Validation of Dry Cured Ham Process for Control of Pathogens

A.E. REYNOLDS, M.A. HARRISON, R. ROSE-MORROW, AND C.E. LYON

ABSTRACT: The dry curing process for hams to control Salmonella spp., Escherichia coli O157:H7, Listeria monocytogenes, and Staphylococcus aureus was evaluated. Fresh hams, surface inoculated with each microorganism, were processed by a commercial style process. There was no significant (p < 0.05) difference in reduction of microbial populations between ham sampling locations (cushion, butt, hock). Interaction of salt concentration (8%), pH (5.5), ham storage temperature (20°C), and ham aw (0.92) limited staphylococcal proliferation. Mean log reduction of Salmonella spp., E. coli O157:H7 and L. monocytogenes populations on inoculated hams after 69 d of curing were 5.5, 5.5, and 4.0 CFU/cm², respectively and after 120 d were 5.7, 5.5, and 4.8 CFU/cm², respectively.

Keywords: dry cured ham, Salmonella, Escherichia coli O157:H7, Listeria monocytogenes, Staphylococcus aureus

Introduction

The USDA, FSIS PATHOGEN REDUCTION: HAZARD ANALYSIS and Critical Control Point System regulations require the validation of ready-to-eat product production procedures to ensure that they are adequate to control pathogenic microorganisms. Presently, any ready-to-eat meat and/or poultry product found contaminated with Salmonella spp., E. coli O157:H7, L. monocytogenes, or staphylococcal enterotoxin is considered adulterated. The dry curing process for country ham or prosciutto has not previously been demonstrated to meet these requirements. It has been long held that the low water activity (aw), high salt concentration and nitrite levels produce a safe, shelf stable, country cured ham product. Rainaldi and others (1991) demonstrated that L. monocytogenes was able to survive for 7 d on stored, dry cured bresaola. Langlois and Kemp (1974, 1979) studied the various microflora of dry cured hams and found the resulting organisms were affected by the initial contamination and the curing process. Huerta and others (1988) demonstrated that the coliform population was greatly reduced in the final stages of the dry curing process. However, little information is available concerning the reduction of E. coli O157:H7, L. monocytogenes, or S. aureus during the dry curing process for hams. To avoid the potential health hazard posed by pathogenic microorganisms, validation of the curing procedures presently used and prescribed by the USDA in dry curing country ham is needed to establish the effectiveness in controlling pathogens. This study was done to evaluate the dry curing process for its efficacy against Salmonella spp., E. coli O157:H7, L. monocytogenes, and S. aureus. Standard commercial methods of processing and good manufacturing practices were followed during the study.

Materials and Methods

Experimental Approach

Dry cured hams made according to a commercial formulation and inoculated with Salmonella spp., E. coli O157:H7, L. monocytogenes, and S. aureus, were processed in a commercial type curing room using commercial conditions and Trichina treatment as per USDA recommendations (Code of Federal Regulations). The challenge study was done following the protocol delineated in 1995 by USDA/FSIS for determining the efficacy of processing of dry cure, fermented sausage. This protocol was modified for the inoculation of the raw product and is described in the following sections. Hams were surface inoculated on the cut surface of the ham cushion (5 cm ventral to the aitch-bone (symphysis pelvis) on the adductor and semimembranosus muscles), butt (gluteus medius muscle), and hock (exposed surface of the tibia and connective tissue). This study did not address injected hams, as this is not a common practice in the dry curing of hams.

Raw Ingredients

Ingredients for the cure mix were purchased locally and fresh hams were obtained from an Atlanta, Ga. distributor. The fresh hams were 6.35 to 7.25 kg and 7 d postmortem. These hams had a packer style cut with line run fat thickness varying from 1.0 to 3.5 cm thick.

