Viability of Escherichia coli O157:H7 in Salami Following Conditioning of Batter, Fermentation and Drying of Sticks, and Storage of Slices

NANCY G. FAITH,1 NELLY PARNIERE,1† TRINA LARSON,1 TIMOTHY D. LORANG,1 CHARLES W. KASPAR,1 AND JOHN B. LUCHANSKY1,2*

1Department of Food Microbiology and Toxicology, Food Research Institute, and 2Department of Food Science, University of Wisconsin, Madison, Wisconsin 53706, USA

ABSTRACT

The fate of Escherichia coli O157:H7 was monitored in salami during conditioning of batter, fermentation and drying of sticks, and storage of slices. The raw batter (75% pork:25% beef, wt/wt, fat content about 20%) was inoculated with a pediococcal starter culture (about 106 CFU/g) and a five-strain cocktail of E. coli O157:H7 (≥2 × 105 CFU/g) and stuffed into 104-mm diameter fibrous casings. After being refrigerated at 4°C or being tempered at 13°C, frozen at -20°C, and thawed at 4°C, or being frozen at -20°C, and thawed at 4°C, the inoculated batter was fermented at 24°C and 90% relative humidity (RH) to pH ≤4.8, dried at 13°C and 65% RH to a moisture/protein ratio of ≤1.9±1, and then stored at 4 or 21°C under air or vacuum. For salami sticks sampled immediately after drying, appreciable differences were evident among the various batter-conditioning treatments; pathogen numbers were reduced from original levels by 2.1, 1.6, or 1.1 log10 units when batter was tempered, frozen, and thawed, frozen and thawed, or refrigerated, respectively. Similarly, regardless of storage temperature or atmosphere, within 7 days salami slices cut from sticks prepared from batter that was tempered, frozen, and thawed (2.7- to 4.9-log10-unit reduction) or frozen and thawed (2.3- to 4.8-log10-unit reduction) displayed a greater impact on pathogen numbers than slices cut from sticks prepared from batter that was refrigerated (1.6- to 3.1-log10-unit reduction). The effects of batter conditioning notwithstanding, a greater reduction in levels of E. coli O157:H7 was observed when slices were stored at 21°C compared to otherwise similar slices stored at 4°C. After storage for 60 days the pathogen was only detected by enrichment in slices stored at 21°C, whereas pathogen levels ranged from 1.4 to 4.5 log10 CFU/g in slices stored at 4°C. Differences related to storage atmosphere were first observed after slices were stored for 21 days. Such differences were more readily demonstrable after 60 and 90 days, with pathogen numbers for treatments that were statistically different ranging from 0.6- to 1.5-log10 units higher on slices stored under vacuum than in air. These data emphasize the need to implement multiple barriers to appreciably reduce numbers of E. coli O157:H7 in salami.

Since early 1995, as one option, processors of dry and semidry fermented sausage have been required by the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) to validate that processing of these products results in at least a 5-log10-units reduction in numbers of Escherichia coli O157:H7 cells (30). This regulatory posture was largely the result of a heightened awareness of serotype O157:H7 strains of E. coli by regulatory agencies, consumers, and processors due to the 1994 Washington and California outbreak, which involved 23 cases, including 6 hospitalized individuals and 2 cases of hemolytic uremic syndrome (HUS) linked with consumption of contaminated salami slices (8). The somewhat surprising identification of dry-cured salami as a "new route of transmission" for E. coli O157:H7 (31) and the USDA FSIS regulatory mandate provided adequate justification for several research groups to conduct validation studies on the fate of E. coli O157:H7 in a variety of fermented meats.

