Validation of Pepperoni Processes for Control of Escherichia coli O157:H7‡

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ABSTRACT

The outbreak of Escherichia coli O157:H7 linked with dry-cured salami in late 1994 prompted regulatory action that required manufacturers of fermented products to demonstrate a 5-log unit reduction in counts of this pathogen during processing. Therefore, pepperoni batter (75% pork:25% beef with a fat content of ca. 32%) was inoculated with a pediococcal starter culture and a five-strain mixture of E. coli O157:H7 (≥2 × 10⁷ CFU/g) and stuffed into 55-mm diameter fibrous casings 47 cm in length. The viability of the pathogen was monitored before stuffing, after fermentation, after thermal processing, and/or after drying. Chubs were fermented at 96°F (36°C) and 85% relative humidity (RH) to pH = 5.0 and then dried at 55°F (13°C) and 65% RH to a moisture/protein ratio of ≤1.6:1 (modified method 6 process). Counts of the pathogen decreased about 1.2 log units after fermentation and drying. In subsequent experiments, heating chubs after fermentation to internal temperatures of 145°F (63°C) instantaneous or 128°F (53°C) for 60 min resulted in a ≥5-log unit decrease in numbers of strain O157:H7 without visibly affecting the texture or appearance of the product. These data revealed that a traditional, nonthermal, process for pepperoni was only sufficient to eliminate relatively low levels (ca. 2 log CFU/g) of E. coli O157:H7, whereas heating to internal temperatures of 145°F (63°C) instantaneous or 128°F (53°C) for 60 min delivered a 5 to 6 log unit reduction in counts of the pathogen in pepperoni.

Key words: Escherichia coli O157:H7, pepperoni, pathogen, fermentation

The Western United States outbreak of Escherichia coli O157:H7 in 1993 due to consumption of contaminated hamburger patties at a fast-food restaurant chain (7) brought considerable focus on this pathogen. Despite the heightened awareness, approximately 1 year after the hamburger outbreak another outbreak of E. coli O157:H7 was linked to meat, this time, a dry fermented pork and beef salami (8). The salami outbreak involved 20 individuals in Washington (age range, 23 months to 77 years; median age, 6 years; 3 hospitalized; one 6-year-old with hemolytic uremic syndrome [HUS]) and 3 individuals in California (age range, 4 to 75 years; 3 hospitalized; one 4-year-old with HUS) (8). E. coli O157:H7 was found on presliced product from delicatessen counters (8). In early 1995 there was another outbreak of HUS linked to semidy (uncooked) fermented sausage (9). This outbreak involved 23 patients (age range, 4 months to 12 years; median age, 4 years; 23 with HUS) in South Australia and was attributed to E. coli O111:NM, another serotype of E. coli that produces a Shiga-like toxin(s). As a result of the California and Washington salami outbreaks the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) developed guidelines (24) for sausage manufacturers to validate processes to ensure a 5-log unit reduction in counts of E. coli O157:H7.

Among the many different categories of fermented sausage, pepperoni is one of the most popular varieties, with annual consumption in the United States exceeding 370 million pounds (Gerry Durnell, personal communication). Pepperoni types and sizes vary greatly, but three basic categories predominate. First, there is the traditional, small-diameter (28 to 36 mm) product found in the deli case or on pegboards in supermarkets. The second type, representing the largest volume consumed, is pepperoni stuffed into 49- to 54-mm diameter casings and then sliced (1 to 2 mm thick) and sold to pizza restaurants. A small portion of this type of product is also sold as sticks and subsequently used as a topping for frozen pizza. The third category of pepperoni is the large-diameter (60 to 80 mm) product used on sandwiches. Differences among manufactures and categories of pepperoni notwithstanding, the use of various combinations of salts, pH, heating, cooling and/or drying ensure that pepperoni is wholesome and safe relative to the parasite Trichinella spiralis and to bacterial pathogens such as Staphylococcus aureus. In contrast, to date, there has been no information available to certify that pepperoni is safe.
relative to *E. coli* O157:H7. To this end, the objective of the present study was to validate the effect of different processing conditions on the fate of *E. coli* O157:H7 in pepperoni.

