Survival of *Escherichia coli* O157:H7 during Manufacture and Storage of White Brined Cheese

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**Abstract:** *Escherichia coli* O157:H7 is a major foodborne pathogen that causes severe disease in humans. Survival of *E. coli* O157:H7 during processing and storage of white brined cheese was investigated. Cheeses were prepared using pasteurized milk inoculated with a 4-strain *E. coli* O157:H7 cocktail (7 log_{10} CFU/g) with or without yogurt starter culture (*Lactobacillus delbrueckii* ssp. bulgaricus and *Streptococcus salivarius* ssp. thermophilus) and stored in 10% or 15% NaCl brine at 10 and 21 °C for 28 d. NaCl concentration, water activity (a_w), pH, and numbers of *E. coli* O157:H7 and lactic acid bacteria (LAB) were determined in cheese and brine. *E. coli* O157:H7 was able to survive in cheese stored in both brines at 10 and 21 °C regardless of the presence of starter LAB, although the latter significantly enhanced *E. coli* O157:H7 reduction in cheese or its brine at 10 °C. *E. coli* O157:H7 numbers were reduced by 2.6 and 3.4 log_{10} CFU/g in cheese stored in 10% and 15% NaCl brine, respectively, in the presence of starter LAB and by 1.4 and 2.3 log_{10} CFU/g, respectively, in the absence of starter LAB at 10 °C. The pathogen survived, but at lower numbers in the brines. The salt concentration of cheese stored in 10% brine remained about 5% during ripening, but in 15% brine, the NaCl level increased 1.6% to 8.1% (w/w) by 28 d. Values of pH and a_w slightly decreased 1 d after exposure to brine and reached 5.5 to 6.6 and 0.88 to 0.94, respectively, in all treatments.

**Keywords:** cheese brine, *E. coli* O157:H7, lactic acid bacteria, NaCl, white brined cheese

**Practical Application:** *E. coli* O157:H7 showed great potential to survive in white brined cheese; therefore, prevention of postpasteurization contamination by the cheese industry and artisanal producers is critical to reduce the risk of *E. coli* O157:H7 in these products.

**Introduction**

*Escherichia coli* O157:H7, a Gram-negative rod, is the most well-known enterohemorrhagic serotype of *E. coli*. In 1982, it was identified as a human pathogen through its production of Shiga-like toxins and hemolysin that result in severe illnesses characterized as hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura. *E. coli* O157:H7 can grow at 7 to 50 °C, in acidic foods at pH 4.4, and in foods with a minimum water activity (a_w) of 0.95 (WHO 2011).

*E. coli* O157:H7 can be transmitted to humans by ingesting contaminated raw or undercooked ground meat and dairy products. The consumption of contaminated cheese was responsible for 0.4% of the total foodborne outbreaks in Europe in 2006 (European Food Safety Authority (EFSA) 2008).

White brined cheese is popular product in Middle Eastern and eastern Mediterranean countries and classified as soft to semihard cheese (Alichanidis and Polychroniadou 2008; Osaily and others 2012). It may serve as an ideal medium for bacterial growth in the absence of a competing starter culture because of its high protein, fat, and water activity. *E. coli* O157:H7 has caused several outbreaks associated with cheese consumption, especially soft cheeses (Kousta and others 2010). Thus, the dairy industry considers eliminating this pathogen from dairy products a major priority. However, *E. coli* O157:H7 and generic *E. coli* can still be isolated from white brined cheeses (De Buyser and others 2001; Öksüz and others 2004; Kursun and others 2011). Several of reports have shown that *E. coli* O157:H7 is not only able to survive, but also grow in various types of cheeses (Vernozy-Rozand and others 2005; Schlesser and others 2006; D’Amico and others 2010). However, there is little information on the viability of *E. coli* O157:H7 during the manufacture and storage of white brined cheese or its brine (Mohammadi and others 2009).

White brined cheese is rennet-coagulated and is eaten fresh or after storage (most often refrigerated but sometimes at room temperature) in brine containing 10% to 24% NaCl (Osaily and others 2012). The salt in curd has many roles; it inhibits undesirable microorganisms, reduces curd moisture, modifies texture and flavor, and contributes to cheese ripening (Laborda and Rubio 1999; Mulet and others 1999). The salt concentrations used in white brined cheese are able to inhibit the growth of most foodborne pathogens, but when proteins and other nitrogenous compounds are leached from the cheese into the brine, survival of pathogens in the brine can be improved. Risks are enhanced when the brine is used repeatedly without pasteurization (Larson and others 1999). Several studies have investigated the *in vitro* effects of salt concentrations on the growth and survival *E. coli* O157:H7 (Conner 1992; Colavita and others 2003; Hrenovic and Ivankovic 2009), but little information is available on the ability of *E. coli* O157:H7 to survive in cheese brine (Ingham and others 2000).

