Behavior of *Escherichia coli* O157:H7 during the Manufacture and Ripening of Feta and Telemes Cheeses

ALEXANDROS GOVARIS,1 DEMETRIOS K. PAPAGEORGIOU,2* AND KONSTANTINOS PAPATHEODOROU3

1Laboratory of Hygiene of Foods of Animal Origin, Veterinary Faculty, University of Thessaly, Karditsa, Greece; 2Department of Food Hygiene and Technology, Laboratory of Milk Hygiene and Technology, School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki 54006, Greece; and 3Agricultural Union of Larisa, “Olympus” Dairy, 41334 Larisa, Greece

ABSTRACT

Pasteurized whole ewe’s and cow’s milk was used in the manufacture of Feta end Telemes cheeses, respectively, according to standard procedures. In both cases, the milk had been inoculated with *Escherichia coli* O157:H7 at a concentration of ca. 5.1 log CFU/ml and with thermophilic or mesophilic starter cultures at a concentration of ca. 5.3 to 5.6 log CFU/ml. In the first 10 h of cheesemaking, the pathogen increased by 1.18 and 0.82 log CFU/g in Feta cheese and by 1.56 and 1.35 log CFU/g in Telemes cheese for the trials with thermophilic and mesophilic starters, respectively. After 24 h of fermentation, a decrease in *E. coli* O157:H7 was observed for all trials. At that time, the pH was reduced to 4.81 to 5.10 for all trials. Fresh cheeses were salted and held at 16°C for ripening until the pH was reduced to 4.60. Cheeses were then moved into storage at 4°C to complete ripening. During ripening, the *E. coli* O157:H7 population decreased significantly (*P* ≤ 0.001) and finally was not detectable in Feta cheese after 44 and 36 days and in Telemes cheese after 40 and 30 days for the trials with thermophilic and mesophilic starters, respectively. The estimated times required for one decimal reduction of the population of *E. coli* O157:H7 after the first day of processing were 9.71 and 9.26 days for Feta cheese and 9.09 and 7.69 days for Telemes cheese for the trials with thermophilic and mesophilic starters, respectively.

*Escherichia coli* O157:H7 is recognized as an important cause of foodborne illness. This pathogen, a gram-negative, facultative anaerobe bacterium, has a low infection dose of 50 to 5 organisms (24). The three distinct virulence factors of this serotype of *E. coli* are the production of the cytoytic Shiga-like toxins (I and II), hemolysin synthesis, and the ability to adhere to and colonize intestinal surfaces (22, 24). *E. coli* O157:H7 can cause hemorrhagic colitis, hemolytic uremic syndrome, and thrombocytopenic purpura and can lead to death (22). Hemolytic uremic syndrome and thrombocytopenic purpura, to which young children and elderly people are most vulnerable, are less common but can lead to the most severe complications.

Since it was first identified in 1982, *E. coli* O157:H7 has been isolated in numerous foodborne outbreaks worldwide, with increasing frequency in the past decade (3). In the United States, it is estimated that *E. coli* O157:H7 outbreaks have resulted in up to 250 deaths over the past decade and that the pathogen causes more than 20,000 infections each year (24). The consumption of undercooked ground beef and that of raw milk have been the most commonly recognized major risk factors for infection (1, 3, 24). In Scotland, one massive outbreak associated with the consumption of contaminated pasteurized milk was recorded that resulted in the infection of 100 people and the hospitalization of 33 (39). Outbreaks due to the consumption of cheese products have also been reported (19, 24, 38).

Dairy cattle are most likely asymptomatic carriers of *E. coli* O157:H7, providing a natural reservoir for this pathogen (19, 40, 43). However, *E. coli* O157:H7 has also been isolated from other animals, such as pigs, sheep (11, 23), and goats (4). Chapman (8) reported that the incidence of *E. coli* O157:H7 isolates was higher in lamb products than in beef products in Sheffield, UK, during surveillance in 1995 and 1996.

