Fate and Thermal Inactivation of *Listeria monocytogenes* in Beaker Sausage and Pepperoni

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**ABSTRACT**

*Listeria monocytogenes* may survive the typical process used to produce sausage such as pepperoni and hard salami that is made from uncooked meat. Studies were done to identify heat treatments that could be applied during sausage-making to inactivate the organism to undetectable levels. Initial studies with beaker sausage revealed that heat treatments of 51.7°C for 8 h or 57.2°C for 4 h reduced *L. monocytogenes* counts by >2 log CFU/g, but for each treatment the organism was detected by enrichment in one of two samples. Heating beaker sausage to an internal temperature of 62.8°C inactivated listeriae to undetectable levels. Studies with pepperoni revealed that heating sausage at 51.7°C for 4 h after fermentation but before the drying cycle killed most *L. monocytogenes*, but the organism was occasionally detected in samples during drying. Heating pepperoni at 51.7°C for 4 h after the drying cycle completely inactivated *L. monocytogenes* in all samples. An additional study done to determine the fate of *L. monocytogenes* in beaker sausage made with and without lactic starter culture revealed that the organism could grow at 32.2°C in sausage during the fermentation period if the lactic starter culture was not added. The organism did not grow, but was reduced by about 1 to 2 log CFU/g during fermentation, in sausage made with lactic starter culture.

Confirmed foodborne outbreaks of listeriosis have been associated with the consumption of vegetable and dairy products (11); however, no such outbreaks have yet been linked to meat and poultry products. Many animal species are known to harbor *L. monocytogenes* (2,4) and the presence of *L. monocytogenes* has been confirmed in meat and poultry products (3,8). Nicolas (8) reported 22% of 18 retail samples of dry sausages were contaminated with *L. monocytogenes*. Johnson et al. (5) established that *L. monocytogenes* can survive up to 3 months in hard salami if the organism is initially present at ≥10⁵ CFU/g.

The normal fermentation and drying process used in sausage-making prevents growth of *L. monocytogenes*, but may not eliminate the organism from the finished product. Hence, additional steps may be needed to produce fermented sausage that is free of *L. monocytogenes*. A mild heat treatment combined with the acidic condition of fermented sausage may be a useful approach to eliminating the pathogen from the product. The objectives of this study were to: (a) determine the effect of low temperature heat treatments on *L. monocytogenes* in beaker sausage, and (b) determine the effect of a selected low temperature (51.7°C, 125°F) heat treatment applied at different stages of processing on *L. monocytogenes* in pepperoni.

**MATERIALS AND METHODS**

**Preparation of bacterial inocula.** A 5-strain mixture of *L. monocytogenes* (including strains Scott A (serotype 4, human isolate), V7 (serotype 1, raw milk isolate), LM101M (serotype 4, meat isolate), LM102M (serotype 1, meat isolate), and LM103M (serotype 1, meat isolate) at approximately equal concentrations) was used to inoculate meat. Each strain was grown in 100 ml of tryptose broth (Difco, Detroit, MI) at 37°C for 24 h. Cells were twice sedimented by centrifugation (2,000 x g at 4°C for 30 min) and resuspended in 10 ml of 0.01 M phosphate-buffered saline solution, pH 7.2 (PBS). The A₅₆₀ of the cell suspension was determined (OD of 0.5, ca. 1 x 10⁵ CFU/ml), and the suspension was diluted appropriately in PBS. Approximately equal concentrations of each cell suspension were combined into a 5-strain mixture and *Listeria* counts were done on each cell suspension both before and after mixing to confirm cell numbers.

A dextrose-fermenting *Pediococcus acidilactici* bacterial culture (LACTACEL 115, Microlife Technics, Sarasota, FL) was used as the starter culture and was added to the meat at levels recommended by the manufacturer (ca. 10⁵ CFU/g).

