**PE6.10 Validation of the Manufacturing Process of Italian Dry-Cured Ham (Prosciutto) for the Inactivation of Listeria Monocytogenes and Salmonella spp. 361.00**

*Silvana Barbuti (1) silvana.barbuti@ssica.it, Maria Silvia Grisenti( 1), Maria Angela Frustoli (1), Giovanni Parolari (1)*
*(1)S.S.I.C.A.*

**Abstract— In order to validate the manufacturing process of Italian typical dry-cured ham, two independent microbial challenge tests (MCT) were performed. The hams were obtained from heavy pigs then processed according to guidelines for Italian typical dried hams. A mixture containing Listeria monocytogenes and Salmonella spp. were inoculated (5-6 log CFU g-1) onto the surface (N. 3 leg areas) of either raw hams (MCT1) or hams at mid ripening time (MCT2) and microbiological analyses were made at several stages of processing. Results from both MCTs show a 4-log reduction for both pathogens over a time span of 4 months (MCT1) and 6 months (MCT2), respectively. The log reduction did not differ (P>0.05) between the three inoculation areas, whose aw values were always <0.92 at the last sampling time. It is concluded that processing hams according to given guidelines is effective to achieve pathogen-free Italian-type dried hams.**

S. Barbuti is with Stazione Sperimentale Industria Conserve Alimentari, 43121 Parma, ITALY. (corresponding author to provide phone: +39-0521 795267; fax: +39 0521 795218; e-mail: silvana.barbuti@ssica.it).

M.S. Grisenti is with Stazione Sperimentale Industria Conserve Alimentari,(e-mail: silvia.grisenti@ssica.it).

M.A. Frustoli is with Stazione Sperimentale Industria Conserve Alimentari,(e-mail: angela.frustoli@ssica.it)

G. Parolari is with Stazione Sperimentale Industria Conserve Alimentari,(e-mail: giovanni.parolari@ssica.it).

Index Terms— Processing validation, Italian dry cured ham, Listeria, Salmonella, microbial challenge test.

## I. INTRODUCTION

ITALIAN typical dry-cured hams have been traditionally manufactured according to given procedures, such as those established by the Parma Ham Consortium Regulation (DPR, 1978). Basic manufacturing steps include salting, resting, washing, drying, maturing and ageing [3], with the first two stages being accomplished under refrigeration (0-4°C), while the subsequent phases require greater temperatures (15-20°C). Because of the long curing time (>12 months since salting) and consequent reduction of aw values, the finished hams are generally regarded as ready-to-eat safe meat products posing no microbial foodborne risk. Apart from investigations performed on other types of hams [4, 5], no systematic work has been done on Italian dried hams to demonstrate the ability of the manufacturing process to inactivate such major pathogens as Salmonella and Listeria monocytogens which are potential contaminants of raw meat or at successive phases of processing. Sea salt, the only additive allowed in manufacturing, is added to the legs by a salting machine and partly manually by sprinkling the salt onto specified leg portions such as the aitch and the femur head bone. Moreover, at six months of processing (end of maturing), the ham surface is manually covered with a spreadable mince made up of ground pork fat and salt (ham fattening) in order to prevent excessive surface drying. Being manually performed, these two steps (salting and fattening) may be regarded as potential sources of microbial contamination, in addition to the contamination of the meat itself. Accordingly, a comprehensive microbial challenge test of traditionally-made dried hams should take into account two independent stages of manufacturing such as salting and fattening, with the former reflecting contamination from meat + salt, and the latter from fat application. Therefore we designed two independent MCTs aimed at validating the manufacturing process of Italian typical dry cured hams as possibly affected by microbial contamination at the salting and fattening steps respectively.

## II. MATERIALS AND METHODS

A. Processing parameters Two MCTs were performed, with the hams prepared or processed as described below. 1st MCT. N. 62 pork hind legs from a local slaughterhouse were selected and manufactured in compliance with requirements

stated by the Parma Ham Consortium Regulation (DPR, 1978). Briefly, the hams were dry cured with no other additives but sea salt then kept cold (salting and resting phase) until 90 days, when they were washed and air-dried and the temperature was raised to 15-18° (maturing phase). 2nd MCT. N. 12 hams were purchased from a Parma ham manufacturer at six months of ageing or at mid-maturing stage. They were treated with ground fat (fattening) then further matured for six months at 18-20°C (ageing) until the end of the process.