The behavior of *E. coli* O157:H7 in cheese products made from cow’s milk (2, 20, 36, 37) has been studied more often than its behavior in ewe’s and goat’s milk. The Greek regulatory standard requires that Feta cheese be made from ewe’s milk or from a mixture of ewe’s and goat’s milk, while cow’s milk is used to produce Telemes cheese. It is important to note that these soft white brined cheeses have occasionally been made from unpasteurized milk. Coliform bacteria are present in raw milk; hence, the purpose of this work was to investigate the behavior of *E. coli* O157:H7 during the manufacture, ripening, and storage of Feta and Telemes cheeses.

MATERIALS AND METHODS

**Bacterial strains.** Two enterohemorrhagic *E. coli* O157:H7 strains, EDL-932 and EDL-933, were kindly provided by Professor Genigeorgis, Aristotle University of Thessaloniki, Greece. Each strain was grown separately in 50 ml of sterile Trypticase soy broth (Oxoid, Basingstoke, UK) for 24 h at 37°C with two consecutive transfers. The inoculum of *E. coli* O157:H7 was prepared according to Govaris et al. (16) with the two strains combined at about equal concentrations. The cheese milk was contam-

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* Author for correspondence. Tel: +031 0999806; Fax: +031 0999803; E-mail: dkpapag@vet.auth.gr.
TABLE 1. Composition of Feta and Telemes cheeses by the end of ripening at 16°Ca

<table>
<thead>
<tr>
<th>Cheese</th>
<th>Starter cultures</th>
<th>Fat in dry matter (%)</th>
<th>Moisture (%)</th>
<th>Sodium chloride (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feta</td>
<td>Thermophilic</td>
<td>44.2 ± 0.15 A</td>
<td>53.4 ± 0.20 A</td>
<td>2.20 ± 0.18 A</td>
</tr>
<tr>
<td></td>
<td>Mesophilic</td>
<td>44.6 ± 0.12 b</td>
<td>52.9 ± 0.15 b</td>
<td>2.27 ± 0.12 A</td>
</tr>
<tr>
<td>Telemes</td>
<td>Thermophilic</td>
<td>43.3 ± 0.12 c</td>
<td>55.2 ± 0.13 c</td>
<td>2.10 ± 0.14 A</td>
</tr>
<tr>
<td></td>
<td>Mesophilic</td>
<td>43.6 ± 0.10 c</td>
<td>54.5 ± 0.11 d</td>
<td>2.15 ± 0.09 A</td>
</tr>
</tbody>
</table>

* a Means ± standard deviations of duplicate samples of two independent trials. Values with the same letter in the same column are not significantly different (P < 0.05).

initated with the inoculum of *E. coli* O157:H7 at a final concentration of 5.6 log CFU/ml.

The mesophilic starter culture was a mixture of commercial lactic acid strains (*Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*, 1:1; R 704, Chr. Hansen A/S, Horlom, Denmark). The thermophilic yogurt-type starter culture was also a mixture of commercial lactic acid strains (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*, 1:1; CH-1, Chr. Hansen). The starter cultures (direct vat set type) were generated in 250 ml of sterile skim milk at 28°C (mesophilic) and 40°C (thermophilic) for 30 min before inoculation into cheese milk.

*E. coli* O157:H7 contamination trials and preparation and ripening of cheeses. Two contamination trials were conducted using the thermophilic and the mesophilic starter cultures for both cheese types. The thermophilic yogurt-type starter culture was used because yogurt (1 to 2%) has traditionally been added to milk and is still used by the Greek cheese industry in the preparation of Feta and Telemes cheeses. The mesophilic starter culture was used because this type of culture is now widely used by the Greek industry. Pasteurization of milk and preparation of cheese types were carried out in a commercial cheese facility under strict hygienic control as described by Papageorgiou et al. (35). Pasteurized whole cow's and ewe's milk (72°C for 16 s) was used in the preparation of Telemes and Feta cheeses, respectively. Feta and Telemes cheeses were prepared according to established methods (33, 35) with some modifications. Both cheeses were prepared in stainless double-jacketed cheese vats with a milk capacity of 150 liters. After pasteurization at 72°C for 16 s, cow's milk (130 kg) and ewe's milk (60 kg) were placed in cheese vats at 35°C.