**Inoculation of treatment of beaker sausage.** Sausage was prepared from meat (23% beef; 77% pork) with a target fat content of 26%. The meat was ground twice through a 3.2-mm plate and inoculated with ca. 5 x 10⁵ CFU of the 5-strain mixture of *L. monocytogenes* (367 ml per 20.9 kg of meat) per gram of meat, and mixed (Buffalo model no. 2VSS mixer, John E. Smith’s Sons Co., Buffalo, NY) for 4 to 5 min. Glucose (131 g), commercial spice premix (627 g), and a commercial cure mixture (390 g), formulated for 156 ppm [156 μg/g] NaNO₂ and 3.3% NaCl, were added and mixed for 4 min. Finally, one half of the sausage batter was removed from the mixer to be used as sausage without starter culture, and LACTACEL 115 starter culture (4.8 g of resuspended culture per 10.4 kg of batter) was added to the remaining batter and mixed for 4 min. The batter (200 g per 250-ml beaker) was stuffed into beakers (90 mm height by 68 mm diameter), covered with aluminum foil (double layer), and heated in water baths using...
different treatments (minimum of 8 beakers of each formulation for each treatment).

The treatment and sampling protocols of sausage inoculated with starter culture were as follows:

(a) 32.2°C (90°F) treatment. Sausage batter was held at 32.2°C until the pH reached 5.2, and then was held at 32.2°C for an additional 10 h. Batter was sampled before heating, when the pH reached 5.2, at pH 4.8, and after 6 h at pH 4.8.

(b) 46.1°C (115°F) treatment. Sausage batter was held at 32.2°C until the pH reached 5.2, and then the temperature of the water bath was increased (8.3°C [15°F] per h) until the water bath temperature reached 48.9°C. Batter was held at 46.1°C for up to 8 h after the internal temperature (measured with a thermometer) reached 46.1°C. Batter was sampled when the internal temperature reached 46.1°C and after 4 and 8 h at 46.1°C.

(c) 51.7°C (125°F) treatment. Sausage batter was held at 32.2°C until the pH reached 5.2, and then the temperature of the water bath was increased (8.3°C per h) until the water bath temperature reached 54.4°C. Batter was held at 51.7°C for up to 8 h after the internal temperature reached 51.7°C. Batter was sampled when the internal temperature reached 51.7°C and after 4 and 8 h at 51.7°C.

(d) 57.2°C (135°F) treatment. Sausage batter was held at 32.2°C until the pH reached 5.2, and then the temperature of the water bath was increased (8.3°C per h) until the water bath temperature reached 60.0°C. Batter was held at 57.2°C for up to 4 h after the internal temperature reached 57.2°C. Batter was sampled when the internal temperature reached 57.2°C and after 4 h at 57.2°C.

(e) 62.8°C (145°F) treatment. Sausage batter was held at 32.2°C until the pH reached 5.2, and then the temperature of the water bath was increased (11.1°C [20°F] per h) until the water bath temperature reached 65.6°C. Batter was held at 62.8°C for up to 1 h after the internal temperature reached 62.8°C. Batter was sampled when the internal temperature reached 62.8°C and after 1 h at 62.8°C.

There was relatively little change in pH of the sausage without lactic starter culture during the duration of the experiment, which can be attributed to the absence of an active lactic acid bacteria population. Therefore, irrespective of pH, samples of this sausage were taken.

Microbiological and chemical analyses of beaker sausage. Two beakers of each formulation, i.e., with and without starter culture, were as follows:

1. Curing protocols described above. The following analyses were done on each sample at each sampling time: (a) L. monocytogenes count and enrichment if the organism was not detected by direct plating, (b) pH, and (c) titratable acidity. A L. monocytogenes count and enrichment was also done on two 25-g samples of meat before the meat was inoculated with listeriae.

L. monocytogenes counts were determined by adding 225 ml of Listeria enrichment broth (LEB: 7) without acriflavin to a 25-g portion of meat in a stomacher bag (Tekmar Co., Cincinnati, OH) and macerated for 2 min in a stomacher (model 400, Tekmar Co., Cincinnati, OH). The homogenate was serially (1:10) diluted in 0.01 M phosphate-buffered saline (PBS) solution, pH 7.2, the dilutions were plated in duplicate on LPM agar (6), and plates were incubated at 30°C for up to 5 d. Plates were counted after 48 h and 5 d. Acriflavin (0.003 g; 0.25 ml of 1.2% filter-sterilized acriflavin) was added to the remaining sample and was incorporated by mixing in a stomacher for 2 min. The sample was then enriched for L. monocytogenes according to the U.S. Department of Agriculture-Food Safety Inspection Service protocol (7). This included incubating the enrichment medium overnight at 30°C, transferring 0.1 ml of enrichment culture to 10 ml of LEB containing 0.1 ml of 2.5% filter-sterilized acriflavin (LEB #2) and incubating the LEB #2 enrichment medium overnight at 30°C. LEB #2 enrichment culture was streaked onto two plates of LPM and 1 ml was added to 4.5 ml of 0.25% KOH, mixed with a Vortex mixer for 2 to 3 sec, and streaked onto two plates of LPM agar. Agar plates were incubated at 30°C for 2 and 3 d. From each pair of LPM agar plates, 10 colonies typical of L. monocytogenes (sparkling blue or white; using 45°C transillumination and a binocular stereoscopic microscope) were selected for confirmation. Isolates were streaked onto tryptose agar, tested for catalase production, and then confirmed as pathogenic L. monocytogenes using the tests described by Johnson et al. (5).