Therefore, the objectives of this study were to determine the effect of temperatures and salt concentrations on the behavior of *E. coli* O157:H7 during manufacture and storage of white brined cheese.
E. coli O157:H7 in white brined cheese

Materials and Methods

Preparation of culture

Four nonpathogenic E. coli O157:H7 strains (3581, 0304, 0627, and 0628) that had become nonpathogenic (verotoxigenic negative) during storage were provided by Rafiq Ahmed, Natl. Microbiology Laboratory, Public Health Agency, Canadian Science Centre for Human and Animal Health, Winnipeg, MB, Canada. E. coli O157:H7 strains were maintained in trypticase soy agar (TSA) (Oxoid Ltd., Basingstoke, Hampshire, U.K.) and subcultured twice in trypticase soy broth (TSB, Oxoid) at 37 °C for 24 to 48 h before use. The cultures were centrifuged (Herolab, Wiesloch, Germany) at 2500×g for 20 min and pellets were resuspended in 1 mL sterile 0.1% peptone water to achieve a concentration of 10^6 CFU/mL. Then, 5 mL from each strain were pooled to obtain equal concentrations of each strain in the cocktail. This mixture of E. coli O157:H7 was used to contaminate milk samples at a final level of approximately 7.2 log10 CFU/mL.

Preparation of cheese and E. coli O157:H7 inoculation tests

Fresh cow’s milk with 12.7 ± 0.7% total solids, 3.5 ± 0.3% protein, and 3.2 ± 0.5% fat was obtained from a local dairy farm. White cheese was manufactured using the method outlined by Al-Holy and others (2012) and Yamani and others (1998) with some modifications. Milk was batch (15 L) pasteurized at 72 °C for 15 s using a Tefal Sensorielle Pot (TEFAL, Berkshire, England) and cooled to 36 to 37 °C. The cocktail of nonpathogenic E. coli O157:H7 strains was added to yield 7.0 log10 CFU/g cheese. In treatments (Table 1) with starter culture, 0.5% (w/v) of lyophilized direct-to-vat mixed thermophilic yogurt starter cultures (Chr. Hansen, Hørsholm, Denmark) containing Streptococcus thermophilus and Lactobacillus delbrueckii sp. bulgaricus was added to the pasteurized milk. After 30 min, the milk was coagulated with single-strength calf rennet (Chr. Hansen), by dilution 30-fold with cold water, added to each batch of milk and held for 30 to 40 min. The coagulum was cut into cubes of 1 cm and agitated gently for 10 min. The sliced curd was carefully transferred from the vat to a stainless steel mold measuring 50 cm × 50 cm × 2 cm (height). After being pressed for 30 min, the curd was cut manually into pieces that weighed 60 to 80 g (4 cm × 3 cm × 1 cm). The cheese pieces were placed in glass beakers containing either 10% or 15% NaCl brine solution and were stored either at 10 or 21 °C (Table 1).

Sampling and microbial analysis

White brined cheese and the brine were sampled at 0, 1, 3, 7, 14, 21, and 28 d storage to enumerate E. coli O157:H7 and lactic acid bacteria (LAB) and to measure NaCl, pH, and NaCl concentration in cheese. For microbiological sampling, 5 g of internal cheese from a cross-sectional sample or 5 mL of brine solution were mixed with 45 mL of 0.1% peptone water and homogenized in a sterile stomacher bag for 2 min with a stomacher model 400 (Seward Ltd., London, U.K.). E. coli O157:H7 numbers were determined by surface plating 100 µL diluted samples on MacConkey agar supplemented with Cefixime and Tellurite (ct-SMAC, Oxoid) and incubated at 37 °C for 24 to 48 h aerobically. To determine the presence of injured E. coli O157:H7 cells, 100 µL diluted samples were surface-plated on TSA and incubated for 4 h at 37 °C, and then the plates were overlaid with an equal volume of ct-SMAC and incubated at 37 °C for 24 to 48 h. The numbers of LAB were determined by surface plating 100 µL diluted cheese or brine sample on deMan Rogosa Sharpe (MRS) agar (Oxoid) and incubated anaerobically at 37 °C for 48 h.

Measurement of water activity (aw)

Approximately 2 g from cross-sectional section was used to measure the aw of white brined cheese with an aw meter (Hygrolab, Rotronic Instrument Corp, Huntington, N.Y., U.S.A.).