**MATERIALS AND METHODS**

**Bacterial strains**

Five strains of *E. coli* O157:H7 were used in this study to inoculate pepperoni: (i) strain SLH21788, human isolate from the Wisconsin daycare outbreak of 1994 (22); (ii) strain C7927, human isolate from the Massachusetts apple cider outbreak of 1991 (6); (iii) strain F-90, sausage isolate from the California dry-cured salami outbreak of 1994 (8); (iv) strain EC204P, pork isolate from the Food Research Institute Culture Collection; and (v) strain C9490, human isolate from the Western States hamburger outbreak of 1993 (7). The *E. coli* strains were maintained at −70°C in 2× Trypticase soy broth (TSB) (Difco Laboratories Inc., Detroit, MI) plus 10% glycerol. Prior to inoculation into pepperoni batter, the cultures were transferred twice in TSB supplemented with 1% glucose at 37°C overnight before use as outlined by the USDA FSIS (24). A commercial *Pediococcus acidilactici* starter culture (Lactacel 115; Quest International, Sarasota, FL) was maintained and propagated according to the manufacturers instructions.

**Preparation of *E. coli* O157:H7 inoculum**

Each of the five strains of *E. coli* O157:H7 were separately grown overnight in 250 ml of TSB plus 1% glucose at 37°C with shaking (100 rpm). The cells were harvested by centrifugation at 1,880 × g for 20 min at 4°C, and washed and resuspended in a small volume of 0.1% peptone (Difco). The five cell suspensions were combined and the final volume was adjusted to 50 ml with 0.1% peptone such that each strain contributed about 20% to the final inoculum of 1 × 10⁸ CFU/ml. The five-strain cocktail was held on ice for ≤30 min before addition to sausage batter as described below. The viable count of the cocktail was confirmed by spread plates on MacConkey sorbitol agar (MSA) (Difco) just prior to inoculation of the batter.

** Manufacture of pepperoni**

A flow diagram for sausage manufacture is provided in Figure 1. The meat block supplied by Doskocil Companies (Jefferson, WI) was maintained at 0°C during batter preparation. The batter (ca. 25 lb [1 lb is ca. 0.45 kg] per trial; 75% [wt/wt] pork and 25% [wt/wt] beef) with a target fat content of ca. 32% (wt/wt) contained 0.63% dextrose (0.16 lb/25 lb of batter; A. E. Staley, Decatur, IL), 3% spice mix (0.75 lb/25 lb of batter; Doskocil house blend), and 2.0% cure mixture (0.5 lb/25 lb of batter; Doskocil house blend). The cure and spice mixtures were formulated to contain garlic, anise, red pepper, clove, and oleoresin of paprika, BHA (28 ppm; Eastman Chemical, Kingsport, TN), BHT (butylated hydroxytoluene) (28 ppm; Eastman), and citric acid (28 ppm; Fuyang Pharmaceutical, China), and to provide a final batter concentration of 156 ppm NaNO₂ and ca. 3.33% NaCl. The batter was inoculated with the five-strain cocktail of *E. coli* O157:H7 (final concentration of ≥2 × 10⁸ CFU/g of batter) and mixed using a Buffalo mixer (model 2VSS; John E. Smith’s and Sons Co., Buffalo, NY) for 2 min. Six milliliters of thawed pediococcal starter culture in 42 ml of sterile dH₂O were added per 25 lb of batter to deliver ca. 10⁶ CFU/g of batter. The commercial spice and cure were added, and mixing was continued for an additional 2 min. A noninhibitory, food grade, green dye (FD&C yellow 5 and FD&C blue 1, McCormick & Co., Inc., Hunt Valley, MD) was added to the batter as an aid to monitor for uniform mixing. A control batter was prepared prior to the experimental batch that was identical to the latter except for the presence of the serotype O157:H7 cocktail. Chemical analyses were performed (see below) on representative chubs prepared from the control batter. The inoculated batter was removed from the mixer and ground through a 0.125 in. (ca. 0.32 cm) die plate using a Hobart grinder (model 84142; Hobart Manufacturing Co., Troy, OH). The batter was then stuffed (ca. 694 g per chub) using a hand stuffer (Koch Supplies, Inc., Kansas City, MO) into 55-mm diameter fibrous casings (TeePak, Inc., Westchester, IL) to a final length of 47 cm and the resulting chubs were sealed using a casing clipper (Poly-clip model SCH 7210; Niedecker, West Germany) loaded with series 7000-VSCC staples (U.S. Clip Corporation, Mundelein, IL). Casings were soaked in tap H₂O at room temperature for 10 min prior to use.