The pH was determined according to the procedure described by Sebranek (9). This involved macerating a 10-g sample of meat with 90 ml of distilled, deionized water for 2 min in a stomacher and then measuring pH with a combination electrode and Corning model 140 pH meter (Corning Glass Works, Corning, NY).

Titratable acidity (TA) was determined according to the procedure described by Sebranek (9). This involved macerating a 25-g sample with 100 ml of hot (ca. 60°C) distilled, deionized water. The homogenate was poured into a 250-ml graduated cylinder and the volume was brought up to 250 ml with water (distilled, deionized) used to rinse the stomacher bag. The mixture was allowed to cool to room temperature and the fat layer was removed by pipetting before the homogenate was filtered through Whatman No. 1 filter paper. The filtrate (100 ml) was titrated with 0.098 N NaOH to pH 8.1 and TA was calculated by the following equation:

\[
%\text{TA} = \left( \frac{\text{N of titrant} \times \text{ml of titrant} \times \text{meq. wt. of acid}}{100} \right) \\
\text{g of sample}
\]

TA was expressed as percent lactic acid; meq. wt. of lactic acid is 0.09.

The following analyses were done only on samples taken at the beginning and end of each process treatment: (a) moisture (AOAC procedure 24.002; 1), (b) nitrite (AOAC colorimetric method 24.044; 1), and (c) salt (AOAC volumetric method 24.010, measured chlorine as sodium chloride; 1).

Preparation and treatment of pepperoni. Very coarsely chopped pepperoni batter (provided by a pepperoni manufacturer) with lactic acid bacteria starter culture (P. acidilactici, LACTACEL 115) added (2.8 x 10^7 lactics/g) was inoculated in two 22.7-kg (50-lb.) batches with ca. 10^6 L. monocytogenes (5-strain mixture as described above) per gram. The listeriae were added in 335 ml of 0.01 M PBS per 22.7-kg batch, and mixed with the batter for approximately 3 min in a Buffalo model 2VSS mixer (John E. Smith’s Sons Co., Buffalo, NY). Fibrous pepperoni casings (5.1 cm diameter; Teepack, Inc., Danville, IL) were made pliable by soaking in hot water (ca. 48.9°C) for 30 min. Excess water was squeezed manually from the casings which were then stuffed with batter using a hand stuffer (F. Dick, Kock Supplies, Inc., Kansas city, MO) and tied by hand (ca. 460 g chubs). Pepperoni was hung in a Vortron model 1000 smokehouse (Vortron, Inc., Beloit, WI) and processed according to one of the following schedules:

(a) Processing schedule No. 1. Heat at 15.6°C for 1 h, at 21.1°C for 1 h, and then at 35.6°C until the product reached pH 4.8 (ca. 13 h).

After fermentation (end of Processing Schedule No. 1), pepperoni was tempered to 24°C to 32°C and hung in a drying room.
Sausages were held at 12.8°C (dry bulb), 10°C (wet bulb), and 70% relative humidity until the moisture: protein ratio of the sausage was ≤1.6:1. Sausages were positioned ≥30 cm apart in the drying room (Biotron Facility; University of Wisconsin-Madison) to allow for proper airflow to prevent mold growth, case shriveling, and case hardening. (The drying cycle required 19 to 26 d in cure.)

(b) Processing schedule No. 2. Heat at 15.6°C for 1 h, at 21.1°C for 1 h, at 35.6°C until the product reached pH 4.8, at 54.4°C until the product internal temperature reached 51.7°C, and then at 51.7°C for 4 h.