To date, relatively little information on the fate of serotype O157:H7 strains of E. coli in fermented meats has been published in peer-reviewed journals. As one example of a study that has been published, Hinkens et al. (22) reported that in pepperoni, fermentation at 96°F to pH ≤5.0 and drying at 13°C and 65% RH was only sufficient to deliver about a 1-log10-unit reduction in pathogen numbers. To deliver the required 5-log10-unit reduction, it was necessary to heat pepperoni sticks after fermentation to internal temperatures of 63°C instantaneous or 53°C for 60 min. These data were in close agreement with results from other studies which reported about a 2-log10-unit reduction in pathogen numbers during manufacture of salami (11, 17, 27) and summer sausage (6). As another example, traditional preparation of beef jerky, including drying for 10 h at 60°C, decreased levels of E. coli O157:H7 by ≥5 log10 units (20). The present study evaluated the effect of three different temperature regimens for conditioning batter on the viability of E. coli O157:H7 in salami fermented at low temperature (24°C) to an intermediate target pH (pH ≤4.8) and then sliced and stored under different temperatures and atmospheres.

MATERIALS AND METHODS

Bacterial strains. The following five strains of E. coli O157:H7 were used in this study to inoculate salami: EC505B, a
beef isolate from the University of Wisconsin Food Research Institute; strain C7927, a human isolate from the Massachusetts apple cider outbreak of 1991 (5); strain F-90, a sausage isolate from the Washington and California dry-cured salami outbreak of 1994 (8); strain EC204P, a pork isolate from the University of Wisconsin Food Research Institute; and strain C9490, a human isolate from the Western States hamburger patty outbreak of 1993 (7). The \textit{E. coli} strains were maintained as outlined by the USDA FSIS (30). A commercial \textit{Pediococcus acidilactici} starter culture (strain HP; Diversitech, Inc., Gainesville, FL) was maintained and propagated according to the manufacturer’s instructions.

**Preparation of \textit{E. coli} O157:H7 inoculum.** Each of the five strains of \textit{E. coli} O157:H7 were separately grown in 750 ml of Trypticase soy broth (TSB; Difco Laboratories Inc., Detroit, MI) supplemented with 1% glucose at 37°C overnight with shaking at 105 rpm. The five cell suspensions were harvested by centrifugation (4°C, 20 min, 1,400 \times g) and combined at equal levels to achieve a final inoculum of about 1 \times 10^9 CFU/ml as described previously (22).

**Manufacture of salami.** A flow diagram for salami manufacture and storage is provided in Figure 1. The commercially supplied meat blocks (23 kg each) contained 75% pork and 25% beef with a target fat content of about 20%. The \textit{E. coli} O157:H7-inoculated batter was conditioned as follows prior to fermentation to evaluate the effect of tempering, freezing, and/or thawing on pathogen viability. For some experiments, the batter was prepared by adding the pediococcal starter culture (12 ml in 88 ml of sterile dH2O per 23 kg of batter to achieve a final concentration of about 10^8 CFU/g) to the raw meat and mixing for 1 min with a Buffalo mixer (model 2VSS; John E. Smith’s and Sons Co., Buffalo, NY). The following nonmeat ingredients were added: 1% dextrose (A. E. Staley, Decatur, IL), 3% spice mix (Diversitech), and 2% cure mixture (Diversitech). Mixing was continued for about 1 min between each addition. After removing a portion of the batter for use as a noninoculated control treatment, the five-strain cocktail of \textit{E. coli} O157:H7 (final concentration of \(\geq 2 \times 10^7\) CFU/g of batter) was added. Mixing was continued for 3 min before the batter was stored at 4°C for 6 to 8 h prior to fermentation (designated “refrigerated”). For other experiments, the meat block was inoculated with the five-strain serotype O157:H7 cocktail as described above, mixed for 2 min with the Buffalo mixer, and then maintained at room temperature for about 3 to 5 h to reach an internal batter temperature of 13°C. The batter was maintained at 13°C for an additional 2 h before being placed at -20°C for 3 days and then thawed at 4°C for 3 days prior to fermentation (designated “tempered, frozen, and thawed”). As a third approach, the meat block was inoculated with the \textit{E. coli} O157:H7 cocktail as described above, placed at -20°C for 3 days, and then thawed at 4°C for 3 days prior to fermentation (designated “frozen and thawed”). After tempering, freezing, and thawing or freezing and thawing, the batter was returned to the Buffalo mixer for the addition of the pediococcal starter culture and the nonmeat ingredients as described above for the “refrigerated” treatment. Whether refrigerated, tempered, frozen, and thawed, or frozen and thawed, the inoculated batter was eventually ground using a 1/8-in. (3.175-mm) plate and commercial grinder (model 84142; Hobart Manufacturing Co., Troy, OH) and then stuffed (about 2 kg per stick) into 104-mm diameter fibrous casings (TeePak, Inc., Westchester, IL) to a final length of about 30 cm. The sticks were transferred to a temperature- and humidity-controlled room (Biotron facility; University of Wisconsin, Madison, WI) and fermentation was conducted at 24°C to a target end point of pH \(\leq 4.8\), which was typically achieved in 60 to 64 h.