For each contamination trial, milk was inoculated with *E. coli* O157:H7, and starter culture prepared as described above was added. After 20 min of incubation at 35°C, 12 g of calcium chloride per 100 liters of milk and 2.5 g of rennet (Hala, Chr. Hansen) per 100 liters of milk were added. The curd was ready to be cut into cubes of 2 to 3 cm after 60 min. Curds were set aside for 15 min and then placed in polypropylene hoops (21 by 11 by 10 cm). A frame (21 by 11 by 5 cm) was used at the top of each hoop and was removed as soon as the curd lowered and reached the upper surfaces of the hoops, after approximately half an hour. The hoops were turned twice at 2-h intervals, and the cheese was sprinkled with coarse dry salt. The cheese blocks (21 by 11 by 8 cm) remained in hoops until the next morning (24 h) at 20 ± 1°C, when they were placed in a tin pack (24 by 24 by 32 cm) in four layers, with two blocks of cheese in each layer. The tin packs were filled with 3 liters of 5.6% salt brine and placed at 16°C. Ripening at 16°C continued until the pH of the cheese blocks decreased to 4.60, at which time cheese packs were stored refrigerated at 4°C to complete the ripening process.

Duplicate samples were taken for composition analysis and enumeration of *E. coli* O157:H7 during the manufacture and ripening of cheeses at the following stages: pasteurized milk, inoculated milk (0 h), curd before cutting (1.3 h), curd in hoops after half an hour of cutting (2 h), curd after salting and drainage (10 h), and cheese after 24 h. Afterward, enumeration of *E. coli* O157:H7 for cheese samples was carried out at 2-day intervals.

**Enumeration of *E. coli* O157:H7 and lactic acid bacteria.** At each designated sampling point, duplicate samples of milk (25 ml), curd (25 g), and cheese (25 g) were placed in sterile stomacher bags and diluted 1:10 in warm (42°C), sterile tryptose broth with 2% sodium citrate. Samples were blended in a stomacher 400 (Seward Medical Ltd., London, UK) for 3 min. After maceration, serial decimal dilutions were carried with 0.1% phosphate-buffered saline (PBS; Oxoid) and samples were assayed for *E. coli* O157:H7 counts by direct plating of 0.1 ml of appropriate dilutions onto sorbitol MacConkey agar (Oxoid). Plates were incubated at 37°C for 48 h.

When *E. coli* O157:H7 cells were not countable by direct plating, surviving cells of the pathogen were detected by enrichment. Cheese samples (25 g) were placed in sterile stomacher bags and diluted 1:10 in modified *E. coli* broth (CM 990, Oxoid) supplemented with novobiocin (SR 181, Oxoid). Enriched samples were incubated at 37°C (16 to 18 h, 150 rpm) and plated (0.1 ml) in duplicate on sorbitol MacConkey agar. Results are reported as the presence or absence of the pathogen in the cheese products.

For the enumeration of lactic acid bacteria, duplicate samples of milk (25 ml), curd (25 g), and cheese (25 g) were macerated as previously described, serially diluted in 0.1% PBS, and plated (0.1 ml) on deMan Rogosa Sharpe agar (Oxoid) as described by Dineen et al. (10). Selected colonies from plates with the higher dilution were confirmed with API 50 CH test strips (API System, Montalieu-Vercieu, France).

**Physicochemical analysis.** The amounts of fat in the dry matter, moisture, and sodium chloride of cheese samples were estimated according to procedures described by Bradley et al. (5).

The pH values of milk, curd, and cheese samples were determined with a pH meter at each sampling time for the enumeration of *E. coli* O157:H7 during manufacture and ripening of cheeses.