Salt determination

The NaCl concentration in cheese was determined using the procedure described by Osali and others (2012). Briefly, 1 to 1.5 g cheese sample was held in a muffle furnace (Labtech Co., Ltd., Namyangjy, South Korea) at 550 °C for 8 h. Then, 25 mL of distilled water and droplets (to approximately 0.5 mL) of 0.5 N potassium chromate (Alpha Chemika, Mumbai, India) were added to the ashed sample and mixed thoroughly. The mixture was titrated against 0.05 N AgNO3 (Alpha Chemika) until the color became brown. The salt concentration was calculated using the following equation:

\[
\text{Salt content} \% = \left(\frac{\text{titrated volume of AgNO}_3}{\text{wt. of sample}}\right) \times 100%
\]

Measurement of pH

The pH values of white brined cheese and brine solution were directly measured using a pH meter (Thermo Electron Co., Waltham, Mass., U.S.A.).

Table 1—Experimental design used for survival studies of E. coli O157:H7 in white brined cheese.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>E. coli O157:H7</th>
<th>Starter culture</th>
<th>NaCl%</th>
<th>Storage temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>−</td>
<td>10</td>
<td>10 °C</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>10</td>
<td>10 °C</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>10</td>
<td>21 °C</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>10</td>
<td>21 °C</td>
</tr>
<tr>
<td>5</td>
<td>−</td>
<td>+</td>
<td>15</td>
<td>10 °C</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>15</td>
<td>10 °C</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>−</td>
<td>15</td>
<td>21 °C</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>15</td>
<td>21 °C</td>
</tr>
</tbody>
</table>

*Approximately 7.40 log CFU/g cheese were added to all treatments.

Table 2—Recovery of E. coli O157:H7 and LAB (and non-LAB) (log10 CFU/mL or g) during cheese processing.*

<table>
<thead>
<tr>
<th>Processing point</th>
<th>E. coli O157:H7 on TSA/ct-SMAC (SD)</th>
<th>LAB and non-LAB on MRS (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw milk</td>
<td>4.26 (±0.36)*</td>
<td>6.07 (±0.43)</td>
</tr>
<tr>
<td>Pasteurized milk</td>
<td>ND*</td>
<td>4.13 (±0.16)</td>
</tr>
<tr>
<td>Inoculated milk</td>
<td>7.16 (±0.13)</td>
<td>6.55 (±0.05)</td>
</tr>
<tr>
<td>Whey</td>
<td>6.73 (±0.58)</td>
<td>6.68 (±0.56)</td>
</tr>
<tr>
<td>Cutting curd</td>
<td>7.29 (±0.33)</td>
<td>7.23 (±0.40)</td>
</tr>
<tr>
<td>Before pressing</td>
<td>7.44 (±0.37)</td>
<td>7.34 (±0.19)</td>
</tr>
<tr>
<td>After pressing</td>
<td>7.40 (±0.08)</td>
<td>7.43 (±0.13)</td>
</tr>
</tbody>
</table>

*Values are the means of 3 experiments (standard deviations).

†The number of Gram-negative bacteria.

ND: Not detected (detection level was ≤ 1 CFU/mL).
**E. coli** O157:H7 in white brined cheese...

### Table 3—Recovery of **E. coli** O157:H7 (log_{10} CFU/g) from white brined cheese made with or without starter culture during storage.

<table>
<thead>
<tr>
<th>Storage time (day)</th>
<th>ct-SMAC agar</th>
<th>TSA/ct-SMAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>7.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.94&lt;sup&gt;bC&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>7.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.40&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>7.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.06&lt;sup&gt;bA&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>7.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>7.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>7.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.51&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>7.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>7.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.38&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Treatments are described in Table 1.

<sup>b</sup>Means from each sampling time in the same row with the same lowercase letters (a to d) are not significantly different (P > 0.05). Means from each sampling time in the same column with the same lowercase letters (a to d) are not significantly different (P > 0.05).

### Results and Discussion

**E. coli** O157:H7 and LAB were detected at each point of cheese processing (Table 2). **E. coli** O157:H7 and other Gram-negative bacteria were not detected in pasteurized milk on TSA/ct-SMAC agar; however, nonstarter LAB and non-LAB survived thermal treatment of milk and were 4.1 log_{10} CFU/mL immediately after pasteurization detected on MRS agar. It has been reported that nonstarter LAB are able to survive pasteurization and are the source of secondary microflora in aseptic cheeses (Beresford and others 2001; Briggiler-Marcó and others 2007). When pasteurized milk was inoculated with 7.2 log_{10} CFU/mL **E. coli** O157:H7, their numbers increased slightly and reached 7.4 log_{10} CFU/g at cheese pressing (Table 2).

