60-Day Aging Requirement Does Not Ensure Safety of Surface-Mold-Ripened Soft Cheeses Manufactured from Raw or Pasteurized Milk When *Listeria monocytogenes* Is Introduced as a Postprocessing Contaminant

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ABSTRACT

Because of renewed interest in specialty cheeses, artisan and farmstead producers are manufacturing surface-mold-ripened soft cheeses from raw milk, using the 60-day holding standard (21 CFR 133.182) to achieve safety. This study compared the growth potential of *Listeria monocytogenes* on cheeses manufactured from raw or pasteurized milk and held for >60 days at 4°C. Final cheeses were within federal standards of identity for soft ripened cheese, with low moisture targets to facilitate the holding period. Wheels were surface inoculated with a five-strain cocktail of *L. monocytogenes* at approximately 0.2 CFU/cm² (low level) or 2 CFU/cm² (high level), ripened, wrapped, and held at 4°C. *Listeria* populations began to increase by day 28 for all treatments after initial population declines. From the low initial inoculation level, populations in raw and pasteurized milk cheese reached maximums of 2.96 ± 2.79 and 2.33 ± 2.10 log CFU/g, respectively, after 60 days of holding. Similar growth was observed in cheese inoculated at high levels, where populations reached 4.55 ± 4.33 and 5.29 ± 5.11 log CFU/g for raw and pasteurized milk cheeses, respectively. No significant differences (*P* < 0.05) were observed in pH development, growth rate, or population levels between cheeses made from the different milk types. Independent of the milk type, cheeses held for 60 days supported growth from very low initial levels of *L. monocytogenes* introduced as a postprocess contaminant. The safety of cheeses of this type must be achieved through control strategies other than aging, and thus revision of current federal regulations is warranted.

According to the Centers for Disease Control and Prevention, *Listeria monocytogenes* infection had the second highest case fatality rate (20%) and the highest hospitalization rate (92.2%) of all infections caused by foodborne pathogens (29). Numerous outbreaks and sporadic cases of listeriosis have been linked to the consumption of fresh and ripened soft cheeses, and a high health risk is associated with the consumption of unripened soft cheese. In 2005, the U.S. Food and Drug Administration (FDA) issued a health advisory warning consumers not to consume soft cheeses made from raw milk (42). Although this warning was focused on illegally produced Mexican-style cheeses, the lack of standards of identity for the various cheese varieties can create much confusion; soft cheeses comprise a wide range of cheese types ranging from Hispanic or Mexican-style cheeses such as unripened queso fresco to surface-ripened varieties such as Camembert types, each with various associated risks. Specific standards of identity are critical to food safety because the characteristics of the specific cheese variety dictate the potential for growth and survival of microbial pathogens. Many hard cheeses made from raw milk and aged for 60 days or more have an excellent food safety record because of the interaction of factors such as pH, salt content, and water activity that render these cheeses microbiologically safe (6). Current regulations (43) permit the manufacture of soft ripened cheeses from raw milk provided that these cheeses are aged for 60 days at ≥1.67°C (35°F) to ensure safety. Raw milk cheeses that have not been properly aged are illegal in the United States and cannot be imported. Because of renewed interest in specialty cheeses, domestic artisan and farmstead producers are manufacturing surface-mold-ripened soft cheeses from raw milk using the 60-day holding standard to achieve safety. In Europe, Protected Denomination of Origin (PDO) cheeses such as Camembert de Normandie are required to be manufactured from raw milk to receive PDO status. Safety is not attained through aging (although these cheeses are aged a minimum of 21 days) but through regulations specified in European Union (EU) directives (e.g., no. 2073/2005), which establish microbiological criteria for cheeses made from raw or thermally treated milk (9).

Although raw milk may contain *L. monocytogenes*, the overall prevalence is low, with sporadic contamination and seasonal variability. When *L. monocytogenes* is present,
levels in bulk tank milk are typically below 3 CFU/ml (27, 30). Because *L. monocytogenes* is primarily an environmental pathogen, the primary risk to cheese safety is from postpasteurization environmental contamination from the cheese making and/or aging environment (19). Pasteurized or otherwise processed milk (4) and cheeses (8, 17, 19, 36) can contain *L. monocytogenes* due to postprocessing contamination that can occur during manufacture, ripening, or washing (19). Recontamination of foods with pathogenic bacteria may be a frequent and important cause of foodborne outbreaks in which postprocessing contamination is believed to be the cause of major outbreaks of listeriosis attributed to the consumption of soft cheese (12, 17). The concern with surface-mold-ripened soft cheese is not necessarily the survival of *L. monocytogenes* but the growth potential during aging and holding following increases in pH.