After processing (end of Processing Schedule No. 2), sausages were showered with cold water for 10 min, then the blowers were turned on to dry the product surface. Pepperoni was tempered to 24°C to 32°C and hung in the drying room. Sausages were dried according to conditions described in Processing Schedule No. 1. (The drying cycle required 22 d in cure.)

(c) Processing schedule No. 3 (Reprocessing cycle). Following drying, one-half of the sausages processed according to Processing Schedule No. 1 were reprocessed by heating product at 52.2°C for 4 h.

After reprocessing, sausages were showered in cold water for 10 min and chilled to ≤4.4°C.

After sausages were processed by the appropriate processing schedule (No. 1, 2, or 3), they were individually vacuum packaged in gas impermeable Curlon bags (nylon-Saran-polyethylene: O₂ transmission of 0.8 to 1.0 cm³/645 cm² per 24 h at 22.8°C; CO₂ transmission of 2.5 to 3.0 cm³/645 cm² per 24 h at 22.5°C; H₂O transmission of 0.5 g/645 cm² per 24 h at 37.8°C and 90% relative humidity; Curwood, Inc., New London, WI) using a Multivac AGW vacuum packaging unit (Sepp Haggemuller KG, Wolfertschwenden, West Germany) and held at 4°C for 60 d.

Microbiological and chemical analysis of pepperoni

Three sausages were taken at each sampling time and tested for: (a) L. monocytogenes count by direct plating and by enrichment if the organism was not detected by direct plating, (b) pH, and (c) titratable acidity. The same procedures were used as described above for the beaker sausage study. Sampling times included: (a) "0" time (end of chopping), (b) end of fermentation (pH 4.8), (c) at 51.7°C internal temperature, (d) after 4 h at 51.7°C internal temperature, (e) after the first day of drying, (f) approximately every 3 d after drying, and (g) approximately every 2 weeks for up to 60 d during storage at 4°C. Sausage casings were wiped with 70% ethanol, cut with a sterile scalpel, and were removed with a sterile forceps. Meat was sampled from different locations within the sausage for microbial analyses. The remaining sausage was ground twice through a 4.76-mm (0.188-in) plate of a Hobart grinder (model 84142: Hobart Manufacturing Co., Troy, OH) and mixed well for pH, titratable acidity, and other chemical analyses.

Other analyses included a lactic acid bacteria count (MRS agar; incubated at 30°C for 48 h) done on samples at "0" time (end of chopping) and chemical analyses of salt (AOAC 24.010; 1) moisture (AOAC 24.002; 1), fat (AOAC 24.005; 1), and protein (AOAC 24.027; 1) done on samples at the end of chopping and at the end of the drying cycle.

RESULTS

Beaker sausage experiment. Results of the chemical composition of and fate of L. monocytogenes in beaker sausage made with lactic starter culture are shown in Table 1. Data indicate that an active fermentation occurred, with the pH of the sausage batter decreasing from 6.3 initially to 4.8 after 16 h at 32.2°C. Concurrently, the lactic acid bacterial population increased tenfold and the TA increased from 0.14% to 0.26%. During this time the sodium nitrite level decreased from 106 ppm to <25 ppm. The L. monocytogenes population decreased by >1 log CFU/g during fermentation, but the organism was still detectable by enrichment in samples held for as long as 6 h after reaching pH 4.8 (22 h at 32.2°C). Treatment of fermented meat at 46.1°C for 8 h or to

![Table 1](image-url)

![Graph](image-url)
an internal temperature of 51.7°C or 57.2°C did not eliminate listeriae, with the organism being detected in all samples assayed but only by an enrichment procedure. Treatment of beaker sausage at 51.7°C for 8 h or 57.2°C for 4 h reduced L. monocytogenes counts by >2 log_{10} CFU/g to below detectable levels (<10 CFU/g) by the direct plating procedure; however, for each treatment the organism was detected by enrichment in one of two samples. Heating sausage to an internal temperature of 62.8°C inactivated listeriae to undetectable levels as determined by both direct plating and enrichment procedures.

Results of the fate of L. monocytogenes in and chemical composition of beaker sausage made without lactic starter culture are shown in Table 2. Data reveal that fermentation did not occur, with the pH and TA of the meat about the same both before and after heating at 32.2°C for up to 22 h. During this time the sodium nitrite concentration decreased from 103 ppm to about 70 ppm, but not to the low level (<25 ppm) observed in the fermented sausage. L. monocytogenes grew (2-log_{10} CFU/g increase) in sausage batter during heating at 32.2°C. Heating sausage batter for 8 h at 46.1°C or 51.7°C after 16 h at 32.2°C reduced the Listeria population about 1 log_{10} CFU/g and 2 log_{10} CFU/g, respectively. Heating sausage batter at 57.2°C for 4 h or to an internal temperature of 62.8°C killed >10^5 L. monocytogenes/g, eliminating detectable lysteriae as determined by both direct plating and enrichment procedures.

