RESEARCH REPORT
PORK SAFETY

Title: Evaluation of the microbial safety and shelf stability of country-cured hams
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ABSTRACT

One hundred sixteen fresh hams each were surface inoculated with a cocktail of either *Listeria monocytogenes*, *Salmonella* spp. (*Salmonella typhimurium, Salmonella enteritidis* and *Salmonella choleraesuis*), *Escherichia coli* O157:H7 or *Staphylococcus aureus*. Hams were Country-cured and analyzed for microbial populations before processing, following inoculation and curing, equalization, smoking or non-smoking and after each 28 days during the six months of aging. The *L. monocytogenes* populations decreased form 7.56 LOG_{10} CFU/cm^2 to below levels of detection after 206 days. *Salmonella* populations decreased from 7.06 LOG_{10} CFU/cm^2 to below levels of detection after 122 days and *E. coli* O157:H7 populations decreased from 8.6 LOG_{10} CFU/cm^2 to non-detectable levels after smoking (67days). There were no significant differences in population reduction between smoked versus non-smoked groups except for *L. monocytogenes* ($P<0.05$) with smoked hams having lower populations at all sample times except after equalization. *S. aureus* populations of 8.57 LOG_{10} CFU/cm^2 decreased 2 logs after curing and were below detection after four months of aging. These data provide strong support that current methods for the production of Country-Cured hams are adequate to produce a safe product. However, further research is needed to determine if additional measures beyond those required by USDA regulations are necessary to further enhance assurance of the safety of Country-Cured Hams.

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Introduction

"Country-cured" or "Country-style" are higher quality, dry cured American hams. The majority of them come from Kentucky, Tennessee, North Carolina, Missouri and Virginia. A wide selection of specially cured hams are also imported from many European countries such as Prosciutto from Italy, Spain's Serrano, German's Westphalian, France's Bayone, and the Yorkshire, Suffolk, Cumberland, Bradenham and Belfast from the United Kingdom.

Pork and pork products are extremely perishable, as are other related meat. Postmortem changes associated with conversion of muscle to meat and subsequent storage and handling are accompanied by some deterioration. Microorganism such as bacteria, yeast and molds cause these changes. However, not all microbial actions are detrimental. Rather than curing, and aging alone, processes in Country-cured hams depend on microorganisms and together with curing ingredients are required to develop the desired flavor, color, texture of a "shelf stable" and "ready to eat" product.

On the other hand, we can not take for granted that food and food practices that have been traditionally safe will remain that way in the future. Therefore, Country-cured ham processors have a tremendous responsibility to ensure the safety of the products they produce. This, combined with the USDA mandated 5-log reduction in microbial pathogens on ready to eat products and consumer concerns has lead to the need to validate the safety of this unique product. In order to assist Country-cured ham producers to produce a safe product, this study was designed to validate a six-log reduction of *Listeria monocytogenes*, *Salmonella* species (*Salmonella enteritidis*, *Salmonella choleraesuis* and *Salmonella typhimurium*), *Staphylococcus aureus* and *Escherichia coli* O157:H7 by use of USDA recommended procedures.

Objective:

Validate a six-log reduction of *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus* and *Escherichia coli* O157:H7 while correlating criteria such as pH, a_w, MPR, and NaCl to determine shelf stability over six months.

Procedures:

For *L. monocytogenes*, swab rinse procedures with UV enrichment *listeria* broth (Difco) was used to take samples from the top and one side of the hams. A series of decimal dilutions with phosphate buffer were plated on modified oxford listeria Agar (MOX)(Difco) and spread with a sterile bent rod. Plates were incubated at 35^{o}C for 48h. Brown colonies surrounded by black precipitate were counted as *L. monocytogenes*. After incubation, 0.1 ml of enrichment broth was transferred to fraser broth, and then streaked onto MOX. Both plates and broths were incubated at 35^{o}C for 24h. Brown colonies surrounded by black precipitate on MOX as well as black color development after fraser supplemented broth was considered positive for *L. monocytogenes*. After 234 days of aging, enrichment procedure was used to determine the presence of *L. monocytogenes* on control hams and on inoculated hams. Fifty grams samples were stomached in 450 ml