**Statistical analysis.** All microbiological assays were performed in duplicate, and the entire study was duplicated. Statistical differences were determined with Student's *t* test after analysis of variance. For statistical analysis of the results obtained after the first day of processing, a reliability and survival analysis was carried out with the MINITAB package (release 11/96).

**RESULTS AND DISCUSSION**

Table 1 shows the fat in dry matter, moisture, and salt contents of Feta and Telemes cheeses after ripening at 16°C.
All cheese samples were in compliance with the Greek State Standard Regulation (14), which requires that Feta and Telemes cheeses contain not less than 43% fat in dry matter and not more than 56% moisture. There is no standard for the salt content of these cheeses; however, the salting procedure is described in this regulation. Commercial Feta and Telemes cheeses produced in Greece contain 2 to 3% salt. A series of analyses of cheese samples showed that the recorded fat in dry matter, moisture, and salt contents did not change significantly (P < 0.01) during subsequent storage at 4°C. The cheese yield for Feta cheese was higher than that for Telemes cheese, with almost 4 kg of ewe’s milk per kg of Feta cheese and 9 kg of cow’s milk per kg of Telemes cheese, because of the higher fat and casein content of ewe’s milk.

The behavior of E. coli O157:H7 during the manufacture of Feta and Telemes cheeses for up to 24 h. Pasteurized milk samples were tested by the enrichment method to ensure that the milk used in the experiments was free of E. coli O157:H7. Figures 1 and 2 show changes in E. coli O157:H7 levels and pH values during the manufacture of Feta and Telemes cheeses, respectively. Our results show that populations of E. coli O157:H7 increased during the manufacture of both types of cheese. In curds that had been placed in molds for almost half an hour after cutting (1.3 h after inoculation) increased by almost 0.3 and 0.4 log CFU/g for both thermophilic and mesophilic starter cultures in the Feta and Telemes cheese trials, respectively. It is also important to note that there was not a significant difference (P < 0.05) between E. coli O157:H7 populations in curds regardless of the cheese type and starter culture used. The pH of curds of both cheese types before cutting was ca. 6.37. Our results show that the growth of E. coli O157:H7 was almost the same in curds before cutting, regardless of the starter culture used or the cheese type. Other investigators have found that the presence of lactic acid bacteria in milk (13) and skim milk (7) has little effect on E. coli O157:H7. Duffy et al. (12) studied the effect of lactic acid bacteria on the growth of E. coli O157:H7 in brain heart infusion broth at 15°C and concluded that the growth of the pathogen was not affected. Dineen et al. (10) found that E. coli O157:H7 could grow in commercial starter cultures and that the relative inhibition of the pathogen in the experimental fermentation systems was strongest for the thermophilic mixture, followed by L. delbrueckii subsp. bulgaricus alone, L. lactis subsp. lactis alone, L. lactis subsp. cremoris alone, and S. thermophilus alone.

In curds that had been placed in molds for almost half an hour after cutting (2 h after inoculation), the populations of E. coli O157:H7 increased from the numbers at the previous sampling time in Feta cheese by ca. 0.55 log CFU/g and in Telemes cheese by ca. 0.56 and 1.35 log CFU/g for the thermophilic and the mesophilic culture trials, respectively. The pH of curds of both
cheese types after cutting was ca. 6.32 and had changed by <0.05 from that recorded at the previous sampling time, indicating that the pH of curds after cutting was almost the same as that before cutting. The average temperature of curds placed in molds was 31 ± 0.7°C. Populations of E. coli O157:H7 between trials with thermophilic and mesophilic starters were not significantly different (P < 0.05) for Feta cheese samples; in contrast, they were significantly different (P > 0.05) for Telemes cheese samples. The increase in the E. coli O157:H7 levels in curds after cutting compared with that at the previous sampling time may be due to the growth of the pathogen after the initial lag of adaptation as well as to the entrapment of the pathogen in the curds after whey drainage. Listeria monocytogenes was also entrapped, and its numbers increased in the curds of Feta cheese after whey drainage (35). Reitsma and Henning (37) also observed that E. coli O157:H7 was more heavily concentrated in the curds of cheddar cheese than in the whey. The larger populations of E. coli O157:H7 in both Telemes cheese trials than in the Feta cheese trials were probably caused by the higher concentration of the pathogen in the curds of Telemes cheese after whey drainage. Telemes cheese had a lower yield with a higher concentration of solid components of milk in curd than did Feta cheese, as mentioned earlier.

