Introduction

Coppa is a typical Italian cured pork meat product obtained from the cervical muscles of the neck of heavy pigs. The traditional areas of production are the provinces of Parma and Piacenza (Emilia Romagna region, Northern Italy); however, it is produced with different recipes in many other Italian regions. Few data exist in literature on the characteristics and on product processing and the most relevant information can be found in the PDO specifications (http://www.salumidoppiacentini.com/coppa-d-p/index.jsp?IdC=160&IdS=168&tipo_cliccato=0&tipo_padr=0&nav=1&css=generico_dop.css&menu=1; http://www.coppadiparmaigp.com/disciplina-re-di-produzione-igp-coppa-parma/) or in the few published papers (Busconi et al., 2014; Zanardi et al., 2000). Coppa is a product consisting of a whole piece of meat, whose manufacturing process includes some peculiar phases. After deboning, half-slicing, and trimming the anatomical cut, salting is carried out: a mixture of salt, additives, and spices is distributed all over the meat, the composition of the ingredients varies according to the tradition and the recipes of production. Meat is then massaged manually or by a meat tumbling machine in order to ensure the homogeneous distribution of the mixture. Generally, one or two salting processes are carried out and followed by storage at low temperatures for a few days on steel trays (cold rest). At the end of the rest period, the meat cuts are wrapped in natural or synthetic casings, tied with a string, and then hung for several days in a drying chamber where they are exposed to higher temperatures and lower relative humidity, in order to reduce moisture. Finally, the ripening takes place for several weeks at a lower temperature and higher relative humidity than drying, until the product reaches the desired characteristics. Dry-cured meat products contamination by food-borne pathogens as Salmonella spp. and L. monocytogenes may result from superficial contamination of the fresh production process. HPP treatment resulted in a significant (P<0.01) superficial and deep decrease in Salmonella spp. enumeration varying from 0.61 to 4.01 log and from 1.49 to 4.13 log. According to the data presented in this study, only the combined approach of coppa manufacturing process followed by HPP treatment always led to a 5-log reduction of Salmonella spp. required by USDA/FSIS guidelines.

Effect of production process and high-pressure processing on viability of Salmonella spp. in traditional Italian dry-cured coppa

Roberta Taddei,1 Federica Giacometti,2 Lia Bardasi,1 Paolo Bonilauri,1 Mattia Ramini,1 Maria Cristina Fontana,1 Patrizia Bassi,1 Sara Castagnini,1 Francesco Ceredi,1 Maria Francesca Pellicioni,1 Andrea Serraino,2 Federico Tomasello,2 Silvia Piva,2 Elisabetta Mondo,2 Giuseppe Meriali1

1Istituto Zooprofilattico Sperimentale delle Lombardia e dell’Emilia Romagna, Sede Territoriale di Bologna; 2Dipartimento di Scienze Mediche Veterinarie, Università di Bologna, Ozzano dell’Emilia (BO); 3Istituto Zooprofilattico Sperimentale delle Lombardia e dell’Emilia Romagna, Sede Territoriale di Reggio Emilia, Reggio nell’Emilia, Italy

Abstract

The aim of the study was to investigate the combined effect of the manufacturing process followed by HPP treatment on the inactivation of Salmonella spp. in artificially contaminated coppa samples, in order to verify the ability of the combined processes to achieve the objective of a 5-log reduction of Salmonella spp. needed for exportation to the U.S. Fresh anatomical cuts intended for coppa production were supplied by four different delicatessen factories located in Northern Italy. Raw meat underwent experimental contamination with Salmonella spp. using a mixture of 3 strains. Surface contamination of the fresh anatomical cuts was carried out by immersion into inoculum containing Salmonella spp. The conditions of the HPP treatment were: pressure 593 MPa, time 290 seconds, water treatment temperature 14°C. Surface and deep samples were performed post contamination (T0), end of the cold phase (T1), end of process (Tend), and after HPP treatment (postHPP) and Salmonella spp. Enumerated. The results of this study show a significant reduction of Salmonella spp. all through the production process (P<0.01) for all companies, followed by an additional reduction of bacterial counts due to HPP treatment (P<0.01), both in superficial and deep contaminations (P<0.01). The superficial overall reduction resulted of 1.58 to 5.04 log CFU/g during the production process. HPP treatment resulted in a significant (P<0.01) superficial and deep decrease in Salmonella spp. enumeration varying from 0.61 to 4.01 log and from 1.49 to 4.13 log. According to the data presented in this study, only the combined approach of coppa manufacturing process followed by HPP treatment always led to a 5-log reduction of Salmonella spp. required by USDA/FSIS guidelines.
used as a final sanitization measure after production and/or packaging procedures. HPP has been successfully applied for the treatment of a wide variety of food such as jams, fruit sauces, yogurt, beef, fruit and vegetable juices, processed poultry products, oysters, cheese and carpaccio (Tao et al., 2016). Several treated RTE dry-cured meat products such as ham and salami are currently available on the market in Europe, U.S.A., Japan, Canada (Tao et al., 2016).