Unlike other pathogens, *L. monocytogenes* can survive and continue to grow during refrigerated storage because of its psychrotrophic nature. The growth of *L. monocytogenes* on the surface of Camembert-type cheese made from pasteurized milk has been previously investigated (1, 13, 25, 32). The use of relatively high inoculation levels (~500 CFU/20 cm², 2 to 4 log CFU per sample) and the repeated sampling and experimental aging conditions, including the sealing of samples in plastic containers or foil, do not represent conditions commonly employed during commercial cheese manufacture. From a risk assessment perspective, realistic inoculation levels and conditions are important because initial concentrations and conditions affect the behavior of pathogens such as *L. monocytogenes* in cheese (13, 25, 32). Unlike pasteurized milk, raw milk may have a protective effect against pathogenic bacteria (6). Gay and Amgar (13) investigated the fate of *L. monocytogenes* added to milk during the manufacture and ripening of Camembert cheese manufactured from either raw or pasteurized milk. These authors found that the lag phase and time to a 10³ CFU increase in *L. monocytogenes* levels were twice as long in raw milk Camembert cheese than in its pasteurized counterpart, likely because of the microbiological composition of raw milk, especially the presence of thermophilic lactobacilli and yeasts.

Although *L. monocytogenes* has been studied extensively in both food and processing environments and extensive risk assessments are available, limited data are available on the survival of *L. monocytogenes* introduced as a postprocessing contaminant on surface-mold-ripened soft cheese. Environmental contamination of food products is an important factor to consider in future assessments such as the Joint FDA–Health Canada Public Health Risk Assessment regarding *L. monocytogenes* in soft ripened cheese, which is currently underway. In the present study, the growth potential of *L. monocytogenes* introduced as a postprocessing contaminant on surface-mold-ripened soft cheese was examined for the effects of (i) two initial inoculation levels, (ii) the use of pasteurized versus raw milk, and (iii) ripening for 70 days.

**MATERIALS AND METHODS**

**Cultures.** Five *L. monocytogenes* strains were used in this study: FSL R2-499, FSL N3-022, and FSL J1-119 (Dr. Martin Wiedmann, Department of Food Science, Cornell University, Ithaca, N.Y.); F5069 and F5027 (Dr. Catherine Donnelly, Department of Nutrition and Food Sciences, University of Vermont, Burlington). Frozen (~78°C) stock cultures were inoculated into 9 ml of Trypticase soy broth with 0.6% yeast extract and incubated at 30 ± 1°C for 18 h for two subsequent transfers before use. Viable numbers of *L. monocytogenes* in suspension for each culture were determined after serial dilution from aerobic plate counts on Petrifilm AC films (3M Microbiology, St. Paul, Minn.) incubated at 32 ± 1°C for 48 ± 2 h. Based on consecutive plate counts, equal proportions of cells from each culture were combined as a cocktail yielding ~3 × 10⁹ cells per ml. Freeze-dried starter cultures of *Lactococcus lactis* subsp. lactis, *L. lactis* subsp. cremoris, *L. lactis* subsp. biavar diacetylactis, and Strep-tococcus salivarius subsp. thermophilus and ripening cultures of *Kluyveromyces lactis*, *Geotrichum candidum*, and *Penicillium candidum* (EZAL MA011 and MA019, MD99, TO50, KL71, GEO17, and SAM3; Danisco, Copenhagen, Denmark) were stored at −35°C until used.

**Milk collection.** Approximately 100 liters each of raw and pasteurized (nonhomogenized) milk were obtained from a local dairy plant in 10-gal (37.8-liter) sanitized stainless steel milk cans. Raw milk samples were collected before entering the pasteurizer, and pasteurized milk samples were high-temperature short-time pasteurized (72°C for 15 s) and collected bypassing the homogenizer. Mean temperatures of pasteurized and raw milk during transport (~90 min) were 4.83 ± 0.70 and 6.76 ± 0.26°C, respectively, with maximum values of 6.89 ± 0.87 and 7.78 ± 0.87°C, respectively. Milk cans were refrigerated (3 ± 1°C) immediately upon arrival until milk was used the next morning (22 ± 2 h). *L. monocytogenes* was not detected in either raw or pasteurized milk through selective enrichment in *Listeria* enrichment broth at 30°C for 24 h and then plating of 0.1 ml on CHROMagar *Listeria* plates (CHROMagar, Paris, France) incubated at 37°C for 24 h.