Ten hours after curdling, the E. coli O157:H7 populations increased by 1.20 and 0.90 log CFU/g in Feta cheese and by 1.56 and 1.35 log CFU/g in Telemes cheese for the trials with thermophilic and mesophilic starters, respectively. The populations of E. coli O157:H7 in cheese blocks in molds of both types of cheese increased by <0.2 log CFU/g from those at the previous sampling time. Populations of E. coli O157:H7 were significantly different (P > 0.05) between samples of trials with thermophilic and mesophilic starters of both cheese types. The pH values of Feta cheese samples were 5.81 and 5.65 and those of Telemes cheese samples were 5.70 and 5.52 for the trials with thermophilic and mesophilic starters, respectively. Ten hours after curdling, the average salt content of all cheese samples was 0.8% (SD = 0.3). The average cheese block temperature was 22 ± 0.5°C. The smaller increase of the E. coli O157:H7 population in cheese blocks may be due to factors like decreased pH and temperature, the addition of salt, or even antagonistic activity of lactic acid bacteria. An inhibitory effect on pathogens by lactic acid bacteria has been attributed to metabolic end products such as organic acids, diacetyl, hydrogen peroxide, and bacteriocins (30). Since bacteriocins offer little protection against gram-negative bacteria, greater inactivation of E. coli O157:H7 populations decreased by 0.84 and 0.96 log CFU/g in Feta cheese and by 1.11 and 1.33 log CFU/g in Telemes cheese for the trials with thermophilic and the mesophilic starter cultures, respectively. Therefore, the decrease was larger for Telemes cheese samples than for Feta cheese samples and was also larger for the trials with mesophilic starter culture than for the trials with thermophilic starter culture for both types of cheese. The pH values were ca. 5.1 for both cheese types for the trials with thermophilic culture and 4.98 and 4.85 for Feta and Telemes cheeses, respectively, for the trials with mesophilic starters. Populations of E. coli O157:H7 were significantly different (P > 0.05) between trials with thermophilic starters and those with mesophilic starters for both cheese types. The salt contents (0.9 ± 0.3) had not changed significantly from the previous sampling time for any type of cheese (P < 0.05), while the average temperature of cheese blocks had dropped to 20°C.

After 24 h of curdling, a decrease in E. coli O157:H7 numbers from the previous sampling time was observed for samples of all types of cheese. E. coli O157:H7 populations decreased by 0.84 and 0.96 log CFU/g in Feta cheese and by 1.11 and 1.33 log CFU/g in Telemes cheese for the trials with thermophilic and the mesophilic starter cultures, respectively. Therefore, the decrease was larger for Telemes cheese samples than for Feta cheese samples and was also larger for the trials with mesophilic starter culture than for the trials with thermophilic starter culture for both types of cheese. The pH values were ca. 5.1 for both cheese types for the trials with thermophilic culture and 4.98 and 4.85 for Feta and Telemes cheeses, respectively, for the trials with mesophilic starters. Populations of E. coli O157:H7 were significantly different (P > 0.05) between trials with thermophilic starters and those with mesophilic starters for both cheese types. The salt contents (0.9 ± 0.3) had not changed significantly from the previous sampling time for any type of cheese (P < 0.05), while the average temperature of cheese blocks had dropped to 20°C.