**Slicing and storage of salami.** After drying at 13°C and 65% RH to a moisture/protein (M/Pr) ratio of \(\geq 1.9:1\) (about 21 days), the salami sticks were sliced (about 15 g per slice) with a Globe slicing machine (Model 500; Stamford, CT). Approximately 50-g (3 to 4 slices) portions of salami were placed into oxygen-impermeable bags (Carlon grade 863, Curwood, Inc., New London, WI) for packaging under air or vacuum (VAC). The vacuum was attained with a Multivac vacuum-packaging machine (Sepp Haggenmüller KG, Allgäu, Germany). Packages containing the salami slices were stored at 4 or 21°C for 7, 14, 21, 28, 60, or 90 days. At each sampling interval, three packages of salami were removed from storage and analyzed as described below.

**Microbiological analyses of salami batter, sticks, and slices.** Salami was tested for viable \textit{E. coli} O157:H7 cells by direct plating prior to stuffing, after fermentation, after drying and slicing, and after storage at different temperatures and atmospheres. When numbers of the pathogen decreased below detection by direct plating (<10^1 CFU/g), the presence or absence of the pathogen was determined by enrichment as previously described (22). The noninoculated batter was tested for background levels of \textit{E. coli} O157:H7 and other non-sorbitol-fermenting bacteria by spread plating onto MacConkey sorbitol agar (MSA; Difco) and for total aerobic bacterial numbers by spread plating onto Trypticase soy agar (TSA; Difco) plates. Immediately after stuffing, the sticks were also tested for viable pediococci by spread plating onto MRS (Difco) agar plates.

At each sampling interval, from each of 3 samples a 25-g
portion of the batter, or a 25-g cross-section from the middle of a stick, or a 25-g composite of salami slices was aseptically transferred to a Stomacher bag (Seward Medical, London, UK) containing 225 ml of 0.85% saline and processed and plated as described previously (22). To achieve a detection level of 10^1 CFU/g, it was necessary to plate 330 µl directly from the stomacher bag onto each of 3 plates. Plates were incubated at 42°C for 18 to 24 h to recover *E. coli* O157:H7 and at 37°C for 48 h to estimate total aerobic bacterial numbers or residual pediococci before colonies were counted. Representative isolates were confirmed to be pediococci or *E. coli* O157:H7 as described (22).

**Chemical analyses of salami sticks.** At each sampling point, 3 control sticks (without inoculated *E. coli* O157:H7) were removed for chemical analyses. The samples were either transported on ice directly to a commercial testing laboratory, or were held at −20°C for up to 7 days and then delivered for testing. Chemical analyses were performed on a 500-g composite sample from the 3 sticks following Association of Official Analytical Chemists (AOAC) procedures. Each composite was tested for fat (AOAC procedure 960.39), moisture (AOAC procedure 950.46), protein (AOAC procedure 928.06), and salt (AOAC procedure 935.47) as reported for meat products by McNeal (25). The water activity (a_w) was determined with an AquaLab water activity meter (model CX-2; Decagon Instruments, Pullman, WA). The pH and titratable acidity (TA) were determined as previously described (24) using a 25-g sample of salami macerated in 100 ml of hot (about 60°C) dH2O. The TA was expressed as the percent lactic acid.