**Cheese manufacture.** Surface-mold-ripened soft cheese was manufactured from 100 liters each of raw and pasteurized milk in separate vats on three different days as three independent trials. Each trial yielded ~35 cheeses of each milk type for a total of ~70 cheeses. From the three trials, a total of ~210 cheeses were manufactured in this study, 120 of which were used for microbiological analyses. A 45-min delay between raw and pasteurized milk cheese manufacture was employed to allow enough time between steps to avoid overlap and to allow thorough cleaning and sanitizing of utensils. Cheeses were manufactured with a slightly lower moisture content to endure the 60-day holding period, extending the ripening so that cheeses were in a consumable state on day 60. Temperature and acidification profiles were monitored and recorded with a temperature and pH data logger (model DO 9505, Delta Ohm, Padua, Italy). Milk samples for compositional and microbiological analyses were taken from the filled vat, chilled and refrigerated (3°C), and used within 24 h. For cheese manufacture, refrigerated raw and pasteurized milk (pH 6.7 to 6.8; titratable acidity, 0.16 to 0.17%) was added to separate sanitized pilot scale double-O style steam-jacketed cheese vats (Kusel Equipment Co., Watertown, Wis.) obtained from the University of Vermont pilot plant. Rehydrated glucono delta-lactone (GDL; 30 g/100 liters; Jungbunzlauer S.A., Marckolsheim, France) and CaCl₂ (10 ml/100 liters) were added to filled vats. GDL is used extensively
throughout the EU as an acidulant in the manufacture of surface-

mold-ripened soft cheeses and was thereby added in the manu-

facture of our experimental cheese to produce a food matrix of

typical chemical composition. In the United States, GDL is a gen-

erally recognized as safe additive (21 CFR 184.1318) and is ap-

typical chemical composition. In the United States, GDL is a gen-

derated for use as a pH control agent, as defined in 21 CFR

170.3(o)(23) in some foods. The action of GDL helps gradually
decrease the pH during the lag period of the freeze-dried culture,

providing consistent and controlled acidity development before
the addition of rennet. Milk temperature was raised to between 36
and 39°C and the lactic starter and ripening cultures were added:
starter culture included 2.25 Danisco culture units (DCU) of
MAO11 and MAO19, 0.25 DCU of MD99, and 1.5 DCU of
TAO54 and TAO50; ripening culture included 0.3 DCU of KL71,
0.2 DCU of GEO17, and 0.6 of DCU SAM3. The temperature
was maintained for 30 to 60 min until the target pH of 6.5 to 6.55
was reached. Once proper pH was attained, calf rennet (20 ml/
100 liters, 1:15,000; New England Cheesemaking Supply, Ash-
field, Mass.) was added to the vat, and the milk mixture was
stirred for approximately 45 s. Cutting time was determined by
multipliying the time of flocculation by 3. Once desired firmness
was reached, the coagulum was cut into curds measuring 2 by
2 cm with sanitized stainless steel curd knives. Curds were allowed
to settle for 5 min, stirred for 5 min, allowed to rest for 5 min, and
stirred for another 5 min. After an additional 5 min of rest,
30% of the whey was removed. The curds were then gently stirred
and transferred to plastic hoops (model M5-3864, Fromagex, Ri-
deaux, Md.). Resulting counts were rounded off to two significant
digits at the time of conversion to SPCs. Cheeses were analyzed
to day 70 to reflect growth during distribution and retail. The
top and bottom surfaces of each cheese were analyzed separately
to increase the sensitivity of detection. Results were then com-
pared for analysis. For example, a cheese was considered positive
for Listeria when either a top or bottom sample was positive;
when both surfaces were negative, the cheese was considered neg-
ative for Listeria. Whole top and bottom 100-cm² surfaces (first
1 cm to 1.5 cm, 100 g) of each cheese were removed using a sterile
cheese cutter, placed in separate sterile Whirl-Pak bags (Nasco,
Fort Atkinson, Wis.), appropriately diluted in sterile Butterfield's
phosphate buffer (BBP), and stomached for 3 min in a Stomacher
400 circulator (Seward Ltd., Worthington, UK). The resulting ho-
medicine was serially diluted (10⁻¹) in BBP to facilitate enu-
meration. To reach a detection limit of ≥5 CFU/g, large (150 by
15 mm) petri dishes (25384-326 VWR) containing 30 ml of
CHROMagar Listeria were inoculated with 1 ml of homogenate
in duplicate. Refrigerated plates were allowed to dry in a laminar
flow hood for ~10 min before inoculation. After incubation at
37°C for 24 h, turquoise colonies surrounded by a white halo were
counted. Sugar tubes were utilized to further discriminate L. mon-
ocytogenes from Listeria ivanovii. Random presumptive L. mon-
ocytogenes colonies were confirmed with an automated PCR assay
(BAX DuPont Qualicon, Wilmington, Del.).