Although **E. coli** O157:H7 was able to survive in cheese stored in 10% or 15% NaCl brine at 10 and 21 °C with or without starter culture, their numbers were significantly reduced by 28 d (Table 3). In the absence of starter LAB, **E. coli** O157:H7 numbers in cheese with 10% NaCl were only reduced by 1.4 log_{10} CFU/g at 10 or 21 °C. However, cheese stored in 15% brine, numbers at...
corresponding temperatures were reduced by 2.3 and 2.7 log_{10} CFU/g, respectively (Table 3). Similarly, other researchers reported that *E. coli* O157:H7 can survive in brined cheeses stored at low temperatures. Mohammadi and others (2009) found that *E. coli* O157:H7 numbers did not change in brined Iranian white cheese (8% NaCl) during 60 d storage at 4 °C.

It was notable in the presence of starter LAB that *E. coli* O157:H7 numbers in cheese stored in 10% or 15% NaCl brine were reduced by 2.6 or 3.4 log_{10} CFU/g, respectively, at 10 °C, but only by 1.0 and 1.6 log_{10} CFU/g, respectively, at 21 °C after 28 d (Table 3). Similarly, Mohammadi and others (2009) found that numbers of *E. coli* O157:H7 were reduced by 3.1 to 3.8 log_{10} CFU/g more in Iranian white cheese made with starter LAB during ripening and 60 d storage at 4 °C than in those prepared without starter cultures. Some, but not all bacteriocin-producing LAB have been found marginally better than bacteriocin-negative cultures for *E. coli* O157:H7 control in raw milk cheese aged 60 d at 12 °C. Rodriguez and others (2005) reported half the bacteriocin-positive strains they tested showed no significant additional inhibitory effects, but the more effective strains caused a ≤0.9 log_{10} CFU/g reduction over bacteriocin-negative starter cultures. In the present study, the largest reduction in *E. coli* O157:H7 numbers was observed in samples with 15% brine in the presence of starter LAB at 10 °C; however, bacterial reductions resulting from 10 °C exposure were inconsistent across treatments and were more likely the result of exposure to the 3% higher concentration of NaCl present in the 15% brined cheese. Certainly, during ripening of cheeses and other dairy products at <10 °C, the numbers of viable *E. coli* O157:H7 decrease during normal storage. This was shown in Cheddar cheese aged 158 d (Reitsma and Henning 1996), in Romano, Colby, and Feta cheeses aged for 30 d (Hudson and others 1997), in buttermilk stored for 35 d and sour cream held 28 d (Dineen and others 1998). Consistently, with all these products, containing *E. coli* O157:H7 was able to survive beyond the end of ripening, and 4 °C storage did not prevent its survival. When salt concentrations in cheese were high (>20%) and pH was low (<4), *E. coli* O157:H7 survival at higher temperature (>13 °C) was reduced. This has often been observed in other systems and is believed due to enhanced lethal effects of higher temperature exposure on the stress caused by acid or salt; however, when the salt concentration was only 4%, as in Greek fresh-cured Galotry cheese, *E. coli* O157:H7 survived better at 13 °C than at 4 °C (Lekkas and others 2006). The tendency toward lower survival of *E. coli* O157:H7 observed here at 10 than 21°C is consistent with the latter result.

*E. coli* O157:H7 from contaminated cheese was found in the brine at maximum numbers on day 1 (3.1 to 4.0 log_{10} CFU/g). Thereafter, *E. coli* O157:H7 viability decreased, but the pathogen survived in 10% brine regardless of the presence of starter LAB at 21 °C or their absence at 10 °C up to 28 d. However, *E. coli* O157:H7 cells were not detected in 10% or 15% brine in the presence of starter LAB after 14 d at 10 °C (Table 4). Conner (1992) also reported that *E. coli* O157:H7 was more susceptible to 4% to 10% NaCl in tryptic soy broth or poultry extract broth at 10 °C than at 37 °C. In contrast, Ingham and others (2000) found that *E. coli* O157:H7 in commercial cheese brines with 22.1% to 29.3% NaCl was reduced by 1.9 to 2.3 log_{10} CFU/g at 4 °C and by 2.1 to 3.0 log_{10} CFU/g at 13 °C after 28 d. As with cheese, at higher salt concentrations in brine, higher temperatures were more antimicrobial. In the current work, with the lower salt concentration used than in some of the studies cited above, the lower temperature plus the starter enhanced the sensitivity of *E. coli* O157:H7 in the cheese and brine.
Injured *E. coli* O157:H7 cells were found in both white cheese and brine (Tables 3 and 4). While the extent of injury seemed higher in brine from some treatments (≤1.6 log CFU/g in brine compared to ≥0.7 CFU/g in cheese), the number of observations was small and injury varied around 0.5 log<sub>10</sub> CFU/g for both types of samples. Waterman and Small (1998) suggested that brine can be more antimicrobial to *E. coli* O157:H7 than cheese since the latter contains fat and protein that may protect the organisms.