**Statistical analyses.** Data were analyzed by general linear modeling using release 6.03 of the Statistical Analysis System (SAS Institute, Cary, NC). Three trials were conducted using three different batches of raw meat and a freshly grown inoculum of the *E. coli* O157:H7 cocktail; triplicate samples (i.e., analyzing 25 g from each of 3 batters, sticks, or packaged slices) of salami at each sampling interval and duplicate plates of each dilution were examined.

**RESULTS**

**Microbiological testing of raw meat.** Analyses of the batter before inoculation with the serotype O157:H7 cocktail and pediococcal starter culture revealed that none of 3 meat blocks tested contained indigenous *E. coli* O157:H7 by direct plating (data not shown). These same 3 meat blocks produced a total aerobic plate count ranging from 1.5 × 10^4 to 2.3 × 10^5 CFU/g of meat (average, 1.0 × 10^5 CFU/g), which indicated that the starting raw materials were of good microbiological quality.

**Microbiological testing of salami batter, sticks, and slices during manufacture and storage.** The number of viable *E. coli* O157:H7 cells were determined following conditioning of the batter, fermentation and drying of the sticks, and storage of slices. Regardless of how the batter was conditioned, fermentation alone resulted in a 0.9- to 1.5-log_{10}-unit reduction in pathogen numbers, with an additional 0.2- to 0.6-log_{10}-unit reduction contributed by drying (Table 1). Differences in pathogen numbers related to batter conditioning were apparent after drying; compared with original levels, pathogen numbers were reduced by 2.1, 1.6, or 1.1 log_{10} units in batter that was previously tempered, frozen, and thawed, frozen and thawed, or refrigerated, respectively. This same trend was observed throughout the 90 days of storage of salami slices. For example, temperature and atmosphere conditions notwithstanding, greater reductions were observed following storage of slices for 7 days when the batter was tempered, frozen, and thawed (2.7-