**Physicochemical analysis of cheese.** Physicochemical anal-
yses were conducted on the milk before cheese manufacture, after
dehooping, and after the drying step. The following analyses were
performed in duplicate: protein (Kjeldahl method), pH (Accumet
Research AR150 with a flat tip electrode; Accumet Reference 13-
solids (TS) after drying to constant weight at 102°C, and chloride
(Chloride Analyzer 926, Nelson Jameson, Marshfield, Wis.). Salt
in moisture phase (SMP), moisture nonfat substance (MNFS), and
fat in dry matter (FDM) were determined using the formulas

\[
\text{SMP} = \frac{[\text{salt}]}{[100 - \text{total solids}]} \times 100
\]

\[
\text{MNFS} = \frac{(100 - \text{TS})}{(100 - \text{fat})} \times 100
\]

\[
\text{FDM} = \frac{[\text{fat}]}{[\text{TS}]} \times 100
\]

Titratable acidity (0.1 M NaOH, phenolphthalein indicator) was
determined for the milk before and throughout manufacturing.
Ripening surface and cheese interior pH measurements also were
taken at each microbiological sampling interval.

**Statistical analyses.** The resulting data were analyzed using
the SPSS for Windows (version 15.0.1; SPSS Inc., Chicago, Ill.).
The general linear models procedure was used to perform univar-
iate analyses of variance (ANOVA) to determine the effect of
milk type on the rate of change in mean log CFU per gram
the effect of time on changes in both pH and mean log CFU per gram. SPCs, cheese composition, temperature, and humidity during drying, aging, and holding were compared between raw and pasteurized milk and cheeses using t tests. Nonparametric comparisons of mean log counts of L. monocytogenes on individual days were made with Mann-Whitney tests. Results with P values <0.05 were considered significant. Data are expressed as mean ± SEM of the three independent trials.

RESULTS

Milk. SPCs for raw and pasteurized milk were significantly different (P < 0.001), with mean counts of 6,900 and 20 CFU/ml, respectively. L. monocytogenes was not detectable in any raw or pasteurized milk samples before cheese manufacture.

Cheese composition. According to U.S. federal standards of identity (43), soft ripened cheeses produced from raw milk must be held for at least 60 days at no more than 35°F (1.67°C) to ensure safety. To produce a 60-day-old cheese with a stabilized paste typically found in a younger surface-mold-ripened soft cheese, cheeses were manufactured with a slightly lower moisture target at dehooping. The federal standards of identity also specify that soft ripened cheese must contain at least 50% FDM. Despite decreased moisture, all trial cheeses from both raw and pasteurized milk contained >50% FDM with a mean (±SD) of 52% ± 0.01% for raw milk cheese and 52% ± 0.02% for pasteurized milk cheese. Overall, no significant differences in general composition were observed between milk types (Table 1).

Drying, aging, and holding conditions. Raw and pasteurized milk cheeses were placed in separate chambers for drying and aging and therefore were subject to variations in conditions. However, no significant differences were observed in average temperature or average relative humidity between cheese types.

Changes in pH. In all trials, the target pH of 4.80 to 4.85 was achieved at dehooping. Figures 1 and 2 illustrate the changes in pH observed on the surface and in the interior of cheeses, respectively, over time at each microbiological sampling interval. The univariate ANOVA revealed that the linear change in interior pH and the surface pH over time was not significantly different between the two cheese types at both inoculation levels (interior high level, P = 0.373; interior low level, P = 0.586; surface high level, P = 0.423; surface low level, P = 0.480). Differences in inoculation level did not significantly affect the rate of pH change (P = 0.691). However, there was a very significant overall change in pH over time (P < 0.001) at both inoculation levels.