The aim of the present study was to investigate the combined effect of the manufacturing process followed by HPP treatment on the inactivation of Salmonella spp. in artificially contaminated coppa samples, in order to verify the ability of the combined processes to achieve the objective of a 5-log reduction of Salmonella spp. needed for exportation to the U.S.

Materials and Methods

Inoculum composition

The Salmonella spp. inoculum culture was prepared using a mixture of 3 strains: 118174/1 (monophasic S. Typhimurium) isolated from fresh pork sausage, 106463/1 (S. Derby) isolated from fresh swine meat, and the reference strain S. Typhimurium ATCC 14028 according to Bonilauri et al., (2019). 100 µl of a stock culture (stored in 20% glycerol at -80°C), each strain was transferred to 10 ml Brain Heart Infusion (BHI) broth and incubated for 24 h at 30°C. Subsequently, an aliquot of 100 µl was transferred to 1000 ml BHI broth and incubated at 30°C for 72 h to reach the stationary phase.

Just before the use, the 3 subcultures of Salmonella spp. were combined in equal volume (one liter each) in order to obtain a multi-strain cocktail of about 109 colony forming units (CFU)/ml and the resulting mixed culture was checked by enumeration on selective agar.

Samples contamination and production process

Fresh anatomical cuts intended for coppa production were supplied by four different small delicatessen factories located in Northern Italy herein named A, B, C and D. Raw meat (weight between 2.5 and 3 kg) underwent experimental contamination with Salmonella spp. Surface contamination of the fresh anatomical cuts was carried out by immersion into inoculum containing Salmonella spp. The immersion lasted for 10 minutes and was followed by drying for 30 minutes.

The four production processes were carried out in IZSLER laboratories following the producers’ standard protocols as summarized in Table 1. One (company A and D) or two salting (companies B and C) were comprised, salting mixtures being supplied by the four companies. In all the protocols meat samples underwent one or more steps in the meat tumbling machine in order to get a homogenous distribution of the salting mixture. After the salting step, coppa samples were singularly packed in polyethylene bags and vacuum sealed. Coppa samples underwent processing steps according to the producer’s specification (see Table 1): a resting phase (14 to 32 days at 1-8°C), a drying phase (3 to 7 days at 12-20°C), and a ripening phase (40 to 69 days at 14-18°C).

HPP treatment

For each contamination study, 5 vacuum-packed coppa samples were exposed to HPP treatment and 5 samples acted as control. The level of contamination before HPP was 1.56 – 5.09 log CFU/g in the superficial samples and 1.60 – 3.06 log CFU/g in the deep samples (see Table 2 Tend values). The conditions of the HPP treatment were: pressure 593 MPa, time 290 seconds, water treatment temperature 14°C, product temperature during treatment 4°C (Bonilauri et al., 2019). The pressure-holding treatment time in this study did not include the pressure increase time or the decompression time. The water temperature during the process started from 14°C, grew until 32°C during the treatment, and immediately returned to 14°C after the end of pressure stress.

Sampling procedure

The protocol of this study included both analysis on the surface and in depth of coppa samples. For superficial sampling, three squares with a length of approximately 3x3 cm and a thickness of about 0.3 cm enough to get a final weight of 25 g, were excided from apical, central and terminal positions of each coppa. Deep sampling was carried out after immersion of coppa samples in boiling water for 60 seconds. A sample unit of 25 g from the depth of coppa was then extracted.