UVM modified *listeria* enrichment broth and incubated at 35°C for 24h. Following incubation 0.1 ml of the pre-enrichment was transferred to fraser broth, incubated at 35°C for 24h and 48h, then streaked onto MOX *listeria* agar with a cotton swab. Plates were incubated at 35°C for 48h. Brown colonies surrounded by black precipitates were considered *Listeria monocytogenes.*

For *Salmonella*, swab rinse procedure with buffered peptone water was used to obtain samples . A series of decimal dilutions were made with phosphate buffer and plated on xylose lysine desoxycholate agar (XLD)(Difco), incubated at 35°C for 24h. The day after 0.1 ml of pre-enrichment was inoculated into selenite cystine (SC)(Difco) and tetrathionate (TT)(Difco) broth at 35°C for 24h. Then, samples were streaked on XLD and incubated at the same conditions explained previously. Red colonies with black centers on XLD either after sampling or after enrichment were counted as *Salmonella* H_2S positive. After the 4th month of aging throughout the 234 days, just the enrichment procedure was used to determine the presence of possible *Salmonella* on inoculated ham. Control hams were also tested at the end of the aging period. Twenty-five gram samples were stomached in 225 ml-lactose broth and incubated at 35°C for 24h. Then 0.1 ml was transferred to tetrathionate and selenite cystine broth and incubated at 35°C for 24h. Each enrichment broth was streaked onto bismuth sulfite agar, xylose lysine desoxycholate agar and Hekton enteric agar. Plates were incubated at 35°C for 24h. Positive growth would be confirmed using biochemical tests.

For *S. aureus*, swab rinse procedure with phosphate buffer was used to take the surface samples. After a series of decimal dilutions in the same buffer, samples were plated on Baird-Parker agar, at 35°C for 24h. Black colonies with an opaque halo and a outer halo were counted as *S. aureus* colonies. A 25 g sample per ham was taken and frozen for further toxin evaluation. Enrichment procedure was followed after plating, using 25 g of sample stomached with 225 ml of tryptic soy broth(TSB)(Difco) with 10% sodium chloride and 1% of sodium pyruvate. Samples were incubated at 35°C for 24h. After incubation, samples were streaked on Baird-Parker agar and incubated at 35°C for 24h. Shiny black colonies with opaque halos were coagulase tested to confirm *S. aureus*.

Typical *S. aureus* colonies were inoculated into 0.5 ml of coagulase rabbit plasma with EDTA and incubated at 35°C for 24h. The tubes in which the clot formed within 24h were considered to be coagulase positive *S. aureus*; while those, which did not form a clot, were considered coagulase negative *Staphylococci.*

For *E. coli* O157:H7, swab rinse procedure with buffered peptone water was used to obtain the samples from the hams. A series of decimal dilutions with phosphate buffer were plated on MacConkey sorbitol agar (MSA) (Difco)and incubated at 35°C for 24h. Pale sorbitol-negative colonies were counted as *E. coli* O157:H7. After the first month of aging, enrichment procedure was used to determine the presence of *E. coli* O157:H7. Twenty-five gram samples were stomached in 225 ml EHEC enrichment broth (Difco) and incubated with shaking at 37°C for up to 24h. After incubation, 0.1 ml was spread on the surface of pre-poured MSA and a loopful streaked onto another plate. Plates were incubated at 35°C for 24h. Pale-gray colonies with smoky centers were considered as *E. coli* O157:H7. Samples for chemical analysis from un-inoculated hams were frozen for further analysis.

Chemical Analysis:

Slices of un-inoculated ham from salt and salt+NO_2 treatment were ground using a Kitchen Aid (KSM90WH, Inc. St. Joseph, Michigan, USA). The ham was divided into well-labeled plastic bags for protein, nitrite, moisture, salt, pH, and a_w analysis. Samples were stored at -74^oC in a CRYOSTAR freezer (Harris, Inc. Asheville, NC, USA) for further analysis. Samples were thawed before analysis.

Approximately 5 g of sample was placed on an aluminum pan and placed into a Programmable Lab Oven at 100-102^oC for 24h. The sample was cooled in a desiccator and weighed. Then percentage of weight loss was calculated and was described as % moisture content.

Formula: %MC = ((Tin + wet sample) - (Tin + dry sample) / ((Tin + wet sample) - (Tin weight) X 100

Two 5 g sample from control un-inoculated ham were placed into a 80 ml beaker and homogenized with 45 ml of distilled water using a Polytron homogenizer (PT10/35, Switzerland Kinematic, AG) until no lumps were observed. The ph of the homogenate was measured using a petentiometric method. (Accumet Basic AB15 pH meter with silver/silver chloride reference, Fisher Scientific).