Survival of L. monocytogenes during drying, aging, and holding. After inoculation, L. monocytogenes was undetectable in all samples tested and remained below the detection limit (≥5 CFU/g) until growth began on day 21 (RL) or day 28 (remaining treatments) with corresponding mean pH values of 5.16 (RL), 5.76 (PL) (Fig. 3A), 5.63 (RH), and 5.91 (PH) (Fig. 3B). In some treatments at the low inoculation level, L. monocytogenes remained undetectable throughout aging and holding even at 70 days of age (Table 2). Because of the very low level of inoculant and the subsequent undetectable nature of cells during the initial 3 to 4 weeks, data from only the initiation of growth to day 70 were used for the univariate ANOVA. Log values of mean counts were used for analysis because of the presence of zeros (null values) in the data. Figure 4A shows the growth of L. monocytogenes over time on both raw and pasteurized milk cheese.

### TABLE 1. Moisture nonfat substance (MNFS), salt in moisture phase (SMP), fat in dry matter (FDM), and protein values for raw (R) and pasteurized (P) surface-mold-ripened soft cheese

<table>
<thead>
<tr>
<th>Trial</th>
<th>MNFS (%)</th>
<th>SMP (%)</th>
<th>FDM (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1R</td>
<td>64.81</td>
<td>3.34</td>
<td>52</td>
<td>ND*</td>
</tr>
<tr>
<td>1P</td>
<td>65.7</td>
<td>3.9</td>
<td>50</td>
<td>ND</td>
</tr>
<tr>
<td>2R</td>
<td>67.13</td>
<td>3.3</td>
<td>51</td>
<td>17.37</td>
</tr>
<tr>
<td>2P</td>
<td>69.65</td>
<td>3.1</td>
<td>51</td>
<td>17.81</td>
</tr>
<tr>
<td>3R</td>
<td>63.74</td>
<td>3.85</td>
<td>54</td>
<td>20.74</td>
</tr>
<tr>
<td>3P</td>
<td>66.15</td>
<td>3.53</td>
<td>53</td>
<td>20.39</td>
</tr>
</tbody>
</table>

* ND, not determined.

![Figure 1](https://via.placeholder.com/150)

**FIGURE 1.** Change in mean (±SD) surface pH of raw (•) and pasteurized (□) milk cheese over time at the low (A) and high (B) L. monocytogenes inoculation levels.
at the high inoculation level. The linear change in log mean CFU per gram over time from the onset of detectable growth again was not significantly different between the two cheese types ($P = 0.962$). Mean counts on individual days did not differ significantly between cheese types ($P < 0.05$) except on day 42, where mean counts of *L. monocytogenes* were moderately higher in the pasteurized milk cheese ($P = 0.1$). For both treatments, there was a very significant change in log mean CFU per gram over time ($P < 0.001$). From the low inoculation level, populations reached maximums of $2.96 \pm 2.79$ and $2.33 \pm 2.10$ log CFU/g after 60 days of holding in raw and pasteurized milk cheeses, respectively. More extensive growth was observed in high-level inoculated cheese, where populations reached $4.55 \pm 4.33$ and $5.29 \pm 5.11$ log CFU/g after 60 days of holding for raw and pasteurized milk cheeses, respectively. Populations of *L. monocytogenes* grew at significantly higher rates from the high inoculation level than from the low inoculation level ($P = 0.032$) despite similar changes in surface and interior pH. This finding seems to suggest population growth potential is dependent on initial contamination levels. However, when cheeses negative for *L. monocytogenes* were eliminated from the analysis and only the positive cheeses were included (Fig. 4), there was no significant effect of initial inoculation level on the rate of growth ($P = 0.096$), although there was still no difference in rate of growth between cheese types ($P = 0.963$).