Bacterial Cultures

Salmonella (S. California, S. Heidelberg, S. Montevideo, S. Muster, Typhimurium 654), E. coli O157:H7 (E009, E0019, 932, 380-94, 204P), L. monocytogenes (Brie, LDCD 81-861, Scott A, V7, 301) and S. aureus (ATCC 12600, 13565, 25923, 27664, 6538) isolates were used. All stock cultures were preserved on Microbank™ beads (Pro-Lab Diagnostics, Austin, Tex., U.S.A.) at -15°C. Each culture was grown in 9 ml of tryptic soy broth (TSB, Difco Laboratories, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.) at 32°C. After 24 h, strains were transferred to a new 9 ml tube of TSB for an additional 24 h at 32°C. The cultures were then centrifuged (1200 x g, 10 min, Sorvall® RC-5B refrigerator centrifuge, DuPont Instruments, Newtown, Conn., U.S.A.), the supernatant removed, the pellet resuspended in 12 ml peptone water (0.1%, pH 7.2) and centrifuged (1200 x g, 10 min) once again. The strains were finally resuspended in 9 ml 0.1% peptone water. Prior to ham inoculation, 2 ml of each strain were combined into a sterile centrifuge tube to achieve a concentration of approximately 10⁶ CFU/ml. This mixture was used to inoculate the hams before cure application.
Validation of Dry Cured Ham Process for Pathogen Control

**Ham Inoculation**

A 5 × 5 cm² sterile template was used as a guide to inoculate the ham cushion surface and butt with 0.1 ml of the pooled microorganism suspension, using a micropipettor. The micropipettor was also used to inoculate the hock by dispensing inoculum between the skin and tibia bone within 2 to 3 cm of the exposed cut. To mark the inoculation area, the corners adjacent to the inoculated areas were marked with food grade marking ink. Visible moisture on the inoculated areas was allowed to dry (approximately 15 min) before application of the cure mix.

**Processing Parameters**

Commercial type processing parameters based on information in the Curing Georgia Hams Country Style bulletin (Christian 1982) were used. Two cure mix formulations were used: (a) 3.63 kg salt, 454 g sugar, 14.2 g sodium nitrite, 56.7 g sodium nitrate and (b) 3.63 kg salt and 454 g sugar. These were applied at the ratio of 42.53 g of cure mixture per 0.45 kg of ham, with a half portion each of this cure mixture applied in 2 separate hand rubs, on d 0 and d 10. Uninoculated control hams for each cure mix group received the same cure treatment as inoculated hams. For safety measures and to prevent cross contamination of the personnel and environment as well as among hams, personnel wore hairnets, disposable lab coats, doubled nitrite gloves, and booties while treating the hams. The gloves and lab coats were changed between ham treatments to prevent cross contamination of the personnel and environment. The hams were cured at 4.4 °C during dry salt curing (35 d), after which the excess salt was brushed off with no water added. The hams were placed in stockettes and held at 4.4 °C for 14 d for salt equalization. Hams were then dry aged for 20 d at 29.4 °C (65% relative humidity) to meet USDA Trichina treatment requirements (Code of Federal Regulations). Dry curing process was considered complete at this point (d 69). At d 69, hams were placed in ambient (20 to 24 °C) storage through d 120.

**Microbial Sampling**

The hams were sampled on d 0, 49, 61, 69, 90, and 120. From inoculated and control hams, samples of the marked 5 × 5 cm² areas of the cushion and butt and the end 5 to 7 cm of skin and muscle at the hock were excised with a sterile knife (approximately 75 g). Samples were placed into 225 ml of 0.1% peptone water and homogenized in a Stomacher knife (approximately 75 g). Samples were placed into 225 ml of peptone water and homogenized in a Stomacher knife (approximately 75 g). Samples were placed into 225 ml of peptone water and homogenized in a Stomacher knife (approximately 75 g). Samples were placed into 225 ml of peptone water and homogenized in a Stomacher knife (approximately 75 g). Samples were placed into 225 ml of peptone water and homogenized in a Stomacher knife (approximately 75 g). Samples were placed into 225 ml of peptone water and homogenized in a Stomacher knife (approximately 75 g). Samples were placed into 225 ml of peptone water and homogenized in a Stomacher knife (approximately 75 g). Samples were placed into 225 ml of peptone water and homogenized in a Stomacher knife (approximately 75 g). Samples were plated onto BSA, XLD agar, and brilliant green agar for possible Salmonella spp. isolates and LSA for possible L. monocytogenes isolates. Plates were incubated at 32 °C for 24 h and examined for presence of representative colonies.