The chubs were transferred to a smokehouse (model 1000; Vortron, Inc., Beloit, WI) and fermentation was conducted at 96°F (36°C) with a relative humidity (RH) of 85 to 90% until reaching a pH ≤ 5.0. After fermentation (ca. 14 to 18 h), the chubs were cold-showered to an internal temperature of ≤80°F (27°C) in the smokehouse and then dried at 55°F (13°C) and 65% RH [wet bulb 50°F (10°C), dry bulb 55°F (13°C)] to a moisture/protein (M/Pr) ratio of 1.6:1 (ca. 15 to 21 days) in an environmentally controlled chamber (Biotron facility; University of Wisconsin, Madison, WI). In addition to the modified method 6 (27) process just described, for some experiments chubs were heated to internal temperatures of 145°F (63°C) instantaneous or 128°F (53°C) for 60 minutes and cold-showered before drying. Three trials were conducted for each of the three processes evaluated: (i) modified method 6, (ii) modified method 6 with a postfermentation heating of chubs to an internal temperature of 145°F (63°C), and (iii) modified method 6 with a postfermentation heating of chubs to an internal temperature of 128°F (53°C) for 60 min.

**Microbiological analyses of pepperoni chubs**

Pepperoni (two chubs per sampling point) was tested for viable *E. coli* O157:H7 by direct plating prior to stuffing, after fermentation, after cooking (when applicable), and after 4, 8, 12, 18, and 21 days of drying. When numbers of the pathogen decreased below detection by direct plating (<10⁵ CFU/g), the presence or absence of the pathogen was determined by enrichment. The noninoculated batter was also tested for background levels of *E. coli* O157:H7 and other non-sorbitol-fermenting bacteria by spreading plates onto MSA and for total aerobic bacterial numbers by spreading plates onto Trypticase soy agar (TSA) (Difco) plates. Immediately after stuffing, the chubs were also tested for

![FIGURE 1. Pepperoni manufacture. Flow diagram depicting the pepperoni processes evaluated. M/Pr, moisture to protein ratio.](image-url)
viable pediococci by spread plating onto MRS (Difco) agar plates. For sampling chubs, the outside of the chub was cleaned with 70% ethanol and the casing was removed with the aid of a sterile scalpel. A 25-g portion of the batter or a 25-g cross-section from the middle of a chub was aseptically transferred to a Stomacher bag (Seward Medical, London, UK) containing 225 ml of 0.1% peptone and the contents stomached (model 400; Tekmar Co., Cincinnati, OH) for 2 min at room temperature. One milliliter of the resulting mixture was serially diluted in 0.1% peptone and spread plated onto duplicate MSA or MRS agar plates. Another portion (10 ml) was transferred to 90 ml of modified EC broth (21) containing novobiocin (final concentration of 20 μg/ml; Sigma Chemical Co., St. Louis, MO) and incubated with shaking (100 rpm) for 18 to 24 h at 37°C to test for the presence or absence of low levels of viable O157:H7 cells. Following enrichment, the samples were diluted in 0.1% peptone and spread plated in duplicate onto MSA. Plates were incubated at 42°C for 48 h to recover E. coli O157:H7 and at 37°C for 24 h to recover pediococci before colonies were counted.

The serotype of six sorbitol nonfermenting (i.e., white) colonies from MSA plates was determined using an O157 latex agglutination test (Unipath Limited, Basingstoke, England). Sorbitol-negative, O157-positive isolates were further confirmed as E. coli using API 20E biochemical test strips (bioMerieux Vitek, Inc., Hazelwood, MO) and for the H7 antigen by agglutination (Difco).

Chemical analyses of pepperoni chubs

At each sampling point, two control chubs (without E. coli O157:H7) were removed for sampling. The control chubs were either transported on ice directly to the Doskocil Companies (Jefferson, WI) for testing or held at -20°C for up to seven days and then delivered to Doskocil. Chemical analyses were performed following Association of Official Analytical Chemists (AOAC) procedures (19). Each chub was tested for fat (AOAC procedure 960.39), moisture (AOAC procedure 950.46), protein (AOAC procedure 928.06), and salt (AOAC procedure 935.47). The water activity (aw) was determined using a Rotronic water activity meter (model DT; Huntington, NY). The pH and titratable acidity (TA) were determined as previously described (18) using a 25-g sample of sausage macerated in 100 ml of hot (ca. 60°C) dH2O. The TA was expressed as the percent lactic acid.