Physicochemical analysis

aw was measured with AcquaLab, series 4 Non-commercial use only
Data analysis

According to EFSA (2010), for statistical analysis, if *Salmonella* spp. was detectable by the presence/absence test but not quantifiable in enumeration analysis (under the limit of quantification: LOQ=10 CFU/g) the value of 9 CFU/g (corresponding to log10 9 = 0.95 log cfu/g) was assigned. If *Salmonella* spp. was not detectable by the presence/absence test, the value of 0.03 CFU/g was assigned (corresponding to less than 1 cell on 25g); log10 0.03 = -1.52 log CFU/g). To compare the level of pathogens observed during processing steps and post HPP treatment, the two-way ANOVA test was chosen; level 1 was Company productive process (A, B, C, D) and level 2 was productive phases post contamination (T0), end of the resting phase (T1), end of ripening phase (Tend), and after HPP treatment (postHPP). When statistically significant differences were detected, one-way ANOVA and post hoc pairwise comparison across levels were performed by using Tukey’s test. Surface and deep contaminations were compared separately.

The statistical analyses were performed using the computer software program STATA 7.0 (STATA Corporation, College Station, TX, USA). Significance was established at p < 0.05.

Results and Discussion

The four production processes were characterized by different numbers of salting, cold and warm phase lengths and temperatures (Table 1) resulting in dry-cured *coppa* with different physicochemical characteristics (a<sub>W</sub> ranging from 0.892 to 0.922, pH ranging from 5.66 to 6.61 on the surface and a<sub>W</sub> ranging from 0.916 to 0.925, pH ranging from 5.61 to 6.13 in the deep part as reported in Table 3). The pH trend was in line with reported variability (5.5-6.5) cited by the PDO Product specification for *coppa* Piacentina (http://www.salumidoppiacentini.com/coppa-

<table>
<thead>
<tr>
<th>pH&lt;sub&gt;Sup&lt;/sub&gt;</th>
<th>Company A</th>
<th>S&lt;sub&gt;Sup&lt;/sub&gt;</th>
<th>C&lt;sub&gt;Sup&lt;/sub&gt;</th>
<th>pH&lt;sub&gt;Deep&lt;/sub&gt;</th>
<th>Company A</th>
<th>S&lt;sub&gt;Deep&lt;/sub&gt;</th>
<th>C&lt;sub&gt;Deep&lt;/sub&gt;</th>
<th>pH&lt;sub&gt;Sup&lt;/sub&gt;</th>
<th>Company A</th>
<th>S&lt;sub&gt;Sup&lt;/sub&gt;</th>
<th>C&lt;sub&gt;Sup&lt;/sub&gt;</th>
<th>pH&lt;sub&gt;Deep&lt;/sub&gt;</th>
<th>Company A</th>
<th>S&lt;sub&gt;Deep&lt;/sub&gt;</th>
<th>C&lt;sub&gt;Deep&lt;/sub&gt;</th>
<th>pH&lt;sub&gt;Sup&lt;/sub&gt;</th>
<th>Company A</th>
<th>S&lt;sub&gt;Sup&lt;/sub&gt;</th>
<th>C&lt;sub&gt;Sup&lt;/sub&gt;</th>
<th>pH&lt;sub&gt;Deep&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>6.04 (0.10)</td>
<td>0.959 (0.09)</td>
<td>6.10 (0.16)</td>
<td>0.959 (0.01)</td>
<td>6.03 (0.18)</td>
<td>0.933 (0.04)</td>
<td>5.92 (0.12)</td>
<td>0.916 (0.03)</td>
<td>6.20 (0.18)</td>
<td>0.933 (0.04)</td>
<td>5.84 (0.15)</td>
<td>0.916 (0.03)</td>
<td>6.40 (0.12)</td>
<td>0.916 (0.03)</td>
<td>5.93 (0.12)</td>
<td>0.916 (0.03)</td>
<td>6.52 (0.15)</td>
<td>0.916 (0.03)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>5.95 (0.07)</td>
<td>0.997 (0.00)</td>
<td>N.D.</td>
<td>N.D.</td>
<td>6.40 (0.12)</td>
<td>0.979 (0.01)</td>
<td>5.93 (0.00)</td>
<td>0.964 (0.00)</td>
<td>5.95 (0.07)</td>
<td>0.997 (0.00)</td>
<td>5.93 (0.00)</td>
<td>0.964 (0.00)</td>
<td>N.D.</td>
<td>N.D.</td>
<td>0.972 (0.02)</td>
<td>0.972 (0.02)</td>
<td>5.54 (0.10)</td>
<td>0.972 (0.02)</td>
<td>5.53 (0.10)</td>
<td>0.972 (0.02)</td>
</tr>
<tr>
<td>Tend</td>
<td>5.95 (0.07)</td>
<td>0.972 (0.00)</td>
<td>6.52 (0.05)</td>
<td>0.955 (0.04)</td>
<td>6.51 (0.05)</td>
<td>0.933 (0.04)</td>
<td>5.92 (0.05)</td>
<td>0.916 (0.03)</td>
<td>6.51 (0.05)</td>
<td>0.933 (0.04)</td>
<td>5.92 (0.05)</td>
<td>0.916 (0.03)</td>
<td>6.52 (0.05)</td>
<td>0.933 (0.04)</td>
<td>5.92 (0.05)</td>
<td>0.916 (0.03)</td>
<td>6.52 (0.05)</td>
<td>0.933 (0.04)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N.D.: Not Determined.