Interestingly, low levels of L. monocytogenes (<10 CFU/g; only detected by the enrichment procedure) were detected in meat before the 5-strain mixture of L. monocytogenes was added, indicating the organism was part of the indigenous microbial flora of the meat used in this study.

**Pepperoni experiment.** Two studies were done to identify and verify treatments effective in killing to undetectable levels L. monocytogenes in pepperoni. In Study No. 1, the fate of L. monocytogenes was determined in pepperoni: (a) during normal processing and storage (Processing Schedule No. 1), (b) during a heat treatment to an internal temperature of 51.7°C for 4 h after fermentation followed by normal drying and storage conditions (Processing Schedule No. 2), and (c) during a heat treatment to an internal temperature of 51.7°C for 4 h after the drying cycle followed by normal storage conditions (Processing Schedule No. 3). In Study No. 2, the fate of L. monocytogenes was determined in pepperoni during normal fermentation and drying followed by a heat treatment to an internal temperature of 51.7°C for up to 4 h (Processing Schedule No. 3 repeated).

In Study No. 1, the pH, TA, lactic acid bacteria count, and L. monocytogenes count of pepperoni batter at “0” time (at end of chopping) were 6.0, 0.31%, 2.8 x 10^2 CFU/g, and

### TABLE 2. Fate of L. monocytogenes in beaker sausage with no lactic starter culture.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>TA (%)</th>
<th>L. monocytogenes</th>
<th>Sodium nitrite</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CFU/g</td>
<td>Enrichment</td>
</tr>
<tr>
<td>Before heating</td>
<td>6.3</td>
<td>0.34</td>
<td>5.5 x 10^3</td>
<td>ND</td>
</tr>
<tr>
<td>32.2°C, pH 5.2</td>
<td>ND</td>
<td>ND</td>
<td>5.0 x 10^6</td>
<td>ND</td>
</tr>
<tr>
<td>32.2°C, pH 4.8</td>
<td>6.2</td>
<td>0.41</td>
<td>3.1 x 10^5</td>
<td>ND</td>
</tr>
<tr>
<td>32.2°C, 6 h</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>after pH 4.8</td>
<td>6.2</td>
<td>0.40</td>
<td>5.2 x 10^5</td>
<td>ND</td>
</tr>
<tr>
<td>46.1°C, 0 h</td>
<td>6.3</td>
<td>0.39</td>
<td>2.7 x 10^7</td>
<td>ND</td>
</tr>
<tr>
<td>46.1°C, 4 h</td>
<td>5.7</td>
<td>0.47</td>
<td>8.1 x 10^7</td>
<td>ND</td>
</tr>
<tr>
<td>46.1°C, 8 h</td>
<td>5.9</td>
<td>0.44</td>
<td>3.2 x 10^8</td>
<td>ND</td>
</tr>
<tr>
<td>51.7°C, 0 h</td>
<td>6.4</td>
<td>0.38</td>
<td>3.5 x 10^5</td>
<td>ND</td>
</tr>
<tr>
<td>51.7°C, 4 h</td>
<td>6.3</td>
<td>0.37</td>
<td>6.5 x 10^5</td>
<td>ND</td>
</tr>
<tr>
<td>51.7°C, 8 h</td>
<td>6.4</td>
<td>0.34</td>
<td>1.7 x 10^7</td>
<td>ND</td>
</tr>
<tr>
<td>57.2°C, 0 h</td>
<td>6.4</td>
<td>0.37</td>
<td>4.4 x 10^5</td>
<td>ND</td>
</tr>
<tr>
<td>57.2°C, 4 h</td>
<td>6.4</td>
<td>0.34</td>
<td>&lt;10</td>
<td>0/2</td>
</tr>
<tr>
<td>62.8°C, 0 h</td>
<td>6.4</td>
<td>0.33</td>
<td>&lt;100</td>
<td>0/2</td>
</tr>
<tr>
<td>62.8°C, 4 h</td>
<td>6.4</td>
<td>0.31</td>
<td>&lt;10</td>
<td>0/2</td>
</tr>
</tbody>
</table>

*Moisture content (%), 61.5 ± 1.1; sodium chloride concentration (%), 3.54 ± 0.12 (mean ± S.D. of 20 determinations).