The identification of representative, presumptive isolates for each ham treatment and each sampling location and time was done. Presumptive Salmonella spp. and E. coli O157:H7 isolates were identified using the Micro-ID™ identification system for Enterobacteriaceae (Remel, Lenexa, Kans., U.S.A.) as per manufacturer’s instructions. Salmonella isolates were serotyped with Salmonella O-antisera and E. coli isolates were serotyped with E. coli O157 and H7 antisera. Listeria isolates were identified using the Micro-ID™ Listeria system (Remel, Lenexa, Kans., U.S.A.) as per manufacturer’s instructions.

In order to estimate the number of coagulase positive S. aureus colonies from other salt tolerant organisms, 80 typi
colony isolates were randomly chosen from Baird-Parker agar plates prepared from d 90 and 120 samples and inoculated onto tryptic soy agar (TSA) slants. After a 24 h incubation period at 32 °C, a coagulase test was performed on each isolate, using the Bacti™ Staph test kit (Remel, Lenexa, Kans., U.S.A.) and following the manufacturer’s instructions.

Ten g samples of randomly chosen hams from d 90 and 120 were tested for the presence of staphylococcal enterotoxin using the Staphylococcal Enterotoxin Visual Immunoassay, (Tecra® Diagnostics, Roseville, Australia) following the manufacturer’s instructions.

**Detection of Injured Organisms**

To facilitate recovery of potentially injured bacteria, 1 ml of the 1:10 dilutions of the stomached ham sample was inoculated into 9 ml tubes of brain heart infusion (BHI) broth and incubated at 32 °C for 4 h (Ray 1979). Subsequent to the 4 h incubation period, appropriate dilutions of the BHI broth were plated onto BSA, SMAC, LSA, and BP agar for enumeration of injured Salmonella spp., E. coli O157:H7, L. monocytogenes, and S. aureus. Plates were incubated at 32 °C for 24 h (PCA, BSA, SMAC, and BP) or 48 h (LSA) and examined for appearance of typical colonies.

**Moisture Content, Salt Content, pH, Titratable Acidity, and Water Activity (a₀)**

Samples were taken 2 to 3 cm from the aitch-bone from the center of the ham in the semimembranosus muscle after surface samples for the microbiological analysis was removed. Titratable acidity was measured by using the AOAC Method 920.124 (AOAC International 1995a). Moisture content was determined by the AOAC Method 935.47 (AOAC In-
Results and Discussion

HAMS HAVE BEEN CURED FOR CENTURIES USING THE TIME
honored process of dry salt curing and drying to pre-
serve the meat for future use. The removal of water from
meat products by dry salt curing and subsequent drying has
successfully served to preserve hams throughout the world.
Water is one of the most important factors governing micro-
bial growth and spoilage in food. The concept of water activ-
ity (aw) has been very valuable in physiological studies of mi-
croorganisms and for predicting the microbial stability and
safety of foods. Measured aw values generally correlate well
with potential microbial growth and metabolic activity
(Gould 1985; Leistner and Rodel 1975; Troller and Christian
1978; Leistner and Rodel 1981; Silverman and others 1983;

Changes in aw and percent moisture in country cured
hams during the present 120 d challenge study are shown in
Figure 1. There was no significant difference (p < 0.05) be-
tween the aw of hams cured with nitrate and nitrite and salt
and sugar cured hams. The hams rapidly lost moisture dur-
ing the dry salting phase (0 to 49 d). The aw closely paral-
leled the moisture loss from the hams, with a mean aw of
0.923 and 0.908 and percent moisture of 57.96 and 54.12 at d
69 after dry aging and after 120 d, respectively. The data
closely parallels the findings of Huerta and others (1988)
showing 61.39% moisture internally after 125 d and a water
activity of 0.909 in dry salted Spanish ham. In that study, the
salt content of the Spanish hams after 125 d was 5.51% inter-
ally, which was lower than the salt content after 120 d in the
present study (8%).