A 100 g sample of frozen ground ham was freeze dried using a freeze dryer (Model 41 Sub Special, PePP. Division of the Virtis Co. Gardiner, New York) from Agricultural Engineering during 3 days at 150oC. Then the dry sample was stored into well-labeled plastic bags at room temperature. Protein and moisture contents were used to calculate a moisture protein ratio (MPR).

Forty-eight samples, distributed into 5 g aliquots were sent the Q Laboratories (Cincinnati, OH) for salt analysis following AOAC (1995) 935.47 method.

Ground ham samples were poured into plastic cups (40mm) in duplicate, and then a_w measurements was done using a Benchtop System-Aw meter. (Rotonic Inc., Huntington, NY).

Results:

Chemical analysis (Water activity, Salt content, Moisture / Protein Ratio, and pH) for each group of hams is shown in Table 1. These data were collected from two control hams during each step during processing and values averaged across curing times.

Listeria inoculated hams averaged 30 % weight loss of their fresh weight at the end of the aging period . The *L. monocytogenes* inoculated ham populations were 7.64 $LOG_{10}CFU/cm^2$ initially and decreased to 1.37 LOG_{10} CFU/cm^2 after 150 days. At each sample time smoked hams had lower populations than non-smoked hams indicating some bactericidal effects of smoking ($P<0.01$) (Table 2).

Salmonella inoculated hams had an average weight of 25 % of their fresh weight at the end of the aging period. *Salmonella* populations decreased more rapidly than *Listeria* hams from an initial population of 7.11 LOG_{10} CFU/cm^2 to 0.48 LOG_{10} CFU/cm^2 after 66days to below levels of detection after 122 days (Table 2).

Mean of *S. aureus* populations were 8.35 LOG_{10} CFU/cm^2 and decreased 2 logs after the curing process to 1.25 LOG_{10} CFU/cm^2 following 94 days and were undetectable

after 150 days.

E. coli O157:H7 inoculated hams averaged 25 % loss of their fresh weight at the end of the aging process . Populations decreased from 8.44 LOG_{10} CFU/cm^2 to below detection levels after smoking (Table 2).

Mean water activity value was 0.92 across all hams with a salt level of 4.32 %.

Conclusions:

The following general conclusions can be drawn from the data generated in the study.

*Water activity and MPR significantly decreased over aging time as expected with microbial populations deceasing as well.

* Country-cured ham processing procedures were effective in producing 6 log reductions of *E. coli* O157:H7, *Salmonella* spp., *Staphylococcus aureus*, and *Listeria monocytogenes*.

Table 1. Result of Chemical Analysis for Country-Cure Hams

Ham Groups	**a_w[y]**	**NaCl (%)[y]**	**MPR[y]**	**pH[y]**
Listeria	0.94±0.01	4.12±1.10	0.94±0.22	6.26±0.65
Salmonella	0.91±0.02	4.35±0.64	0.90±0.12	5.93±0.19
S. aureus	0.93±0.01	3.91±1.51	0.98±0.19	6.20±0.39
E. coli O157:H7	0.91±0.01	4.90±1.14	0.82±0.17	6.06±0.83

[y] Mean of 6 samples

Table 2. Mean Pathogen Population on Country-Cured Hams

	Processing Time (Days)								
	0	45	66	94	122	150	178	206	234
Inoculated Groups	**LOG_{10} CFU/cm^2**								
L. monocytogenes Non-smoked	7.64	8.61	5.34	2.01	3.10	2.21	0.91	0.38	0
L. monocytogenes Smoked			4.04	3.40	0.91	0.52	0.33	0.17	0
Salmonella spp. Non-smoked	7.11	3.94	0.45	0	0	0	0	0	0
Salmonella spp. Smoked			0.51	0.59	0	0	0	0	0
S. aureus Non-smoked	8.35	6.24	4.85	1.58	0.4	0.6	0	0	0
S. aureus Smoked			0.77	0.91	0.38	1.75	0	0	0
E. coli O157:H7 Non-smoked	8.44	4.87	0.85	0	0	0	0	0	0
E. coli O157:H7 Smoked			0	0	0	0	0	0	0