**TABLE 1. Escherichia coli O157:H7 population during manufacture of salami**

<table>
<thead>
<tr>
<th>Type of batter</th>
<th>Before fermentation</th>
<th>After fermentation</th>
<th>After drying</th>
<th>Temperature</th>
<th>Atmosphere</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>60</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refrigerated</td>
<td>7.7 ± 0.2</td>
<td>6.8 ± 0.16</td>
<td>6.6 ± 0.2</td>
<td>21°C</td>
<td>Air</td>
<td>4.6 ± 0.5 c</td>
<td>4.0 ± 0.2 d</td>
<td>3.2 ± 1.1 d</td>
<td>2.2 ± 1.6 c</td>
<td>&lt;1.0 log_{10} CFU/g</td>
<td>&lt;1 per 25 g(\times)E</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>VAC</td>
<td>5.0 ± 0.4 c</td>
<td>4.2 ± 0.4 d</td>
<td>3.1 ± 1.2 d</td>
<td>2.1 ± 1.3 c</td>
<td>&lt;1.0 log_{10} CFU/g</td>
<td>&lt;1 per 25 g(\times)E</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4°C</td>
<td>Air</td>
<td>6.0 ± 0.4 c</td>
<td>5.8 ± 0.5 AB</td>
<td>5.7 ± 0.6 AB</td>
<td>5.8 ± 0.5 A</td>
<td>4.5 ± 1.0 A</td>
<td>3.0 ± 1.0 B</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>VAC</td>
<td>6.1 ± 0.5 A</td>
<td>6.1 ± 0.3 A</td>
<td>6.1 ± 0.3 A</td>
<td>5.4 ± 0.2 A</td>
<td>4.4 ± 1.1 A</td>
<td>3.6 ± 0.4 A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4°C</td>
<td>Air</td>
<td>4.7 ± 0.9 c</td>
<td>4.2 ± 0.9 d</td>
<td>4.4 ± 1.2 c</td>
<td>4.3 ± 1.3 B</td>
<td>2.2 ± 1.6 B</td>
<td>1.6 ± 0.9 D</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>VAC</td>
<td>4.8 ± 0.9 c</td>
<td>4.5 ± 1.0 CD</td>
<td>4.7 ± 1.0 c</td>
<td>4.3 ± 1.2 B</td>
<td>2.3 ± 1.0 B</td>
<td>2.3 ± 1.5 C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4°C</td>
<td>Air</td>
<td>5.0 ± 0.7 BC</td>
<td>4.7 ± 0.6 CD</td>
<td>5.0 ± 0.4 BC</td>
<td>4.3 ± 0.8 B</td>
<td>1.4 ± 1.0 log_{10} CFU/g</td>
<td>&lt;1 per 25 g(\times)E</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>VAC</td>
<td>5.4 ± 0.9 BC</td>
<td>5.1 ± 0.4 BE</td>
<td>4.9 ± 0.2 BC</td>
<td>4.4 ± 0.3 B</td>
<td>2.9 ± 0.3 B</td>
<td>1.4 ± 0.6 log_{10} CFU/g</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Raw batter was inoculated with *E. coli* O157:H7 and (i) held at 4°C for about 6 to 8 h (refrigerated), or (ii) held at 13°C for 2 h and then frozen at −20°C for 3 days and thawed at 4°C for 3 days (tempered/frozen/thawed), or (iii) frozen at −20°C for 3 days and then thawed at 4°C for 3 days (frozen/thawed).

\(^b\)Values in a column that are not followed by the same letter are significantly different (\(P < 0.05\)).

\(^c\)VAC, vacuum.

\(^d\)1 of 3 trials negative by direct plating but positive with enrichment.

\(^e\)2 of 3 trials negative by direct plating but positive with enrichment.

\(^f\)3 of 3 trials negative by direct plating but positive with enrichment.

\(^g\)3 of 3 trials negative by direct plating; 1 of 3 trials positive with enrichment.

\(^h\)3 of 3 trials negative by direct plating and enrichment.
TABLE 2. Chemical composition of salami during manufacture and storage