The effect of pH on the growth of microorganisms in dry
sausage is well-documented (Messier and others 1989; Nissen
and Holck 1998). However, the changes in pH of country ham are more subtle than those found in dry fermented sau-
Figure 1—Changes in the average water activity (aw), percent moisture, and percent sodium chloride during processing of dry cured hams which were processed using a cure mix either with or without sodium nitrite and sodium nitrate. Cure Mix 1: 3.63 kg salt, 454 g sugar, 14.2 g so-
dium nitrite, and 56.7 g sodium nitrate. Cure Mix 2: 3.63 kg salt and 454 g sugar. (n = 3)
genes surviving as the curing process progressed through d 61 (Table 3). Listeria populations on both the uninoculated control and inoculated hams decreased to the direct plating detection level or below by d 120 of the process. In the case of the Listeria inoculated hams, this was at least a 4.8 log reduction in the populations. For one sampling location on d 120, for the inoculated hams without nitrite, presumptive Listeria was noted. However, Listeria identification by biochemical testing was not confirmed. When samples with direct Listeria counts below the plating detection level were analyzed after selective enrichment, all were negative for Listeria.

Nissen and Holck (1998) found that S. Kentucky, E. coli O157:H7, and L. monocytogenes populations decreased in fermented dry sausage during fermentation and maturation. After 5.5 mo of storage at 4 °C there was approximately a 90% reduction of the original populations of E. coli O157:H7, and L. monocytogenes, while S. Kentucky populations were reduced to below detectable limits. However, after 5.5 mo of storage at 20 °C all pathogen populations decreased below the detection limit. This suggests that a higher storage temperature may enhance pathogen inactivation. The results of the present study show L. monocytogenes populations declined after the hams completed the dry-aging process (d 69) and continued to decline to undetectable levels by 120 d (Table 3). This data is in agreement with Nissen and Holck (1998), as no additional growth of L. monocytogenes was noted after the hams reached an aw of 0.929, and populations continued to decline to undetectable levels during storage at 20 °C.

L. monocytogenes has been shown to survive in low aw

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**Table 1—Mean Salmonella population values (log CFU/cm²) on dry cured hams during processing*.** Hams were either inoculated with Salmonella or left uninoculated at initiation of process.

<table>
<thead>
<tr>
<th>Day</th>
<th>Uninoculated with Cure Mix 1b</th>
<th>Uninoculated with Cure Mix 2</th>
<th>Inoculated with Cure Mix 1</th>
<th>Inoculated with Cure Mix 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.03 ± 1.17</td>
<td>3.18 ± 1.31</td>
<td>7.28 ± 1.28</td>
<td>7.31 ± 1.32</td>
</tr>
<tr>
<td>61</td>
<td>&lt; 1.80 ± 0.00</td>
<td>&lt; 1.80 ± 0.00</td>
<td>2.00 ± 0.92</td>
<td>1.96 ± 0.55</td>
</tr>
<tr>
<td>69</td>
<td>1.80 ± 0.34 d,e,f</td>
<td>1.80 ± 0.34 d,e,f</td>
<td>1.80 ± 0.33 d,f</td>
<td>1.80 ± 0.33 g,f</td>
</tr>
<tr>
<td>90</td>
<td>&lt; 1.57 ± 0.00</td>
<td>&lt; 1.57 ± 0.00</td>
<td>&lt; 1.57 ± 0.00</td>
<td>&lt; 1.57 ± 0.00</td>
</tr>
<tr>
<td>120</td>
<td>&lt; 1.57 ± 0.00</td>
<td>&lt; 1.57 ± 0.00</td>
<td>&lt; 1.57 ± 0.00</td>
<td>&lt; 1.57 ± 0.00</td>
</tr>
</tbody>
</table>

*Since there was no significant difference (p < 0.05) in ham sample types (cushion, butt, and hock), the values are the means of 3 replications of the 3 sample locations. Typical colonies forming on bismuth sulfite agar were counted as presumptive Salmonella.

b Cure Mix 1: 3.63 kg salt, 454 g sugar, 14.2 g sodium nitrite, and 56.7 g sodium nitrate. Cure Mix 2: 3.63 kg salt and 454 g sugar.

c One butt sample was Salmonella positive after selective enrichment.

d One hock sample was E. coli O157:H7 positive after selective enrichment.

e One cushion sample was E. coli O157:H7 positive after selective enrichment.