*Reported values are average of duplicate determinations.

*TA, titratable activity.

*No. of samples L. monocytogenes-positive/No. of samples tested.

*1D 0: lactic acid bacteria count, 2.8 x 10^2 CFU/g; moisture content, 53.5%; fat content, 27.1%; sodium chloride concentration, 3.57%; protein content, 13.9%. All values are average of duplicate determinations.

*2End of fermentation; required 12 h.

*3Twelve h after start of drying cycle.

*4At d 22 the moisture; protein ratio was >1.6:1.

*5D 26 (end of drying cycle): moisture content, 23.5%; fat content, 48.3%; sodium chloride concentration, 4.51%; protein content, 16.7%. Values are average of duplicate determinations.

*6Moisture:Protein Ratio, 0.71:1.

### TABLE 3. pH, titratable acidity, L. monocytogenes count (or presence), and chemical composition of pepperoni during processing (normal procedure) and storage at 4°C (Study No. 1, Processing Schedule #1).

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>pH</th>
<th>TA (%)</th>
<th>L. monocytogenes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CFU/g</td>
</tr>
<tr>
<td>D 0</td>
<td>6.0</td>
<td>0.31</td>
<td>7.5 x 10^6</td>
</tr>
<tr>
<td>pH 4.8</td>
<td>4.7</td>
<td>0.54</td>
<td>3.5 x 10^5</td>
</tr>
<tr>
<td>D 1</td>
<td>4.6</td>
<td>0.53</td>
<td>1.7 x 10^4</td>
</tr>
<tr>
<td>D 5</td>
<td>4.5</td>
<td>0.73</td>
<td>&lt;10</td>
</tr>
<tr>
<td>D 7</td>
<td>4.5</td>
<td>0.77</td>
<td>&lt;10</td>
</tr>
<tr>
<td>D 10</td>
<td>4.5</td>
<td>1.03</td>
<td>&lt;10</td>
</tr>
<tr>
<td>D 13</td>
<td>4.5</td>
<td>0.80</td>
<td>&lt;10</td>
</tr>
<tr>
<td>D 16</td>
<td>4.5</td>
<td>0.75</td>
<td>&lt;10</td>
</tr>
<tr>
<td>D 19</td>
<td>4.5</td>
<td>0.74</td>
<td>&lt;10</td>
</tr>
<tr>
<td>D 22</td>
<td>4.5</td>
<td>0.85</td>
<td>&lt;10</td>
</tr>
<tr>
<td>D 26</td>
<td>4.6</td>
<td>0.83</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Wk 2</td>
<td>4.6</td>
<td>0.83</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Wk 4</td>
<td>4.6</td>
<td>0.97</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Wk 6</td>
<td>4.6</td>
<td>0.94</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Wk 8</td>
<td>4.5</td>
<td>0.95</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

*TA, titratable activity.

*No. of samples L. monocytogenes-positive/No. of samples tested.

*1D 0: lactic acid bacteria count, 2.8 x 10^2 CFU/g; moisture content, 53.5%; fat content, 27.1%; sodium chloride concentration, 3.57%; protein content, 13.9%. All values are average of duplicate determinations.

*2End of fermentation; required 12 h.

*3Twelve h after start of drying cycle.

*4At d 22 the moisture; protein ratio was >1.6:1.

*5D 26 (end of drying cycle): moisture content, 23.5%; fat content, 48.3%; sodium chloride concentration, 4.51%; protein content, 16.7%. Values are average of duplicate determinations.

*6Moisture:Protein Ratio, 0.71:1.