<table>
<thead>
<tr>
<th>Manufacturing step</th>
<th>pH</th>
<th>TA (%)</th>
<th>M/Pr</th>
<th>aₙ</th>
<th>Salt (%)</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batter</td>
<td>5.81 ± 0.12</td>
<td>0.42 ± 0.08</td>
<td>3.82 ± 0.03</td>
<td>0.93 ± 0.00</td>
<td>3.04 ± 0.16</td>
<td>60.00 ± 0.72</td>
<td>15.71 ± 0.10</td>
<td>19.71 ± 0.89</td>
</tr>
<tr>
<td>After fermentation</td>
<td>4.80 ± 0.02</td>
<td>0.75 ± 0.07</td>
<td>3.62 ± 0.02</td>
<td>0.93 ± 0.00</td>
<td>3.23 ± 0.19</td>
<td>58.57 ± 0.41</td>
<td>16.18 ± 0.18</td>
<td>20.92 ± 0.58</td>
</tr>
<tr>
<td>After drying/slicing</td>
<td>4.80 ± 0.03</td>
<td>1.37 ± 0.49</td>
<td>1.99 ± 0.07</td>
<td>0.92 ± 0.01</td>
<td>4.41 ± 0.21</td>
<td>44.51 ± 1.95</td>
<td>22.37 ± 0.21</td>
<td>26.75 ± 1.34</td>
</tr>
<tr>
<td>After storage</td>
<td>4.82 ± 0.15</td>
<td>1.50 ± 0.17</td>
<td>1.78 ± 0.13</td>
<td>0.90 ± 0.01</td>
<td>4.65 ± 0.09</td>
<td>40.84 ± 1.65</td>
<td>22.91 ± 0.78</td>
<td>29.04 ± 1.12</td>
</tr>
<tr>
<td>Refrigerated</td>
<td>4.82 ± 0.02</td>
<td>1.73 ± 0.04</td>
<td>1.66 ± 0.24</td>
<td>0.90 ± 0.01</td>
<td>4.60 ± 0.44</td>
<td>39.02 ± 3.25</td>
<td>23.54 ± 1.43</td>
<td>30.50 ± 1.73</td>
</tr>
<tr>
<td>Tempered/frozen/thawed</td>
<td>4.79 ± 0.04</td>
<td>1.53 ± 0.08</td>
<td>1.81 ± 0.07</td>
<td>0.90 ± 0.00</td>
<td>4.79 ± 0.04</td>
<td>40.60 ± 0.18</td>
<td>22.50 ± 0.76</td>
<td>29.91 ± 0.33</td>
</tr>
<tr>
<td>Frozen/thawed</td>
<td>4.80 ± 0.02</td>
<td>0.75 ± 0.07</td>
<td>3.62 ± 0.02</td>
<td>0.93 ± 0.00</td>
<td>3.23 ± 0.19</td>
<td>58.57 ± 0.41</td>
<td>16.18 ± 0.18</td>
<td>20.92 ± 0.58</td>
</tr>
</tbody>
</table>

a TA, titratable acidity as % lactic acid.

b M/Pr, moisture to protein ratio.

c Values are the averages of 3 trials for sticks prepared from refrigerated batter.

d Values are the averages of 2 trials for storage at 21°C in air.

to 4.9-log₁₀ reduction) or frozen and thawed (2.3- to 4.9-log₁₀ reduction) than when refrigerated (1.6- to 3.1-log₁₀ reduction). The data also revealed that, in general, greater reductions in pathogen numbers were observed when slices were stored at 21°C than at 4°C and when slices were stored under air than when under VAC. For example, for all three regimens used to condition batter, the number of cells of the pathogen were reduced to below detection and were recoverable only by enrichment after 60 days when stored at 21°C. In contrast, with the exception of slices prepared from batter previously frozen and thawed and stored at 4°C under air, the pathogen was recovered by direct plating from slices stored at 4°C under air or VAC after 60 days. Differences in pathogen levels attributed to atmosphere were first demonstrable after 21 days of storage and were subsequently observed after 60 and 90 days of storage, wherein greater reductions were observed when slices were stored under air compared to under VAC for some treatments. Lastly, in addition to microbiological analyses, empirical inspection of salami sticks and slices did not reveal any apparent defects in color, texture, or odor (data not shown) or in chemical composition (Table 2) directly attributable to batter conditioning. Likewise, the proximate composition of the salami displayed the expected levels for pH and aₙ, as well as salt, protein, moisture, and fat concentrations (Table 2).

**DISCUSSION**

Since bovines are a primary reservoir of *E. coli* O157:H7 and since foods derived from cattle have been incriminated in a majority of illness episodes (18), much of the research performed and published on *E. coli* O157:H7 has focused on characterizing and controlling this pathogen in meats. In this regard, the highly publicized outbreaks in the 1990s related to contaminated hamburger patties (7), pork and beef salami (8), meat pies (2), and Mettwurst (9), as well as the USDA FSIS mandate for a 5-log₁₀ reduction in fermented sausage, provided the impetus and justification for conducting the present study to monitor the fate of this pathogen during salami manufacture and storage.