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>E. coli O157:H7 (log CFU/g ± SD)</th>
<th>pH</th>
<th>TA (%)a</th>
<th>M/Prb</th>
<th>aw</th>
<th>Salt (%)</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0 Emulsion</td>
<td>6.89 ± .86</td>
<td>6.82 ± .24</td>
<td>4.85 ± .17</td>
<td>0.62 ± .08</td>
<td>3.46 ± .07</td>
<td>.96 ± .00</td>
<td>3.17 ± .09</td>
<td>48.83 ± 0.46</td>
<td>14.07 ± .29</td>
</tr>
<tr>
<td>Day 0 Post fermentationc</td>
<td>6.59 ± .29</td>
<td>4.72 ± .09</td>
<td>.77 ± .03</td>
<td>.25 ± .11</td>
<td>.95 ± .00</td>
<td>3.63 ± .09</td>
<td>41.77 ± 0.60</td>
<td>16.13 ± .61</td>
<td>38.10 ± 2.20</td>
</tr>
<tr>
<td>Drying day 4</td>
<td>6.50 ± .47</td>
<td>4.64 ± .08</td>
<td>.86 ± .04</td>
<td>2.12 ± .12</td>
<td>.93 ± .01</td>
<td>3.83 ± .17</td>
<td>37.17 ± 1.16</td>
<td>17.57 ± .49</td>
<td>39.73 ± 0.92</td>
</tr>
<tr>
<td>Drying day 8</td>
<td>6.14 ± .37</td>
<td>4.73 ± .05</td>
<td>.88 ± .04</td>
<td>1.78 ± .17</td>
<td>.91 ± .00</td>
<td>3.77 ± 1.12</td>
<td>32.73 ± 2.17</td>
<td>18.43 ± .97</td>
<td>42.93 ± 1.00</td>
</tr>
<tr>
<td>Drying day 12</td>
<td>5.97 ± .38</td>
<td>4.72 ± .09</td>
<td>.88 ± .04</td>
<td>1.53 ± 1.17</td>
<td>.89 ± .00</td>
<td>3.90 ± .00</td>
<td>29.43 ± 1.89</td>
<td>19.33 ± .82</td>
<td>45.00 ± 1.08</td>
</tr>
<tr>
<td>Drying day 18</td>
<td>5.69 ± .23</td>
<td>4.74 ± .09</td>
<td>.93 ± .09</td>
<td>1.35 ± .14</td>
<td>.87 ± .02</td>
<td>4.40 ± .29</td>
<td>27.47 ± 1.97</td>
<td>20.33 ± .73</td>
<td>46.60 ± 1.44</td>
</tr>
</tbody>
</table>

a TA, titratable acidity as % lactic acid.
b M/Pr, moisture to protein ratio.
c Smokehouse settings were dry bulb 85°F and wet bulb 80°F during conditioning for 1 h and dry bulb 96°F and wet bulb 92°F during fermentation to ≤pH 5.0.

**Statistical analyses**

Data were analyzed using the Statistical Analysis System (SAS Institute, Cary, NC).

## Results

**Microbiological testing of raw meat and batter**

Analyses of meat blocks before inoculation with the five-strain cocktail and pediococcal starter culture revealed that none of 7 meat blocks tested contained E. coli O157:H7 by direct plating (data not shown). In addition to counts of serotype O157:H7, a portion of these same 7 meat blocks was also tested to determine the total aerobic plate count. Levels ranged from <10^2 to 4 × 10^4 CFU/g of meat (average, 1.3 × 10^4 CFU/g), indicating that the raw materials were of excellent microbiological quality.

Prior to addition of the pediococcal starter culture, the antimicrobial capabilities of the spice and cure mixtures on the five-strain mixture of E. coli O157:H7 was also ascertained. The data revealed that the spice and cure mixtures used in this study had no significant effect on the numbers of O157:H7 (α < 0.05; P = 0.9945). The average count of E. coli O157:H7 from emulsions prepared from 6 of the 7 meat blocks tested prior to the addition of spice and cure was 2.5 × 10^7 CFU/g of batter (range, 1.1 × 10^6 to 1.4 × 10^8 CFU/g). The average count from these 6 emulsions tested after the addition of the spice and cure mixtures and grinding was 2.2 × 10^7 cfu per gram of batter (range, 1.5 × 10^5 to 1.3 × 10^8 cfu/gram).

**Fermentation at 96°F (36°C) to pH ≤ 5.0 and drying at 55°F (33°C) and 65% RH to a moisture/protein (M/Pr) ratio of ≤1.6:1**

The first manufacture process evaluated was the modified method 6 process for pepperoni (Table 1), wherein the pathogen cell numbers remained essentially the same during the 14- to 18-h fermentation. Although a 1.2 log CFU/g...
TABLE 2. E. coli O157:H7 count and chemical composition of pepperoni during fermentation at 96°F to pH <5.0, heating to an internal temperature of 145°F, and drying at 53°F and 63% RH to a moisture to protein ratio of ≤1.6:1.