The behavior of E. coli O157:H7 during the ripening of Feta and Telemes cheeses. Fresh cheeses were salted and held at 16°C for ripening until their pH was reduced
Changes in E. coli O157:H7 level and pH during ripening of Feta cheese prepared with thermophilic or mesophilic starter cultures at 16 and 4°C. ○, populations of E. coli O157:H7 in Feta cheese with thermophilic starter; ▲, populations of E. coli O157:H7 in Feta cheese with mesophilic starter; ○, pH of Feta cheese with thermophilic starter; △, pH of Feta cheese with mesophilic starter. Values of <2 log CFU/g were estimated by enrichment; positive values of <2 and >0; negative = a value of 0. Arrows show when the temperature changed from 16 to 4°C.

Changes in E. coli O157:H7 level and pH during ripening of Telemes cheese prepared with thermophilic or mesophilic starter cultures at 16 and 4°C. ○, populations of E. coli O157:H7 in Telemes cheese with thermophilic starter; ▲, populations of E. coli O157:H7 in Telemes cheese with mesophilic starter; ○, pH of Telemes cheese with thermophilic starter; △, pH of Telemes cheese with mesophilic starter. Values of <2 log CFU/g were estimated by enrichment; positive values of <2 and >0; negative = a value of 0. Arrows show when the temperature changed from 16 to 4°C.

to 4.6. These cheeses were then moved into storage at 4°C to complete the ripening process. The changes in the E. coli O157:H7 levels and pH values of Feta and Telemes cheeses during ripening at 16°C and during subsequent storage at 4°C are shown in Figures 3 and 4. The ripening times required at 16°C until the pH of Feta cheese decreased to <4.6 were 16 and 8 days for the trials with the thermophilic and the mesophilic starters, respectively, and those required for Telemes cheese were 12 and 6 days for the trials with the thermophilic and the mesophilic starters, respectively. During ripening at 16°C, the pH values of all cheese samples decreased by almost 0.5, while during storage at 4°C, the pH values of all cheese samples stabilized. Thermophilic lactic acid bacteria increased in both types of cheese from an initial population of ca. 5.3 log CFU/ml to maximum populations of ca. 8.8 log CFU/g at almost the middle of the 16°C ripening time and decreased to ca. 7.8 log CFU/g by the end of the 16°C ripening time. Mesophilic lactic acid bacteria increased from an initial population of ca. 5.6 log CFU/ml to maximum populations of ca. 8.3 log CFU/g by the end of the 16°C ripening time for both types of cheese. Populations of thermophilic and mesophilic lactic acid bacteria stabilized during storage at 4°C for both types of cheese.

The population of E. coli O157:H7 decreased significantly (P < 0.001) to <100 CFU/g in Feta cheese after 32 and 26 days and in Telemes cheese after 28 and 22 days for the trials with the thermophilic and the mesophilic starters, respectively. The pathogen was not detectable in Feta cheese after 44 and 36 days or in Telemes cheese after 40 and 30 days for the trials with the thermophilic and the mesophilic starters, respectively. The decrease in the population of E. coli O157:H7 followed the pH decrease and was faster in trials with mesophilic starters than in trials with thermophilic starters for both types of cheese (Figs. 3 and 4).

The estimated times required for one decimal reduction of the population of E. coli O157:H7 after the first day of processing were 9.71 and 9.26 days for Feta cheese and 9.09 and 7.69 days for Telemes cheese for the trials with thermophilic and mesophilic starters, respectively. The slopes of the death curves in Figures 3 and 4 were estimated from a reliability and survival analysis of the data with the MINITAB package (release 11/96). The functional relationships between the log of E. coli O157:H7 populations (C2) and the time of ripening (C1) as derived from slopes were C2 = 4.88 − 0.103C1 and C2 = 4.24 − 0.108C1 for Feta cheese and C2 = 4.66 − 0.110C1 and C2 = 4.19 − 0.130C1 for Telemes cheese for the trials with thermophilic and mesophilic starters, respectively.