B. Microorganisms for inoculation The microorganisms used in this study were L.monocytogenes (Scott A, ATCC 7644, PE 425 (4ab), PE 429 (1/2b) e PE 10977 1/2a), Salmonella (Typhimurium PE 11001, Typhimurium PE 10999, Typhimurium IS 7080, Derby IS 7265, Typhimurium ATCC 14028). Microorganisms were transferred twice in brain hearth infusion (BHI) broth (OXOID) prior to preparing the inoculating cocktails. Five-strain inoculating cocktails were prepared for each species by growing each strain individually in the BHI broth. The 18h cultures were combined in plastic centrifuge tubes and centrifuged at 5,000 x g at 4°C for 20 min. The supernatant in the tubes was decanted and the pellets were resuspended in equivalent amounts of 0.1% peptone water. The resulting cocktails were serially diluted and plated to determine cell concentration. Equal volumes of individual suspensions were mixed to achieve the final five-strain cocktails for ham inoculation.

C. Ham inoculation 1st MCT. Raw hams, except the controls, were open-surface inoculated with 200 ìl of the pooled microorganism suspensions, using a micropipettor. The micropipettor was also used to inoculate the hock by dispensing the inoculum between the tibia bone and the skin of the exposed cut. At this site only Listeria cocktail was inoculated because a large inoculum of Salmonella was likely to cause deep putrefactive spoilage of hams, which was outside the scope of this investigation. Levels of about 105 cfu g-1 for each species were used to account for an appropriate reduction in the challenge organisms. 2nd MCT. Twelve hams were obtained from a processor at six monthes of age; eight of them were inoculated just before fattening using the same procedure as for fresh hams.

D. Microbial sampling and analysis All hams were sampled at two sites (A and B, Figure 1) of the open surface by a 10x10 mask, and at the knock level (C); all the samples were taken by excision. Enumeration of surviving pathogen bacteria was done by direct plating method after appropriate dilution using ALOA (Biolife) for Listeria and Rambach agar (Biolife) for Salmonella, respectively. All plates were inoculated at 37°C for 24-48h before typical colonies forming units were enumerated.

E. Chemical and Physical measurements Water activity (aw) (Novasina Aw-Center) and pH (electrode direct insertion) were determined according to standard procedures. Salt at the end of the salting phase was measured by standard AOAC method as total NaCl absorbed into the ham. Weight loss of hams were obtained after the salting, resting and drying stages respectively.

F. Statistical analysis All microbial data were converted to log10 cfu g-1 and submitted to analysis of variance (one-way ANOVA, SPSS 13.0). As the initial sample dilution was 1:3, the minimum detectable count (detection limit) in this study was 3 cfu g-1. Because final counts for Listeria and Salmonella were very low and/or below detection limit, a value corresponding to half detn. limit (1.5 cfu g-1 or 0.18 log cfu g-1) was used for statistical analysis when no population could be detected [2].

## III. RESULTS AND DISCUSSION

Preliminary microbial data revealed no statistical difference ($p>0.05$) between the testing areas (cushion, knuckle, hock); therefore the values were reported as means of the 3 sample locations. Two replicates were made for testing of Salmonella and Listeria; and non-inoculated hams were used for chemical and physical analysis. Typical colonies on Aloa agar were counted as presumptive Listeria; typical colonies on Rambach agar were counted as presumptive Salmonella strains. 1st MCT Data for both pathogens showed no growth at any of the sampling times (tables 1-2). Rather, there was a similar, strong decrease in their number, resulting (at 108 days or end of drying) in 4.48 and 4.06 log reductions (n.D) for L. monocytogenes and Salmonella, respectively.

However the two pathogens exhibited differing behaviour, with Listeria undergoing a steady linear

decrease until 108 days, whereas Salmonella dropped after 40 days to values that remained unchanged at successive sampling phases. The weight losses measured at each sampling time were in agreement with values typically reported for Italian dried hams [3], with a tendency to upper range losses for hams at the end of resting (weight loss=18.2%) which could be related to relatively low weight of green legs. Water activity values (surface measurements) denote a regular salt effect at the end of the salting stage, and a normal dehydration at subsequent times (half resting and end of resting respectively).

Finally, data from salt analysis (average value=5.1, N=3) were in accordance with reference values for Italian typical dried hams, whose regulatory range is set between 4.5 - 6.7. Further comments on pathogen inactivation patterns Listeria – This pathogen decreased by 1.33 log units over the time (42 days) needed for the accomplishment of the salting and half-resting stages (Table 1). The number of surviving cells was 2.35 log at the end of the resting or cold phase, which means a 3.75 log decrease after inoculation. It is noteworthy that in 14 out of 24 samples assayed at the end of resting there was a detectable number of surviving Listeria; however, ten of these samples were below the plating detn limit. The number of positive units was further reduced during the drying phase, with a number of five positive samples out of the ten examined. Salmonella

– Data in Table 2 show that Salmonella was inactivated in a non-linear fashion, suggesting that the first-order kinetic model commonly used for describing microbial survival data [1] may be unsuitable to describe the behaviour of Salmonella in dried hams. Unlike Listeria, this pathogen was reduced rather quickly during the first part of the cold stage, with 2.28 log cells surviving (of the 6.23 inoculated), corresponding to 4 log reduction after 42 days. 2ndMCT This test was based on hams purchased at six months of age, then inoculated at our laboratory and allowed to mature for 6 more months until the accomplishment of 1 year since salting (finished hams).