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>log CFU/g ± SD</th>
<th>Presence</th>
<th>pH</th>
<th>TA (%)a</th>
<th>M/Prb</th>
<th>aw</th>
<th>Salt (%)</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td></td>
<td></td>
<td>4.90 ± 1.0</td>
<td>.68 ± .07</td>
<td>2.67 ± .05</td>
<td>.96 ± 1.0</td>
<td>3.53 ± .06</td>
<td>43.50 ± 1.66</td>
<td>16.20 ± 1.0</td>
<td>35.07 ± 1.06</td>
</tr>
<tr>
<td>Emulsion</td>
<td>6.81 ± .82</td>
<td>1/3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After fermentationd</td>
<td>6.75 ± .29</td>
<td></td>
<td>4.90 ± .20</td>
<td>.79 ± .09</td>
<td>2.06 ± .27</td>
<td>.93 ± .01</td>
<td>3.90 ± .36</td>
<td>37.77 ± 1.97</td>
<td>18.50 ± 2.01</td>
<td>38.60 ± 1.73</td>
</tr>
<tr>
<td>After heat processinge</td>
<td>&lt;2.00 ± 0.0</td>
<td></td>
<td>4.91 ± .19</td>
<td>.64 ± .09</td>
<td>2.67 ± .05</td>
<td>.96 ± 1.0</td>
<td>3.53 ± .06</td>
<td>43.50 ± 1.66</td>
<td>16.20 ± 1.0</td>
<td>35.07 ± 1.06</td>
</tr>
<tr>
<td>Drying day</td>
<td></td>
<td></td>
<td>4.90 ± .11</td>
<td>.68 ± .07</td>
<td>3.31 ± .10</td>
<td>.97 ± .00</td>
<td>3.47 ± .06</td>
<td>50.50 ± 1.31</td>
<td>15.27 ± 0.59</td>
<td>30.13 ± 2.29</td>
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<tr>
<td>4</td>
<td>&lt;2.00 ± 0.0</td>
<td>2/3</td>
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<tr>
<td>8</td>
<td>&lt;2.00 ± 0.0</td>
<td>1/2</td>
<td>4.95 ± .15</td>
<td>.81 ± .06</td>
<td>1.97 ± .12</td>
<td>.92 ± .00</td>
<td>3.87 ± .23</td>
<td>34.93 ± 1.57</td>
<td>17.77 ± 1.27</td>
<td>41.10 ± 0.89</td>
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<td>12</td>
<td>&lt;2.00 ± 0.0</td>
<td>0/2</td>
<td>4.90 ± .06</td>
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<td>1.53 ± .10</td>
<td>.91 ± .10</td>
<td>4.07 ± .35</td>
<td>31.17 ± 2.72</td>
<td>19.97 ± 0.85</td>
<td>43.37 ± 1.08</td>
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<td>18</td>
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<td>0/1</td>
<td>4.90 ± .11</td>
<td>.82 ± .24</td>
<td>1.48 ± .02</td>
<td>.89 ± .01</td>
<td>4.10 ± .28</td>
<td>28.95 ± 0.64</td>
<td>19.60 ± 0.71</td>
<td>44.30 ± 0.28</td>
</tr>
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</table>

a TA, titratable acidity as % lactic acid.
b M/Pr, moisture to protein ratio.
c No. positive/no. of chubs tested by enrichment.
d Smokehouse settings were dry bulb 85°F and wet bulb 80°F during conditioning for 1 h and dry bulb 96°F and wet bulb 92°F during fermentation to pH ≤5.0.
e Postfermentation settings were dry bulb 115°F and wet bulb 104°F for 1 h for cycle 1, dry bulb 120°F and wet bulb 102°F for 1 h for cycle 2, dry bulb 125°F and wet bulb 115°F for 1 h for cycle 3, dry bulb 135°F and wet bulb 126°F for 1 h for cycle 4, and dry bulb 157°F and wet bulb 148°F until 128°F internal temperature reached for cycle 5.

decrease was observed after 21 days of drying, the desired 5-log CFU/g reduction was not achieved. The proximate composition of the pepperoni displayed the expected levels for pH, aw, salt, protein, moisture, and fat (Table 1). In related experiments, the dextrose concentration was adjusted from 0.63% to 0.90% to extend the fermentation to 24 h and lower the pH to <4.7. As in the 14- to 18-h fermentation, counts of the pathogen only decreased ca. 0.5 log CFU/g after fermentation and another 1.41 log CFU/g after 12 days of drying in the single trial conducted (data not shown). These data revealed that the modified method 6 process for pepperoni was not sufficient to eliminate high levels of serotype O157:H7 strains of E. coli following 14 to 24 h of fermentation and subsequent drying.