The decreases in the population of E. coli O157:H7 ranged from 2 to 2.33 log CFU/g during ripening at 16°C and from 0.8 to 0.95 log CFU/g during storage at 4°C for all cheese samples, as counted by direct plating on sorbitol MacConkey agar. The addition of 5.6% pasteurized salt brine at the beginning of the ripening process may also have affected the death rate of the pathogen. An increased
survival of *E. coli* O157:H7 at refrigeration temperatures (0 to 4°C) relative to that at room temperatures was also found in other low-acid food products, such as mayonnaise (41, 42), apple cider (29), soy sauce (27), and yogurt (20). The same phenomenon of better survival at refrigerated temperatures than at room temperatures was also observed for *L. monocytogenes* in salted whey and skim milk (34) and for *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* Kentucky in fermented dry sausage (31). A high inactivation rate of *E. coli* O157:H7 as the pH decreased to 4.6 has been observed in other fermented food products, such as sausage (15, 31), a smear-ripened cheese (25), cultured milk (7), and yogurts (26).

Our results show that *E. coli* O157:H7 can survive in the acidic environment of Feta and Telemes cheeses during ripening at 16°C and during the following refrigerated ripening at 4°C. Survival times were 34 to 42 days for Feta cheese (Fig. 3) and 28 to 38 days for Telemes cheese (Fig. 4). By inoculating milk with high (10³ CFU/ml) and low (1 CFU/ml) concentrations of *E. coli* O157:H7, Reitsma and Henning (37) also found that the pathogen survived up to 158 days during the manufacture and storage of Cheddar cheese at 4°C. Hudson et al. (20) found a 3-log cycle reduction in counts of *E. coli* O157:H7 after 27, 30, and 27 days for Colby, Romano, and Feta cheeses, respectively. They also observed a final pH of 4.80 for Feta cheese. Other investigators (36) found that *E. coli* O157:H7 survived in Feta cheese made from cow’s milk and was present in larger numbers at the end of the storage period (75 days) than in the initial inoculum. The pH of this cheese was consistently lower (ca. 5.0 or higher) than the pH of that in our study. The survival of *E. coli* O157:H7 as a prefermentation (26) or a postfermentation contaminant (20) in yogurts with a pH below 4.6 was also reported. Govaris et al. (17) observed that *E. coli* O157:H7 was detectable in Feta cheese with a pH of 4.6 to 4.4 for up to 50 days with 5.3 log CFU/ml of the pathogen inoculated in brine. The results of another study indicated that *Yersinia enterocolitica* survived for <5 days in Feta cheese with a pH of 4.5 (21). *Aeromonas hydrophila* was also inactivated during the manufacture and ripening of Feta cheese as soon as the pH dropped below 4.8 and at least 5 days had passed since the time of curdling (28). *Brucella melitensis* was inactivated in Feta cheese in <30 days when it was inoculated at 4 × 10⁸ CFU/ml in milk (32). *L. monocytogenes* survived for >90 days in Feta cheese (35).

In conclusion, our results show that the survival of *E. coli* O157:H7 was higher in Feta cheese than in Telemes cheese. *E. coli* O157:H7 survival was also higher in trials with thermophilic starters than in trials with mesophilic starters for both types of cheese (Figs. 3 and 4). Our results also show that *E. coli* O157:H7 can grow in Feta and Telemes cheeses during manufacture and survive for 1 to 1.5 months during ripening and storage at 4°C. If Feta and Telemes cheeses are consumed within the first 30 to 44 days of production, illness due to *E. coli* O157:H7 is possible. The cheese industry should apply control measures to avoid postpasteurization contamination of milk with this pathogen. Good manufacturing practices and hazard analysis critical control point programs in the manufacture of cheese can help to prevent contamination of cheese products with pathogens like *E. coli* O157:H7. Finally, the results of this study indicate that even initial populations of *E. coli* O157:H7 as high as 5.3 to 5.6 log CFU/ml of milk are destroyed during Feta and Telemes cheese ripening in <60 days, which is the minimum ripening time required by Greek law, regardless of the use of raw or pasteurized milk.

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