Starter and nonstarter (present in raw milk) LAB numbers decreased slightly (by 1.2 to 1.9 log<sub>10</sub> CFU/g) in white cheese stored at 10% or 15% NaCl brine after 28 d at 10 °C. However, the number remained constant in white cheese with 10% NaCl brine stored at 21 °C (Table 5). However, in the brines, numbers of starter and nonstarter LAB gradually declined and reached 1.6 to 2.3 log<sub>10</sub> CFU/g by 28 d at 10 °C. In contrast, at 21 °C in 10% NaCl brine, starter and nonstarter LAB grew and reached ≥7.0 log<sub>10</sub> CFU/g by 28 d (Table 6). Other work has shown that LAB can grow in MRS containing 10% NaCl (Nanasonamb and others 2012) and survived up to 10 d in brine containing 13.2% NaCl at 4 or 12 °C (Boyier and others 2009). Although nonstarter LAB grow poorly in milk, they are able to grow in cheese and may affect its quality either positively or negatively; therefore, they are considered a major uncontrolled factor in cheese manufacture (Crow and others 2001; Breggler–Marcó and others 2007). Interestingly, nonstarter LAB numbers were controlled at 21 °C in 15% brine in both the cheese (where they were reduced by 4.7 log<sub>10</sub> CFU/g, Table 5), and in brine alone where they were not detected on day 28 (Table 6). It is probable that the reduction of cheese a<sub>0</sub> to 0.88 (Table 7) at 15% NaCl was inhibitory to these organisms, reducing lactose consumption and yielding reduced growth (Basit and others 1993; Liu and others 1998).

The cheese at day 1 in 10% and 15% brine contained 4.4% to 5.4% and 5.5% to 6.7% NaCl (w/w), respectively, and although little further change occurred in 10% brined samples, the salt content of samples stored in 15% brine increased by 1.6% to 8% by 28 d (Table 8). In all treatments, the pH of cheese and brine remained unchanged or slightly declined during storage. These values were similar for cheese and brine and ranged from 6.4 to 6.8 at 10 °C and 5.5 to 6.7 at 21 °C by 28 d. The pH values of both cheese and brine at 10% NaCl were slightly lower than at 15% NaCl (Tables 9 and 10). This was expected since the higher salt level influenced LAB viability, and probably affected
microbial lactose metabolism, as well as development of proteolytic and lipolytic activities during ripening. Salting is a major step in the manufacture of white brined cheese that influences pH and ensures development of its characteristic properties (Turhan and Kaletruc 1992). The pH values observed here were typical of semisoft white brined cheeses that range from pH 5.2 to 6.5 (Tarakci and Tun Bunduk 2008; Osami and others 2012). It is unlikely that lower pH (<5.0) will enhance the safety of these cheeses with respect to _E. coli_ O157:H7 contamination because the pathogen can tolerate such conditions, although low pH usually affords some protection from most other pathogens and reduces spoilage rates of these types of cheese (Alcaindis and Polychroniadou 2008).

**Conclusions**

Although _E. coli_ O157:H7 numbers were reduced ≤3.4 log10 CFU/g by 15% brine plus starter LAB at 10 °C, the pathogen was able to survive in white brined cheese aged 28 d in 10% or 15% brine at 10 or 21 °C. It is apparent that starter culture LAB and the low _a_w had inhibitory effects against _E. coli_ O157:H7, but that the lower temperatures used (10 °C) that was more lethal to the pathogen than 21 °C. Starter and nonstarter LAB numbers remained constant or slightly decreased in cheese stored in both brines by 28 d at 10 °C. However, nonstarter LAB numbers were reduced by 4.7 log10 CFU/g in cheese samples stored in 15% brine at 21 °C. Since these results indicate that _E. coli_ O157:H7 can survive white brined cheese ripening, control measures including good manufacturing practices and hazard analysis critical control point programs during its processing are necessary to reduce the risk of _E. coli_ O157:H7 contamination.

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**References**