Fermentation at 96°F (36°C) to pH ≤5.0, heating to an internal temperature of 145°F (63°C), and drying at 53°F (13°C) and 63% RH to a M/Pr of ≤1.6:1

Since the modified method 6 process did not produce a 5-D kill as required by the USDA/FSIS, experiments were conducted to evaluate postfermentation heating for eliminating the pathogen. In the first of two such experiments, after reaching pH ≤5.0, pathogen numbers remained essentially the same as starting levels (Table 2). The smokehouse air

TABLE 3. E. coli O157:H7 count and chemical composition of pepperoni during fermentation at 96°F to pH <5.0, heating to an internal temperature of 128°F for 60 min, and drying at 53°F and 63% RH to a moisture to protein ratio of ≤1.6:1.

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>log CFU/g ± SD</th>
<th>Presencec</th>
<th>pH</th>
<th>TA (%)a</th>
<th>M/Prb</th>
<th>aw</th>
<th>Salt (%)</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
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</thead>
<tbody>
<tr>
<td>Day 0</td>
<td></td>
<td></td>
<td>4.72 ± 0.9</td>
<td>.70 ± 0.3</td>
<td>3.52 ± 0.4</td>
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<td>3.10 ± 0.8</td>
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<td>13.97 ± 0.48</td>
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<td>Emulsion</td>
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<tr>
<td>After fermentationd</td>
<td>6.89 ± 0.34</td>
<td></td>
<td>4.74 ± 0.12</td>
<td>.81 ± 0.04</td>
<td>2.64 ± 0.30</td>
<td>.95 ± 0.02</td>
<td>3.50 ± 0.29</td>
<td>44.80 ± 2.49</td>
<td>17.20 ± 1.34</td>
<td>34.33 ± 2.04</td>
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<tr>
<td>After heat processinge</td>
<td>&lt;2.00 ± 0.0</td>
<td></td>
<td>4.78 ± 0.15</td>
<td>.71 ± 0.01</td>
<td>3.38 ± 0.03</td>
<td>.96 ± 0.01</td>
<td>3.37 ± 0.21</td>
<td>51.07 ± 2.01</td>
<td>15.73 ± 0.21</td>
<td>29.13 ± 3.03</td>
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<tr>
<td>Drying day</td>
<td></td>
<td></td>
<td>4.74 ± 0.12</td>
<td>.81 ± 0.04</td>
<td>2.64 ± 0.30</td>
<td>.95 ± 0.02</td>
<td>3.50 ± 0.29</td>
<td>44.80 ± 2.49</td>
<td>17.20 ± 1.34</td>
<td>34.33 ± 2.04</td>
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<td>4</td>
<td>&lt;2.00 ± 0.0</td>
<td>3/3</td>
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<td>8</td>
<td>&lt;2.00 ± 0.0</td>
<td>3/3</td>
<td>4.76 ± 0.06</td>
<td>.84 ± 0.04</td>
<td>2.12 ± 0.05</td>
<td>.93 ± 0.01</td>
<td>3.87 ± 0.46</td>
<td>38.37 ± 2.17</td>
<td>18.40 ± 0.57</td>
<td>36.80 ± 3.47</td>
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<tr>
<td>12</td>
<td>&lt;2.00 ± 0.0</td>
<td>2/3</td>
<td>4.76 ± 0.06</td>
<td>.80 ± 0.03</td>
<td>2.01 ± 0.36</td>
<td>.94 ± 0.03</td>
<td>3.93 ± 0.21</td>
<td>36.23 ± 5.48</td>
<td>17.73 ± 0.40</td>
<td>39.67 ± 4.60</td>
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<tr>
<td>18</td>
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<td>4.79 ± 0.05</td>
<td>.91 ± 0.08</td>
<td>1.61 ± 0.25</td>
<td>.90 ± 0.03</td>
<td>4.43 ± 0.25</td>
<td>29.93 ± 4.46</td>
<td>19.17 ± 1.10</td>
<td>43.23 ± 4.61</td>
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a TA, titratable acidity as % lactic acid.
b M/Pr, moisture to protein ratio.
c No. positive/no. of chubs tested by enrichment.
d Smokehouse settings were dry bulb 85°F and wet bulb 80°F during conditioning for 1 h and dry bulb 96°F and wet bulb 92°F during fermentation to pH ≤5.0.
e Postfermentation settings were dry bulb 106°F and wet bulb 98°F for 1 h for cycle 1, dry bulb 120°F and wet bulb 102°F for 1 h for cycle 2, dry bulb 130°F and wet bulb 115°F for 1 h for cycle 3, dry bulb 140°F and wet bulb 123°F for 1 h for cycle 4, and dry bulb 140°F and wet bulb 130°F until 128°F internal temperature reached and then hold for 60 min for cycle 5.
temperature was then raised at a rate of 12 to 15°F (7°C to 8°C) per h until an internal chub temperature of 145°F (63°C) was reached (Table 2). Heating to 145°F (63°C) reduced counts of the pathogen below detection (> 5.0-log unit decrease); however, it was possible to recover viable cells of serotype O157:H7 by enrichment after heating and up to 8 days of drying. Nevertheless, heating chubs to an internal temperature of 145°F (63°C) delivered the required 5-log unit decrease in counts of the pathogen without causing any visible textural or color changes to the final product (data not shown).