Results (Table 3) show a strong inactivation of both pathogens, with Listeria and Salmonella decreased respectively by 4.63 and 4.77 log over the time elapsed after inoculation. These hams, analyzed for some major physical properties, exhibited regular surface aw values (Table 4) as well as normal pH data, with values comprised in a rather narrow range (5.7-5.8). Also, their salt content at the end of the process (finished hams) matched the requirements established for Italian typical dried hams. It is to be noted that aw values increased after the addition of the fat mince, reaching final values of 0.91-0.92, which is below the threshold values for the growth of both pathogens. aw values were obtained by using salt in such an amount that finished hams were in the lower range of values enabled for Italian typical hams (NaCl>4.5%). Because hams in current practice achieve an average final value of 5.5-6.0 (salt in the inner muscle), it can be concluded that pathogens, when present on the ham surface, are likely affected by salt contents (and inversely, by aw values) greater or equal to those tested in this work.

## V. CONCLUSION

Results demonstrate that, even with an extremely large contamination of around 106 pathogens per gram of fresh meat (green legs), a regularly conducted manufacturing process can lead to as many as 5 log reductions within less than 3 months of processing (end of the drying phase). Such results were achieved with both the pathogens which are likely to contaminate raw pork meat, i.e. Listeria and Salmonella. A similar outcome was obtained when mid-matured hams, instead of raw legs, were used to mimic potential ham contamination from the fat mince used to cover the lean surface. Results from this latter study showed total inactivation of both pathogens at 400 days of processing, with final values below the detn limit for these bacteria.

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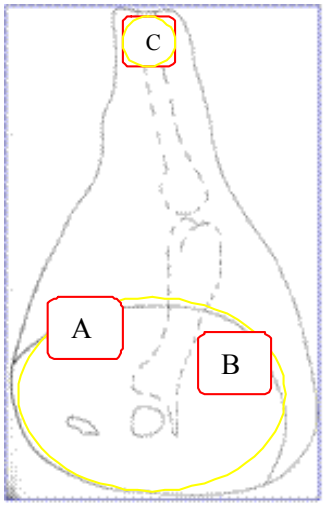
Figure 1 – Inoculation areas (circled) of hams and the three sampling areas: A-cushion, B-knuckle, and C-shank.



Tab. 1 – Fate of *L. monocytogenes* during the 1st MCT

| Processing steps | n. hams/n. samples | *Listeria monocytogenes* | | |
|---|---|---|---|---|
| | | mean log cfu/g (n. positive samples) | St.dev. | n. D |
| Fresh hams | 3/9 | 6.10 (9) | 0.26 | |
| End of salting | 4/12 | 5.61 (12) | 0.28 | 0.49 |
| Half resting | 6/12 | 4.77 (12) | 0.85 | 1.33 |
| End of resting | 8/24 | 2.35 (14) | 0.67 | 3.75 |
| End of drying | 10/10 | 1.62 (5) | 0.54 | 4.48 |

Tab. 2 – Fate of *Salmonella* during the 1st MCT

| Processing steps | n. hams/n. samples | *Salmonella* | | |
|---|---|---|---|---|
| | | mean log cfu/g (n. positive samples) | St.dev. | n. D |
| Fresh hams | 3/6 | 6.23 (6) | 0.23 | |
| End of salting | 4/8 | 5.04 (8) | 0.30 | 1.19 |
| Half resting | 6/6 | 2.22 (6) | 0.48 | 4.01 |
| End of resting | 8/16 | 2.28 (15) | 0.70 | 3.95 |
| End of drying | 10/10 | 2.17 (9) | 0.31 | 4.06 |

Tab. 3- Fate of *L. monocytogenes* and *Salmonella* during the 2nd MCT

| Processing steps | n. hams | *Listeria monocytogenes* | | | *Salmonella* | | |
|---|---|---|---|---|---|---|---|
| | | Average log cfu/g | Dev. st | n. D | Average log cfu/g | St.dev. | n. D |
| Fattening | 8 | 5.63 | 0,37 | | 5.77 | 0.20 | |
| End of seasoning | 8 | < 1 | | > 4.63 | < 1 | | > 4.77 |

Tab. 4 – Physical and chemical analysis of hams in the 2nd MCT

| Processing step | Average weight (kg) | Weight loss (per-cent) | Surface $a_w$ | pH | NaCl (grams/100 g) |
|---|---|---|---|---|---|
| Fattening | 8.1 | | 0.86 - 0.85 | 5.7 | 4.6 - 3.9 |
| End of seasoning | 7.9 | 2,5 | 0.92 - 0.91 | 5.8 | 5.0 - 4.6 |