**DISCUSSION**

Although no standards exist in the United States for compositional characteristics of soft ripened cheese beyond FDM, the Codex Alimentarius (21) classifies our experimental cheese as a semisoft full-fat surface-mold-ripened cheese. The Codex Alimentarius international individual standard for Camembert (22) states that with a minimum FDM between 45 and 55%, moisture must not exceed 57% with a corresponding minimum dry matter of 43%. All conditions were met in our experimental cheese. With no differences in compositional characteristics or in drying, aging, holding conditions, or initial inoculation level, the assumption was made that the only variable between cheeses was milk type. Variance between cheeses within a vat cannot be controlled for and may contribute to any error that occurred when whole individual cheeses were sampled for bacteriological analysis. According to the FDA Center for Food Safety and Applied Nutrition Food Compliance Program for Domestic and Imported Cheese and Cheese Products (44), samples from both domestic and imported cheeses must come from intact units from the same lot. In the present study, individual cheeses from the same lot were
tested at each sampling point rather than taking multiple samples from the same cheese over time to overcome the heterogeneous pattern of pathogen distribution in cheese and because the fluid and soft texture of soft ripened cheeses make them difficult to cut and rewrap without altering the product.

During the initial 3 to 4 weeks of aging, populations of *L. monocytogenes* were below our detection limit. The inability to enumerate also was observed by Ryser and Marth (38) when pasteurized milk Camembert-type cheese was surface inoculated. One strain failed to grow but was detected after cold enrichment. However, Ryser and Marth (38) applied *L. monocytogenes* on top of the Penicillium *camemberti* lawn on the surface of the cheese, meaning that the organism had to penetrate the rind to enter the cheese before growth could occur. Decreases in populations on or near the surface of Camembert-type cheese during the initial stages of ripening also have been reported (1, 26). Some cells were likely transferred to the aging racks during the initial stages of ripening in our study; viable cells were detected through environmental sampling of the aging racks (data not shown). We were unable to determine quantitatively the extent of this transfer.

When examining the survival of *L. monocytogenes* in Camembert-type cheese at different pH values, Ryser and Marth (38) found populations decreased at pH 4.6 and failed to grow at pH 5.5. Cheese pH at dehooping (4.8 to 4.9) increases during assimilation of lactate by *K. lactis* and *G. candidum*, decreasing the rind acidity, and because of increased ammonia concentration within the cheese. Surface pH increases more rapidly through the proteolytic and peptidase activity of ripening fungi, notably *P. camemberti* (23). In our study, growth initiated at pH values between 5.16 and 5.91, and once detectable the *L. monocytogenes* population growth paralleled the gradual increase in pH, as found in other studies (31, 33, 38). The delayed onset of growth is likely due to microbial injury incurred from the presence of salt and the low pH environment of the fresh cheese surface (14). When *L. monocytogenes* (strains Scott A, OH, and CA) was inoculated into milk used for Camembert-type cheese manufacture, counts in wedge and interior samples decreased 10- to 100-fold during the first 17 days of ripening, an observation attributed to the low pH (<5.5) and low storage temperature (15 to 16°C) (38). Gay et al. (14) found that *L. monocytogenes* Scott A populations declined and were unable to grow in Richard’s broth adjusted to pH 4.8 used to simulate Camembert cheese at the beginning of ripening.

Increased lag times as a result of microbial injury have been previously reported for *L. monocytogenes* (3, 7, 18, 46). The duration of the lag phase of *L. monocytogenes* under stresses such as high salt (35) or conditions simulating soft cheese ripening (14) also is affected by inoculum size. In our study, in some replicates at the low inoculation level, cells remained undetectable through day 70. Under very unfavorable growth conditions, cells will die during the extended lag phase (32). With a very small inoculum size, heterogeneity in cellular stress responses within the population can result in the death of all the cells in the inoculum and thus no growth (32), or a few cells could survive and thus initiate growth, as was observed in other replicates within the same inoculum set. The presence of null values and the variability in the duration of lag periods

### Table 2. Number of cheese samples positive for *L. monocytogenes* by sampling day

<table>
<thead>
<tr>
<th>Cheese type</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>35</th>
<th>42</th>
<th>49</th>
<th>56</th>
<th>63</th>
<th>70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw high</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Pasteurized high</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Raw low</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Pasteurized low</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

a Three samples were tested for each cheese type on each sampling day.
affected the growth potential for the two types of cheeses, thus contributing to the high level of variation in the log mean counts reported. The effect of inoculation level on the growth—no growth barrier was evident when cheeses negative for \textit{L. monocytogenes} were eliminated from the analysis and included counts from only the positive cheeses. After elimination of the no-growth data, there was no significant effect of initial inoculation level on the rate of growth, suggesting that once population growth commences, growth rates will be similar regardless of the initial inoculum. Storage of these cheeses at 4°C also could have restricted growth \citep{1, 39}. \textit{Little and Knochel} \citep{25} found that only the psychrotrophic pathogen \textit{Yersinia enterocolitica} was able to grow when introduced as a postprocessing contaminant on stabilized Brie, whereas populations of both \textit{Salmonella} and \textit{Bacillus cereus} declined at 4 and 8°C. Because of the difficulty and variability in the detection of \textit{L. monocytogenes} from mold-ripened soft cheeses in general \citep{34, 40}, especially in raw milk cheeses with very low inoculation levels \citep{20, 34}, samples that were negative by direct plating did not undergo standard enrichment procedures.