Fermentation at 96°F (36°C) to pH ≤ 5.0, heating to an internal temperature of 128°F (53°C) for 60 min, and drying at 55°F (13°C) and 65% RH to a M/P of 1:6:1

On the basis of the encouraging results with the 145°F (63°C) heating process, a second heating regimen was evaluated. As in the previous experiments, fermentation alone had no appreciable effect on the pathogen (0.9-log cfu/g decrease) (Table 3). However, holding the chubs at an internal temperature of 128°F (53°C) for 60 min (Table 3) resulted in at least a 5-log CFU/g reduction in numbers of O157:H7. After heating, it was only possible to recover the pathogen by enrichment. Also, heating for 60 min at 128°F (53°C) did not visibly affect the appearance of the resulting product and did not cause “greasing out” (i.e., there was no residual fat layer under the casing) (data not shown).

DISCUSSION

Escherichia coli O157:H7 emerged as a foodborne pathogen of primary health concern in the 1990s. During this period, serotype O157:H7 strains were incriminated in numerous sporadic cases and several outbreaks involving a variety of foods (6, 7–9, 22, 29). Foods of bovine origin in general, and ground beef in particular, were most often identified as the food vehicles (16). To better understand the response of this bacterium to intrinsic (e.g., pH) and extrinsic (e.g., temperature) conditions associated with foods, studies were conducted using apple cider (6, 20), cottage cheese (4), ground beef (13, 17, 26), mayonnaise and/or dressings (23, 29), and salad vegetables (1). In general, these studies revealed E. coli O157:H7 is very tolerant to acid. Although there is significant variation in the degree of acid tolerance from strain to strain, most O157:H7 strains are more acid tolerant than other E. coli (3, 5, 11, 20). However, E. coli O157:H7 does not appear to be particularly heat tolerant (13). Although there have been reports on the behavior of non-O157:H7 strains of E. coli in fermented meats (12, 28), with the exception of a previous study conducted at the Food Research Institute using salami (15), no information has been published on the behavior of serotype O157:H7 strains in fermented sausages.

Several factors associated with pepperoni batter were evaluated for possible effects on the viability of the serotype O157:H7 cocktail. For example, high levels of indigenous bacteria could have a negative impact on the behavior of E. coli O157:H7. However, the relatively low numbers of aerobic bacteria in the raw materials suggest that indigenous bacteria were probably not a significant factor in this study. Likewise, microbiological analyses of the batter revealed that the spice and cure mixtures had no significant effect on the O157:H7 cocktail. Due to the limitations of the facility housing the smokehouse, it was not possible to evaluate the effect of smoke in this study. Smoking is presumably most effective against surface contamination of sausage and, as such, was not expected to have an appreciable effect on cells of E. coli within pepperoni chubs. The addition of smoke could enhance the kill achieved by fermentation, but it would probably not be sufficient to deliver a 5-D kill. Among the variety of other factors that could influence the viability of serotype O157:H7 strains in pepperoni, the pH and acid type, as well as the time held at a given temperature, probably present the greatest potential for impacting pathogen numbers (2). For example, E. coli O157:H7 is more sensitive to acid conditions (pH 3.6 to 4.0) at abuse (15 to 20°C or 60 to 77°F) rather than refrigeration (4 to 8°C or 39 to 46°F) temperatures (6, 20). It is also significant that survival of serotype O157:H7 strains can also be affected by the type of acid. In a study by Abdul-Raouf et al. (2) evaluating the effect of acidulants on E. coli O157:H7 in beef slurries, the order of effectiveness for inhibiting growth was acetic > lactic > citric acid at all pH values and temperatures tested. However, Conner and Kotrola (10) reported that organic acids and salts enhanced the survival of serotype O157:H7 strains during storage at 4°C in a synthetic medium. These data underscore the importance of conducting validation studies in bona fide fermented meats rather than microbiological media and/or beaker sausage to more precisely establish the viability of E. coli O157:H7 for a given formulation and manufacturing process.