*7Number of weeks of storage at 4°C.
7.5 \times 10^4 \text{ CFU/g}, respectively (Table 3). In pepperoni processed according to Processing Schedule No. 1, there was about a 2 \log_{10} \text{ CFU/g} decrease of \textit{L. monocytogenes} during 12 h of fermentation, and a further decrease to <10 \text{ CFU/g} by d 5 of drying (Table 3). Although \textit{L. monocytogenes} was not detected by direct plating (<10 \text{ CFU/g}) in any pepperoni samples taken from d 5 of drying through 8 weeks of refrigerated storage, the organism was consistently detected by the enrichment procedure in samples taken through d 26 of drying and again at 4 and 8 weeks of refrigerated storage. The pH of the pepperoni during drying decreased to about 4.5 and the TA increased to >0.8\%. The moisture:protein ratio reached the desirable level (\leq 1.6:1) for acceptable pepperoni by d 26, but not at d 22. Hence, sausages were vacuum packaged at d 26 of drying and then stored refrigerated.

Heating pepperoni to an internal temperature of 51.7°C after fermentation but before drying (according to Processing Schedule No. 2) had relatively little effect on \textit{L. monocytogenes} (Table 4). The \textit{L. monocytogenes} counts of pepperoni at the end of fermentation and after heat treatment to 51.7°C were 3.5 \times 10^2 and 1.3 \times 10^2 \text{ CFU/g}, respectively. Heating pepperoni at 51.7°C for 4 h reduced the \textit{L. monocytogenes} population to undetectable levels by both direct plating and enrichment procedures. However, \textit{L. monocytogenes} was isolated sporadically by the enrichment procedure from pepperoni during the drying cycle, starting at d 5 and up to d 22. The organism was not isolated from sausage during refrigerated storage. The pH of the pepperoni during drying decreased to about 4.5 and the TA increased to >0.8\%. The moisture:protein ratio reached 1.4:1 by d 22.

One-half of the pepperoni produced using Processing Schedule No. 1 was reprocessed (according to Processing Schedule No. 3) after the drying cycle by heating to an internal temperature of 51.7°C for 4 h. At d 26 of processing (end of drying cycle), the \textit{L. monocytogenes} count of the sausage was <10 \text{ CFU/g}, but the organism was detected by the enrichment procedure in two of three samples (Table 5). After heating pepperoni to an internal temperature of 51.7°C, and after 4 h at this temperature, \textit{L. monocytogenes} was not detected in sausages, including after refrigerated storage for 8 weeks.

A second study (Study No. 2) was done to verify the effectiveness of reprocessing after drying in eliminating detectable \textit{L. monocytogenes} from pepperoni and to determine if heating pepperoni during the reprocessing cycle to an internal temperature of 51.7°C for a full 4 h was needed to completely inactivate the organism (Table 6). Pepperoni was made according to Processing Schedule No. 1 and drying was completed at d 19 when the moisture:protein ratio was 1.49:1. The \textit{L. monocytogenes} count was 5.3 \times 10^5 \text{ CFU/g} at d 19, which was considerably more listeriae than was observed at a similar stage of processing in pepperoni prepared in Study No. 1 (D 26; Table 3). Heating sausage at d 19 to an internal temperature of 51.7°C reduced the \textit{L. monocytogenes} count to <10 \text{ CFU/g}, but the organism was still detectable by the enrichment procedure (Table 6). \textit{L. monocytogenes} was detected in sausage heated at 51.7°C for up to 3 h but not for 4 h.


**TABLE 6. pH, titratable acidity, L. monocytogenes count (or presence), and chemical composition of pepperoni during processing and reprocessing (heated to internal temperature of 51.7°C for 1, 2, 3 and 4 h) after drying. (Study No. 2, Processing Schedule #3),**

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>pH</th>
<th>TA (%)</th>
<th>L. monocytogenes CFU/g</th>
<th>Enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5</td>
<td>0.65</td>
<td>5.3 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1/3</td>
</tr>
<tr>
<td>Internal temp.</td>
<td>51.7°C</td>
<td>4.9</td>
<td>0.81</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Internal temp.</td>
<td>51.7°C, 1 h</td>
<td>4.6</td>
<td>0.97</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Internal temp.</td>
<td>51.7°C, 2 h</td>
<td>4.9</td>
<td>0.91</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Internal temp.</td>
<td>51.7°C, 3 h</td>
<td>4.6</td>
<td>0.85</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Internal temp.</td>
<td>51.7°C, 4 h</td>
<td>4.6</td>
<td>0.89</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

<sup>a</sup>TA, titratable acidity.

<sup>1</sup>No. of samples L. monocytogenes-positive/No. of samples tested.

<sup>2</sup>Lactic acid bacteria count at d 0 was 5.6 x 10<sup>8</sup> CFU/g.

<sup>3</sup>End of fermentation.