**Table 2—Mean Escherichia coli O157:H7 population values (log CFU/cm²) on dry cured hams during processing*.** Hams were either inoculated with E. coli O157:H7 or left uninoculated at initiation of process.

<table>
<thead>
<tr>
<th>Day</th>
<th>Uninoculated with Cure Mix 1b</th>
<th>Uninoculated with Cure Mix 2</th>
<th>Inoculated with Cure Mix 1</th>
<th>Inoculated with Cure Mix 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.71 ± 1.20</td>
<td>3.84 ± 1.24</td>
<td>7.13 ± 0.87</td>
<td>7.72 ± 1.40</td>
</tr>
<tr>
<td>49</td>
<td>2.81 ± 1.14</td>
<td>2.28 ± 0.92</td>
<td>5.50 ± 0.77</td>
<td>6.07 ± 1.07</td>
</tr>
<tr>
<td>61</td>
<td>2.13 ± 0.84</td>
<td>1.80 ± 0.00</td>
<td>2.13 ± 0.82</td>
<td>1.89 ± 0.47</td>
</tr>
<tr>
<td>69</td>
<td>1.94 ± 0.68</td>
<td>1.91 ± 0.59</td>
<td>1.80 ± 0.00</td>
<td>1.85 ± 0.45</td>
</tr>
<tr>
<td>90</td>
<td>1.73 ± 0.00</td>
<td>1.57 ± 0.00</td>
<td>&lt; 1.57 ± 0.00</td>
<td>&lt; 1.57 ± 0.00</td>
</tr>
<tr>
<td>120</td>
<td>&lt; 1.57 ± 0.00</td>
<td>&lt; 1.57 ± 0.00</td>
<td>&lt; 1.57 ± 0.00</td>
<td>&lt; 1.57 ± 0.00</td>
</tr>
</tbody>
</table>

*Since there was no significant difference (p < 0.05) in ham sample types (cushion, butt, and hock), the values are the means of 3 replications of the 3 sample locations. Typical colonies forming on sorbitol MacConkey agar were counted as presumptive E. coli O157:H7.

b Cure Mix 1: 3.63 kg salt, 454 g sugar, 14.2 g sodium nitrite, and 56.7 g sodium nitrate. Cure Mix 2: 3.63 kg salt and 454 g sugar.

c One butt sample was E. coli O157:H7 positive after selective enrichment.

d One hock sample was E. coli O157:H7 positive after selective enrichment.

e One cushion sample was Salmonella positive after selective enrichment.

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**Figure 2—Changes in the average pH in dry cured hams which were processed using a cure mix either with or without sodium nitrite and sodium nitrate.** Cure Mix 1: 3.63 kg salt, 454 g sugar, 14.2 g sodium nitrite, and 56.7 g sodium nitrate. Cure Mix 2: 3.63 kg salt and 454 g sugar.
products (Rainaldi and others 1991). Like most bacterial species associated with food, *L. monocytogenes* grows optimally at an aw of approximately \( \geq 0.97 \), however, this pathogen has the ability to multiply at aw values as low as 0.92 (Petran and Zottola 1989). Skovgaard (1987) estimated the minimum aw that *L. monocytogenes* can tolerate to be approximately 0.93.

In fermented hard salami containing 5.0 to 7.8% NaCl and 156 ppm sodium nitrite with a pH of 4.3 to 4.5 and an aw between 0.79 and 0.86, Johnson and others (1988) found *L. monocytogenes* populations decreased with time, but they were able to recover the organism after at least 84 d at 4°C. They concluded that the conditions would not allow an increase in the population of the pathogen.

Bacteria of public health concern are not normally associated with dry cured ham. The rapid decline of *Salmonella* spp. and *E. coli* O157:H7 in this study indicates the effectiveness of a low aw in reducing populations of these pathogenic microorganisms.