Although lower pH and/or higher fermentation temperatures may result in a greater decrease in pathogen numbers, it is unlikely that these parameters could be adjusted sufficiently to deliver a 5-D kill in pepperoni. More specifically, at pH ≤ 4.3, pepperoni may be too tart for most consumers. Similarly, due to intrinsic limitations of the starter culture, it may not be possible to conduct a fermentation at temperatures much in excess of 120°F (49°C). For these reasons, it was necessary to evaluate postfermentation heating of chubs as a method to deliver the mandated 5-D reduction in counts of E. coli O157:H7. The first thermal process tested, 145°F (63°C) internal, was selected because it meets the requirements for trichinae destruction and because it approximates the time, temperature, and humidity guidelines established by the USDA for cooked and/or roast beef (27). The cooking requirements for cooked and/or roast beef were also approved by the USDA FSIS as an option to control serotype O157:H7 strains of E. coli in fermented sausage (25). The other thermal process tested, 128°F (53°C) internal for 60 min, was selected because it also meets the requirements for trichinae control, but more important, it is less harmful to the texture and appearance of pepperoni than the 145°F (63°C) cooking regimen (Steve Seideman, personal communication). This approach is also not without limitations, as some sausage varieties are not amenable to postfermentation heating due to possible unde-
sirable textural effects on the product and/or some manufacturers are not capable of including such a heating step due to the volume of product produced or the cost of the attendant equipment and facilities. From a practical standpoint, heating may cause “greasing out” and may yield a product more prone to cupping when used as a pizza topping (Steve Seideman, personal communication). Further studies may be warranted to identify heating regimens that are sufficient to eliminate the pathogen without producing untoward effects on the finished product and that are cost effective.

The present study evaluated the modified method 6 pepperoni process, as well as postfermentation heating, to inactivate *E. coli* O157:H7. A comprehensive sampling and screening of raw materials to account for low levels of this pathogen in raw materials, as well as adherence to good manufacturing procedures, would also contribute to the safety of fermented meat products. As another consideration, the sampling plan for raw materials that will undergo subsequent heat processing, such as pepperoni used as pizza topping, is less stringent (i.e., 15 samples per lot compared to 30 samples per lot (25)), because the ensuing cooking is presumably sufficient to eliminate the pathogen. Perhaps a more serious threat to consumer safety would result from contaminated pepperoni used for sandwiches or salad bars. For these uses, it is necessary to include a postfermentation heating step sufficient to deliver the 5-D kill. Thus, it would also be informative to substantiate the fate of the pathogen after frozen or refrigerated storage of pepperoni targeted for use in sandwiches, on pizza, or for salad bars.

The results of the present study revealed that the nonthermal process (modified method 6) for pepperoni manufacture was not sufficient to effect a 5-log unit reduction in counts of *E. coli* O157:H7. The observed <2-log unit decrease in counts of the pathogen following fermentation of pepperoni at 96°F (36°C) to pH ≤ 5.0 and drying was in agreement with a previous report of a 1- to 2-log unit decrease in counts of the pathogen under similar process conditions for salami (i.e., fermentation at 96°F (36°C) to pH 4.8, drying to a M/Pr of ≤1.9:1, and storage at 4°C for 2 weeks) (15). In the present study, postfermentation heating achieved a 5-D kill of this pathogen. Although both heating regimens evaluated were effective for eliminating *E. coli* O157:H7, cooking at 128°F (53°C) for 60 min was less harmful to the product than cooking to an internal temperature of 145°F (63°C). These data represent the first of several reports expected to be published on the fate of *E. coli* O157:H7 in fermented meats by our group and by other investigators. In addition to a thermal processing step, we are evaluating the effect of fermentation temperature and pH, a 3- to 7-day postfermentation storage at low pH, and various heating regimens for eliminating this pathogen in ready-to-eat fermented sausage. It is possible that slight increases in the fermentation temperature and/or extended storage at low pH would result in a greater reduction in counts of the pathogen and further reduce the time and temperature required for postfermentation heating. A comprehensive effort to screen raw materials for the pathogen, to follow good manufacturing practices, and to monitor finished product would significantly reduce the likelihood of illness due to *E. coli* O157:H7 in fermented sausage. In the interim, the results of the present study will assist manufacturers in prioritizing options for validating the safety of their respective manufacturing processes.

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