Table 4. Mean value log cfu/g (standard deviation) of *Salmonella* spp. (S) enumeration analyses carried out in superficial (Sup) and deep (Deep) Samples.

<table>
<thead>
<tr>
<th>pH&lt;sub&gt;Sup&lt;/sub&gt;</th>
<th>Company A</th>
<th>S&lt;sub&gt;Sup&lt;/sub&gt;</th>
<th>C&lt;sub&gt;Sup&lt;/sub&gt;</th>
<th>pH&lt;sub&gt;Deep&lt;/sub&gt;</th>
<th>Company A</th>
<th>S&lt;sub&gt;Deep&lt;/sub&gt;</th>
<th>C&lt;sub&gt;Deep&lt;/sub&gt;</th>
<th>pH&lt;sub&gt;Sup&lt;/sub&gt;</th>
<th>Company A</th>
<th>S&lt;sub&gt;Sup&lt;/sub&gt;</th>
<th>C&lt;sub&gt;Sup&lt;/sub&gt;</th>
<th>pH&lt;sub&gt;Deep&lt;/sub&gt;</th>
<th>Company A</th>
<th>S&lt;sub&gt;Deep&lt;/sub&gt;</th>
<th>C&lt;sub&gt;Deep&lt;/sub&gt;</th>
<th>pH&lt;sub&gt;Sup&lt;/sub&gt;</th>
<th>Company A</th>
<th>S&lt;sub&gt;Sup&lt;/sub&gt;</th>
<th>C&lt;sub&gt;Sup&lt;/sub&gt;</th>
<th>pH&lt;sub&gt;Deep&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>6.04 (0.10)</td>
<td>0.959 (0.09)</td>
<td>6.10 (0.16)</td>
<td>0.959 (0.01)</td>
<td>6.03 (0.18)</td>
<td>0.933 (0.04)</td>
<td>5.92 (0.12)</td>
<td>0.916 (0.03)</td>
<td>6.20 (0.18)</td>
<td>0.933 (0.04)</td>
<td>5.84 (0.15)</td>
<td>0.916 (0.03)</td>
<td>6.40 (0.12)</td>
<td>0.933 (0.04)</td>
<td>5.93 (0.12)</td>
<td>0.916 (0.03)</td>
<td>6.52 (0.15)</td>
<td>0.916 (0.03)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>5.95 (0.07)</td>
<td>0.997 (0.00)</td>
<td>N.D.</td>
<td>N.D.</td>
<td>6.40 (0.12)</td>
<td>0.979 (0.01)</td>
<td>5.93 (0.00)</td>
<td>0.964 (0.00)</td>
<td>5.95 (0.07)</td>
<td>0.997 (0.00)</td>
<td>5.93 (0.00)</td>
<td>0.964 (0.00)</td>
<td>N.D.</td>
<td>N.D.</td>
<td>0.972 (0.02)</td>
<td>0.972 (0.02)</td>
<td>5.54 (0.10)</td>
<td>0.972 (0.02)</td>
<td>5.53 (0.10)</td>
<td>0.972 (0.02)</td>
</tr>
<tr>
<td>Tend</td>
<td>5.95 (0.07)</td>
<td>0.972 (0.00)</td>
<td>6.52 (0.05)</td>
<td>0.955 (0.04)</td>
<td>6.51 (0.05)</td>
<td>0.933 (0.04)</td>
<td>5.92 (0.05)</td>
<td>0.916 (0.03)</td>
<td>6.51 (0.05)</td>
<td>0.933 (0.04)</td>
<td>5.92 (0.05)</td>
<td>0.916 (0.03)</td>
<td>6.52 (0.05)</td>
<td>0.933 (0.04)</td>
<td>5.92 (0.05)</td>
<td>0.916 (0.03)</td>
<td>6.52 (0.05)</td>
<td>0.933 (0.04)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N.D.: Not Determined.