The population of *S. aureus* organisms on the inoculated hams was reduced by at least a 1.5 log reduction by d 90 (Table 4). There was a subsequent 0.5 log CFU/cm² increase during the last 30 d at ambient storage without humidity control. Huerta and others (1988) also found an increase in the halotolerant bacteria during the salting and drying stage of processing, but did not find any coagulase positive *S. aureus* using similar test methods as used in this study. In a random sampling of 80 presumptive *S. aureus* colonies from hams on d 90 and 120, it was found that 20% of them were coagulase positive. While this was not a complete survey, it is possible that the majority of the staphylococci enumerated during the final stages of processing the hams were coagulase negative. Since staphylococcal populations increased to a greater magnitude on the control hams compared to the inoculated hams, randomly chosen control hams were tested for the presence of staphylococcal enterotoxin. None of the hams tested were positive for enterotoxin.

An extensive study has been made of *S. aureus* growth and toxin production in food products (Genigeorgis and others 1969; Tatini, 1973; Troller and Stinson 1975; Boylan and others 1976; Tatini and others 1976; Lee and others 1981; Notermans and Heuvelman 1983; Halpin-Dohnalek and Marth 1989; Messier and others 1989). In precooked bacon with an aw of 0.99, enterotoxin was produced when stored aerobically at 20°C (Lee and others 1981). In laboratory media at pH 5.2 and at 25°C, Notermans and Heuvelman (1983) reported enterotoxin A, but not B, was produced at an aw of 0.90. Troller and Stinson (1975) found that the production of enterotoxin was not a result of the number of *Staphylococcus* present, but was probably related to the moisture available in the system with enterotoxin production at an aw 0.93, but not at aw, of 0.88. These, and other studies indicate a variety of conditions which are required for *S. aureus* growth and enterotoxin production, including: chemical composition of the food, pH, aw, other bacteria present, composition of the atmosphere, time and temperature of storage, other ingredients, and processing. Despite the attainment of high *S. aureus* populations, enterotoxin production may be inhibited by the independent and interactive effects of temperature, pH, oxygen tension, aw, and competitive growth of other microorganisms (Tatini 1973). The interactions that occur dur-

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**Table 3—Mean *L. monocytogenes* population values (log CFU/cm²) on dry cured hams during processing*. Hams were either inoculated with *L. monocytogenes* or left un inoculated at initiation of process.**

<table>
<thead>
<tr>
<th>Day</th>
<th>Uninoculated with Cure Mix 1</th>
<th>Uninoculated with Cure Mix 2</th>
<th>Inoculated with Cure Mix 1</th>
<th>Inoculated with Cure Mix 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.52 ± 1.33</td>
<td>3.41 ± 1.63</td>
<td>6.40 ± 1.18</td>
<td>6.59 ± 1.39</td>
</tr>
<tr>
<td>49</td>
<td>2.80 ± 1.06</td>
<td>2.03 ± 0.34</td>
<td>4.58 ± 1.61</td>
<td>5.24 ± 1.48</td>
</tr>
<tr>
<td>61</td>
<td>1.83 ± 0.69</td>
<td>2.24 ± 1.04</td>
<td>1.86 ± 0.44</td>
<td>2.14 ± 0.78</td>
</tr>
<tr>
<td>69</td>
<td>&lt; 1.80 ± 0.00</td>
<td>2.36 ± 1.14</td>
<td>2.47 ± 1.29</td>
<td>2.66 ± 1.92</td>
</tr>
<tr>
<td>90</td>
<td>&lt; 1.57 ± 0.00</td>
<td>&lt; 1.57 ± 0.00</td>
<td>1.80 ± 0.53</td>
<td>1.75 ± 0.56</td>
</tr>
<tr>
<td>120</td>
<td>&lt; 1.57 ± 0.00</td>
<td>&lt; 1.57 ± 0.00</td>
<td>&lt; 1.57 ± 0.00</td>
<td>1.57 ± 0.00</td>
</tr>
</tbody>
</table>

*aSince there was no significant difference (p < 0.05) in ham sample types (cushion, butt, and hock), the values are the means of 3 replications of the 3 sample locations. Typical colonies forming on Listeria selective agar, Oxford formulation agar were counted as presumptive *L. monocytogenes*. bCure Mix 1: 3.63 kg salt, 454 g sugar, 14.2 g sodium nitrite, and 56.7 g sodium nitrate. Cure Mix 2: 3.63 kg salt and 454 g sugar. c<: indicates below direct plating detection level.