There are several intrinsic (e.g., salts, fat, moisture) and extrinsic (e.g., refrigerated or frozen storage, modified-atmosphere packaging, thermal processing) factors that can impact the growth and survival of undesirable bacteria in foods, including serotype O157:H7 strains of *E. coli*. Previous findings (22, 27) indicated that intrinsic factors alone were probably not sufficient to appreciably reduce high levels of *E. coli* O157:H7 during fermentation. Several investigators have published studies on the effect of extrinsic factors, such as refrigeration, on the growth of *E. coli* O157:H7 in foods. For example, some strains of this pathogen grew in synthetic media at 8°C (28, 29), but growth in cheese (23) and on salad vegetables (1) occurred at 10 to 12°C. Other studies revealed that the pathogen remains viable for several weeks during storage at about 4°C on vegetables (1) and in fresh sausage (15), as well as in relatively high-acid foods such as mayonnaise (pH 3.65 to 3.97) (21, 32) and fermented meats (pH 4.63 to 5.0) (6, 11, 16, 17). Several investigators reported that this pathogen is acid tolerant (3, 4) and that its survival at low pH is further enhanced at colder temperatures (3, 12, 26). Freezing of foods is also an extrinsic factor detrimental to several pathogenic and spoilage microorganisms (14). Although *E. coli* O157:H7 survived in frozen ground beef patties for 9 months (13), little information is available on the effects of freezing in combination with other extrinsic or intrinsic factors on the viability of this pathogen in foods. More recent studies at the Food Research Institute (10) demonstrated that tempering inoculated ground beef at 15°C for 4 hours followed by freezing and thawing compared to freezing and thawing without tempering sensitized *E. coli* O157:H7 to subsequent challenge with acid. Also germane to the present study, relatively little has been published on the effect of modified-atmosphere packaging (MAP) on growth of serotype O157:H7 strains of *E. coli*. In synthetic media, none of 6 CO₂, O₂, N₂ gas combinations affected growth of one strain of *E. coli* O157:H7 at 5, 10, or 20°C or its tolerance to 3% salt (19). Likewise, packaged salad vegetables stored under an atmosphere of 3% O₂ and 97% N₂ did not appreciably affect the pathogen (1). Another study reported that vacuum-packaged fermented sausage stored at 4°C supported viability of this pathogen for 8 weeks (17). From the above discussion, it is readily apparent that further studies are warranted to validate the effects of refrigeration, freezing and thawing, and MAP, alone and in combination, on the behavior of *E. coli* O157:H7 in foods.

The present study affirmed other reports (6, 11, 16, 17,
that fermentation and drying were only sufficient to effect a 1- to 2-log_{10} reduction of E. coli O157:H7 in fermented meats. Although pathogen numbers may be further reduced somewhat by modifications to product formulations (e.g., varying the types and levels of spices, smoke, and acids), more aggressive methods (e.g., postfermentation heating, extended fermentation and/or drying times at lower pH and/or higher temperature) are necessary to appreciably reduce pathogen numbers. The results of the present study with salami compliment our companion study with pepperoni (16) and demonstrate that storage of salami slices at ambient temperature under air was more effective at reducing levels of E. coli O157:H7 than refrigeration or storage under VAC. In contrast to our study with pepperoni slices, storage of salami slices under air for 28 days at ambient temperature did not cause discoloration or contribute to the appearance of yeasts or molds. The present study also demonstrated that tempering, freezing, and thawing or freezing and thawing butcher had an appreciable effect on the pathogen without affecting the chemical composition of salami slices. Additional studies are underway to optimize the temperatures and times used to condition butcher, to evaluate other atmospheres, temperatures, and/or times for storage of sliced sausages, and to evaluate select food-grade antimicrobial chemicals/proteins to achieve the desired 5-log_{10} unit reduction. Future studies could also be directed at developing media that are better suited than MSA for recovering injured or stressed cells of E. coli O157:H7. The results of the present study should find immediate applications for enhancing the safety of salami targeted for use on sandwiches or salads, as well as add to the growing body of knowledge on the growth and survival of E. coli O157:H7 in fermented meats.

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