 ± 0.56</td>
<td>3.3 ± 0.29</td>
<td>4.4 ± 0.05</td>
<td>2.8 ± 0.15</td>
</tr>
<tr>
<td>Without starter</td>
<td>6</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>6.7 ± 0.04</td>
<td>3 ± 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 h</td>
<td>36.6 ± 0.42</td>
<td>ND</td>
<td>6.7 ± 0.09</td>
<td>6.0 ± 0.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 h</td>
<td>40.3 ± 0.92</td>
<td>4.0 ± 0.15</td>
<td>6.5 ± 0.04</td>
<td>5.8 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 d</td>
<td>34.0 ± 0.89</td>
<td>4.1 ± 0.23</td>
<td>6.2 ± 0.09</td>
<td>6.6 ± 0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 d</td>
<td>34.3 ± 0.79</td>
<td>4.4 ± 0.16</td>
<td>6.1 ± 0.06</td>
<td>6.3 ± 0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45 d</td>
<td>38.6 ± 0.30</td>
<td>4.1 ± 0.17</td>
<td>5.4 ± 0.09</td>
<td>6.2 ± 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 d</td>
<td>42.6 ± 0.55</td>
<td>4.3 ± 0.19</td>
<td>5.7 ± 0.09</td>
<td>5.9 ± 0.09</td>
</tr>
<tr>
<td>With starter</td>
<td>8</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>6.7 ± 0.03</td>
<td>3 ± 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 h</td>
<td>36.6 ± 0.42</td>
<td>ND</td>
<td>6.7 ± 0.09</td>
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<td>5.7 ± 0.09</td>
<td>5.9 ± 0.09</td>
</tr>
</tbody>
</table>

ND: Not determined. Values are means of triplicate experiments (n = 3). The means with different letters in each column are significant at P<0.05, using Duncan’s multiple range test. SEM: Standard error of the mean.

Fig. 1: *Escherichia coli* O157:H7 counts during the manufacture and storage of cheese made with starter

A similar antagonistic effect of lactic acid bacteria on food-borne pathogens was reported by Larson *et al.* (1993) in whey. They reported a significant growth of *Listeria monocytogenes* and *salmonella Heidelberg* in whey containing no lactic acid bacteria.

Fig. 2: *Escherichia coli* O157:H7 counts during the manufacture and storage of cheese made without starter

A study by Glass *et al.* (1992) showed that *E. coli* O157:H7 can grow in TSB culture compared to the samples containing lactic acid bacteria.
containing ≤6.5% NaCl or at a pH of 4.5 to 9.0, adjusted with HCl. When TSB was acidified with lactic acid, the organism grew at pH 4.6 but not at pH 4.5.

According to a study by Leyer et al. (1995), the organism was adapted to acid by culturing for one to two doubling at pH 5.0. Acid adapted cells had an increased resistance to lactic acid, and their survival was better than non-adapted cells during the fermentation of sausage. Also, there were no differences in the salt content of the cheeses, given that increased salt (4%) may protect E. coli O157:H7 from acid (Casey and Condon, 2002; Lekkas et al., 2006).

Since the populations of E. coli O157:H7 was later decreased during storage period, one of the main factors causing the initial decrease in E. coli O157:H7 might be attributed to the storage temperature of 4°C. Optimum growth temperature of E. coli O157:H7 is around 37°C in several foods (Govaris et al., 2001) and incubating the milk at this temperature during preripening procedures contributed to the excessive growth rate.

Results presented in this study may suggest that the manufacturing procedure of Iranian white cheese in brine do not eliminate E. coli O157:H7, emphasizing the tolerance of this pathogen to acid produced by starter culture. Although the population of E. coli O157:H7 was high (2.3 log cfu/g), no signs of spoilage were observed from the day of inoculation until day 60 of storage at 4°C. Since no spoilage was visible in the cheese containing high numbers of the E. coli O157:H7, consumer health could be endangered.

References


Leuschner, RGK and Boughtflower, MP (2002). Laboratory-scale preparation of soft cheese artificially contaminated with low levels of Escherichia coli O157, Listeria...