**Table 4—Mean *Staphylococcus* population values (log CFU/cm²) on dry cured hams during processing*. Hams were either inoculated with *Staphylococcus aureus* or left un inoculated at initiation of process.**

<table>
<thead>
<tr>
<th>Day</th>
<th>Uninoculated with Cure Mix 1</th>
<th>Uninoculated with Cure Mix 2</th>
<th>Inoculated with Cure Mix 1</th>
<th>Inoculated with Cure Mix 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.53 ± 1.20</td>
<td>4.84 ± 0.92</td>
<td>6.55 ± 1.34</td>
<td>6.81 ± 1.95</td>
</tr>
<tr>
<td>49</td>
<td>5.25 ± 0.91</td>
<td>5.55 ± 1.02</td>
<td>7.11 ± 0.76</td>
<td>6.95 ± 0.87</td>
</tr>
<tr>
<td>61</td>
<td>6.31 ± 1.46</td>
<td>5.98 ± 1.74</td>
<td>6.72 ± 1.15</td>
<td>6.42 ± 1.19</td>
</tr>
<tr>
<td>69</td>
<td>4.88 ± 2.16</td>
<td>6.08 ± 1.39</td>
<td>6.21 ± 1.18</td>
<td>6.58 ± 1.39</td>
</tr>
<tr>
<td>90</td>
<td>4.83 ± 2.43</td>
<td>5.41 ± 1.90</td>
<td>4.91 ± 2.62</td>
<td>4.98 ± 2.25</td>
</tr>
<tr>
<td>120</td>
<td>5.14 ± 1.77</td>
<td>5.37 ± 1.61</td>
<td>5.29 ± 1.65</td>
<td>5.52 ± 1.67</td>
</tr>
</tbody>
</table>

*aSince there was no significant difference (p < 0.05) in ham sample types (cushion, butt, and hock), the values are the means of 3 replications of the 3 sample locations. Typical colonies forming on Baird-Parker agar were counted as presumptive *Staphylococcus*. bCure Mix 1: 3.63 kg salt, 454 g sugar, 14.2 g sodium nitrite, and 56.7 g sodium nitrate. Cure Mix 2: 3.63 kg salt and 454 g sugar.
ing curing could be responsible for the lack of staphylococcal enterotoxin production on dry cured hams. In this study, *S. aureus* did not grow during the initial curing stages when water activity and pH conditions were appropriate for its growth since the temperature was at 4.4 °C. Since there was no increase in the population of *Staphylococcus* on the inoculated hams during this time, but actually a decrease, it indicates that by the time the hams were transferred to a more appropriate temperature for the growth of this pathogen, the interactions of the other environmental factors became limiting. The *Staphylococcus* populations on the uninoculated hams did increase by d 61; however, none of the samples tested contained enterotoxin indicating the increase was not sufficient to pose a problem related to enterotoxin production. This is reasonable considering there is no evidence of staphylococcal enterotoxin food poisoning in the general population due to consumption of country ham.

Another factor to consider related to the potential for staphylococcal enterotoxin production concerns the percent brine concentration of a cured product. According to Jay (2000) and Tatini and others (1976), staphylococcal enterotoxin production is inhibited at brine concentrations above 10%, especially when the pH is below 5.45. In addition, it is likely that the surface concentration of salt in dry cured ham is 1 to 3% higher than the mean salt concentration during the equilibration and drying phase (Christian 1982). At d 90, the percent brine concentration of the hams was 10.14% and 10.04% for Cure Mix 1 and Cure Mix 2, respectively. By d 120, the brine concentration was 13.81% and 12.33% for Cure Mix 1 and Cure Mix 2, respectively. Since the percent brine concentration of the hams from d 90 onward was above the 10% level, staphylococcal enterotoxin production on the hams was not an issue of concern.