<sup>4</sup>At d 19 (end of drying cycle); moisture content, 27.6%; protein content, 18.5%; Moisture:Protein Ratio, 1.49:1.

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**DISCUSSION**

*Listeria monocytogenes* does not grow in sausage batter during the processing of properly fermented pepperoni (this study) or hard salami (5) prepared with lactic starter culture. The importance of adding lactic starter culture to control the growth of listeriae was clearly demonstrated by our studies with beaker sausage. In salami batter without lactic starter culture *L. monocytogenes* grew prolifically during the 16-h fermentation period at 32.2°C, whereas under the same conditions but with lactic starter culture added the organism not only did not grow but populations were reduced.

The sole presence of 3.5% sodium chloride and 103 ppm (initial determined) sodium nitrite in meat at pH 6.3 did not control the growth of *L. monocytogenes* at 32.2°C; acid development, and perhaps the production of other antimicrobial factors such as bacteriocins, by lactic acid bacteria was essential to prevent growth of listeriae in sausage batter. Although acid production to pH 4.8 during fermentation of sausage batter greatly reduces the *L. monocytogenes* population, these conditions do not completely kill all contaminating listeriae. Results of studies by Shahamat et al. (10) on the influence of sodium chloride, pH, and temperature on the inhibitory activity of sodium nitrite on *L. monocytogenes* in broth revealed that the antimicrobial activity of 100 ppm sodium nitrite in the presence of 3% sodium chloride was achieved only at pH ≤5.5 and refrigeration temperature (4°C).

The normal process used to manufacture pepperoni is not sufficient to kill all *L. monocytogenes* if the organism is initially present at 7 x 10<sup>6</sup> cells per gram of pepperoni batter. *L. monocytogenes* does not grow during the process, and in one instance (Study No. 1), was inactivated to low numbers (<10 CFU/g) during the drying cycle. In pepperoni prepared for Study No. 2, there was only about a 1-log<sub>10</sub> CFU/g reduction of *L. monocytogenes* during fermentation and relatively little inactivation of the organism during drying, indicating a substantial number of listeriae may survive in finished product if large numbers are initially present.

Heating pepperoni to an internal temperature of 51.7°C for 4 h immediately after fermentation reduced the *L. monocytogenes* population considerably but did not eliminate the organism. Low levels (<10 CFU/g) of *L. monocytogenes* were detected in pepperoni throughout the drying cycle; however, the organism was not in any sausage that was stored refrigerated after drying. Perhaps the combined stress induced by acid produced during fermentation, heating at 51.7°C, and drying sufficiently injure the listeriae so that the additional stress of cold storage inactivates the bacteria.

Reprocesssing pepperoni by heating to an internal temperature of 51.7°C for 4 h after the drying cycle completely inactivated detectable listeriae. When the number of *L. monocytogenes* at the end of drying was high (5 x 10<sup>8</sup> cells/g), heating to an internal temperature of 51.7°C for 3 h or less greatly reduced the number of surviving listeriae, but did not kill all of them. Heating to an internal temperature of 51.7°C for 4 h yielded no surviving listeriae, including by the enrichment procedure.

It appears that reprocessing pepperoni after drying by heating it to an internal temperature of 51.7°C for 4 h is the best of the procedures evaluated for producing product free of viable *L. monocytogenes*.

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**REFERENCES**

sensitive to gentamicin, in this study 5 (21.7%) were resistant. The apparent increase in resistance to most of the antibiotics tested could be due to resistance transfer which has been reported amongst salmonellae including isolates in Nigeria (18,19). Also, uncontrolled use and misuse of antibiotics in the environment could be a factor. Ojo (16) stated that resistance to antibiotics, particularly tetracycline, could be due to their use as additives in food given to animals as concentrates or supplements to grass. Resistance to antimicrobial agents like penicillin, erythromycin and methicillin detected in this study should, however, not elicit concern since these are generally used against gram-positive bacteria.

In conclusion, the widespread contamination of slaughter and dressing areas of Zaria abattoir with salmonellae and the discharge of untreated effluents into the environment could pose a health hazard from the viewpoint of food hygiene and environmental contamination. Also, resistance of Salmonella isolates to antibiotics like chloramphenicol, gentamicin and tetracycline, which appear to be on an increase in Nigeria, could be a health risk when therapy of salmonellosis in humans is considered.

REFERENCES