After 60 days of ripening, maximum population levels differed between cheeses of the two initial inoculation levels. \textit{Ryser and Marth} \citep{38} observed 2- to 3-log increases in three strains of \textit{L. monocytogenes} surface inoculated onto 10-day-old Camembert-type cheese at 6°C. Maximum observed populations of approximately 3 to 5 log CFU/g were similar to the 4.55 and 5.29 log CFU/g observed in the present study \citep{high level} despite differences in initial inoculation level between these two studies. These contamination levels also are consistent with \textit{L. monocytogenes} levels found in contaminated surface-mold-ripened soft cheeses at retail \citep{17} and \textit{Listeria innocua} levels under similar conditions of inoculation and ripening \citep{39}. \textit{Back et al.} \citep{1} found an approximately 2-log increase in \textit{L. monocytogenes} on the surface of laboratory manufactured Camembert-type cheese after 40 days at 3 and 6°C, although their results were not reported. Comparisons with data reported by \textit{Georgiades et al.} \citep{15} also are difficult because of differences in methodology, storage conditions, and the units reported (log CFU per sample). Based on our results, the use of raw milk rather than pasteurized milk does not seem to provide enhanced protection when \textit{L. monocytogenes} is introduced as a postprocessing contaminant in surface-mold-ripened soft cheese. Other investigators have reported that the growth of \textit{L. monocytogenes} during the manufacture and ripening of Camembert is unaffected by the use of a nisin-producing starter or raw milk when compared with the use of pasteurized milk \citep{33}.

Based on our data and results previously reported, surface-mold-ripened soft cheeses support the growth of \textit{L. monocytogenes} when introduced postprocessing at levels \( \geq 0.2 \) CFU/g, independent of the milk type used for cheese manufacture. The use of pasteurized milk in the manufacture of surface-mold-ripened soft cheese does not provide protection from \textit{L. monocytogenes} introduced as a postprocess contaminant. Despite low pH, lower moisture, and the presence of salt, \textit{L. monocytogenes} introduced as a post-processing contaminant survived and grew on soft ripened cheeses held for \( \geq 60 \) days. The 60-day aging rule encourages extended refrigerated holding of these cheeses, which could inadvertently contribute to risk through the proliferation of psychrotrophic pathogens. The safety of cheeses of this type must be achieved through control strategies other than a 60-day holding period, and revision of current federal regulations are warranted.

Under U.S. Department of Agriculture (USDA) Food Safety and Inspection Service rules, manufacturers of ready-to-eat products at high risk of \textit{L. monocytogenes} contamination must operate under hazard analysis critical control point (HACCP) systems, where products are considered adulterated if they contain \textit{L. monocytogenes} and if they have been in direct contact with a surface contaminated with \textit{L. monocytogenes}. Producers must address control of \textit{L. monocytogenes} in their HACCP plan, sanitation standard operating procedures, or other prerequisite programs whose effectiveness is verified through frequent environmental and end product testing \citep{41}. Similar systems also may be effective for preventing the contamination of high-risk cheeses.

Canada is currently seeking to develop a policy to replace the 60-day aging requirement for soft and semisoft cheeses after the occurrence of outbreaks of listeriosis in Canada in 2002 that were linked to cheeses made from pasteurized or heat-treated milk. Although not in line with domestic policy, the Canada Food Inspection Agency has allowed the importation of certified raw milk soft and semi-soft cheese from France. The accompanying certificate verifies that the cheese was manufactured in compliance with French national standards and verifies that the products meet the microbiological criteria of both Canada and France.

Epidemiologic evidence and the results of scientific investigation make it clear that neither pasteurization nor 60 days of aging are sufficient to ensure the safety of surface-mold-ripened soft cheese. Cheese safety can be better assured through a combination of stringent raw milk production and microbiological quality, improved process controls, the use of performance criteria, and aggressive environmental monitoring and finished product testing.

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