Large numbers of aerobic microorganisms survived the curing process but with a population reduction of up to 2 log CFU/cm² (Table 5). The largest reduction in aerobic microorganisms occurred when the hams were placed in ambient storage and the surface moisture and temperature were reduced due to equilibration with the ambient dry air (approximately 50% relative humidity).

The method of Ray (1979) was used in an attempt to recover injured cells during the ham curing process. Compared to the direct plating methods used, the recovery methods did not detect any additional numbers of *Salmonella*, *E. coli* O157:H7, *L. monocytogenes*, *S. aureus*, nor total aerobes at any sampling time.

The growth of *Clostridium botulinum* on country cured ham was not addressed in the present study. However, it is well documented in the literature (Johnston and others 1969; Tompkin 1976) that either the use of sodium nitrite (50 ppm) or an aw of 0.92 is sufficient to ensure that *C. botulinum* will not grow. These values were obtained in this study. The combined interactions of dry curing and aging also provide assurance against the survival of *Trichinella spiralis* (Pinedo and others 1987; Smith and others 1989; Lin and others 1990). The higher temperature during dry aging ensures lethality for *Trichinella*, but also may be effective in reducing the residual levels of other microorganisms such as *L. monocytogenes* (Nissen and Holck 1998).

In this study, the mean log reduction of populations for *Salmonella* spp., *E. coli* O157:H7, *L. monocytogenes*, and *S. aureus* after the 69 d curing process, were 5.5, 5.5, 4.0, and 0.3 log, respectively. Analysis of data showed no significant (p < 0.05) difference in the reduction of microbial populations between ham sampling locations. At d 120, the mean log reduction for *Salmonella* spp. and *L. monocytogenes* was 5.7 and 4.8, respectively, with neither detected when the appropriate enrichment media was used. For *E. coli* O157:H7, 1 hock and 1 cushion were positive after selective enrichment at d 120. However, the mean log reduction for this microorganism was greater than 5 logs. The aw of the hams was below 0.91 by d 120. The interaction of this reduced aw, salt concentration (8%), pH (5.5), and ham storage temperature (20 °C) was sufficient to prevent or limit proliferation of staphylococci. Of the samples tested for staphylococcal enterotoxin, none were found to be positive.

### Table 5—Mean aerobic plate count population values (log CFU/cm²) on dry cured hams during processing a

<table>
<thead>
<tr>
<th>Day</th>
<th>Uninoculated with Cure Mix 1</th>
<th>Uninoculated with Cure Mix 2</th>
<th>Inoculated with Cure Mix 1</th>
<th>Inoculated with Cure Mix 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>49</td>
<td>61</td>
<td>69</td>
</tr>
<tr>
<td>Uninoculated with Cure Mix 1 a</td>
<td>6.26 ± 0.50</td>
<td>6.72 ± 1.20</td>
<td>7.00 ± 1.40</td>
<td>5.48 ± 1.93</td>
</tr>
<tr>
<td>Uninoculated with Cure Mix 2</td>
<td>5.74 ± 0.98</td>
<td>7.16 ± 1.52</td>
<td>6.73 ± 1.48</td>
<td>6.21 ± 1.36</td>
</tr>
<tr>
<td>Inoculated with Cure Mix 1</td>
<td>7.35 ± 1.29</td>
<td>7.76 ± 0.83</td>
<td>6.57 ± 1.31</td>
<td>6.69 ± 1.44</td>
</tr>
<tr>
<td>Inoculated with Cure Mix 2</td>
<td>7.45 ± 1.07</td>
<td>7.83 ± 0.71</td>
<td>7.07 ± 1.16</td>
<td>7.04 ± 1.04</td>
</tr>
</tbody>
</table>

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**Table 5—Mean aerobic plate count population values (log CFU/cm²) on dry cured hams during processing**. Hams were either inoculated with *Salmonella*, *E. coli* O157:H7, *Listeria*, and *Staphylococcus* or left uninoculated at initiation of process.

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