Table 3. Results of chemico-physical analysis differentiated for manufacturing company carried out in superficial (Sup) and deep (Deep) samples: it is reported the mean value of the obtained measurements followed by the standard deviation into brackets.

[Italian Journal of Food Safety 2020; 9:8445] [page 143]
reduction) for company D (see Table 5); for company D a noticeable shorter duration of all the phases was observed; it was reported that Salmonella count reduction during seasoning is related not only to $a_c$ reached at the end of the process but in particular to the duration of seasoning (Pin et al., 2011).

In this study, HPP treatment resulted in a significant (P<0.01) superficial and deep decrease in Salmonella spp. enumeration varying from 0.61 to 4.01 log and from 1.49 to 4.13 log, respectively; the results show that HPP treatment of coppa samples has proven to be effective against both superficial and deep contamination. The generally lower decreases in superficial contamination correlate with the lower $a_c$ values that are proven to have a protective effect on microbial inactivation by HPP (Black et al., 2007; Black et al., 2007; Hayman et al., 2008; Patterson et al., 2005). HPP has demonstrated to be able to reduce Salmonella load in different types of food like raw chicken meat, poultry sausage, RTE meat (Anthoula et al., 2018; Hayman et al., 2004; Lerasle et al., 2014; Tananuwong et al., 2012; Yuste et al., 2000) and fermented pork sausages (Garriga et al., 2003); in particular in dry-cured ham (Bover-Cid et al., 2017; Garriga et al., 2004); when an HPP treatment of 600 MPa for 5 min was used on artificially contaminated sliced cured ham ($a_c$ ca 0.92), the reduction of S. enterica ranged from 3.72 to 5.04 log (Bover-Cid et al., 2017). In general, lower values of microbial reductions during HPP treatment were observed in the present study, but the comparison of this kind of data appears to be problematic as regards the possible differences in the characteristics of the treated products, in experimental design, HPP treatment conditions and baroresistance of the strains used for contamination.

The results of this study confirm that HPP treatment can be successfully used as an effective supplemental intervention strategy for controlling Salmonella spp. contaminations in dry-cured meat products such as coppa. In the case of products intended for exportation to countries with a zero tolerance policy for Salmonella spp., specifically the United States, HPP treatment used as a final sanitization measure after production, resulted to be a determining factor for the achievement of the USDA/FSIS requisites in establishments B, C and D, resulting particularly relevant in establishment D. According to the data presented in this study, only the combined approach of coppa manufacturing process followed by HPP treatment always led to a 5-log reduction of Salmonella spp. required by USDA/FSIS guidelines. Results suggest that the three establishments B, C, D should review their entire production process (especially for establishment D) either by adding the HPP step or, as additional option, by reviewing the time/temperature of the other decontamination steps of resting, drying and ripening.

### Table 5. Logarithmic unit reductions of Salmonella spp. (S) in superficial samples after each sampling step.

<table>
<thead>
<tr>
<th></th>
<th>Company A</th>
<th></th>
<th>Company B</th>
<th></th>
<th>Company C</th>
<th></th>
<th>Company D</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$S_{Sup}$</td>
<td>$S_{Deep}$</td>
<td>$S_{Sup}$</td>
<td>$S_{Deep}$</td>
<td>$S_{Sup}$</td>
<td>$S_{Deep}$</td>
<td>$S_{Sup}$</td>
<td>$S_{Deep}$</td>
</tr>
<tr>
<td>Resting - $\Delta$(T0-T1)</td>
<td>1.22</td>
<td>N.D.</td>
<td>2.03</td>
<td>N.D.</td>
<td>1.67</td>
<td>N.D.</td>
<td>2.66</td>
<td>N.D.</td>
</tr>
<tr>
<td>Drying and Ripening - $\Delta$(T1-Tend)</td>
<td>3.82</td>
<td>N.D.</td>
<td>2.83</td>
<td>N.D.</td>
<td>3.02</td>
<td>N.D.</td>
<td>-1.08</td>
<td>N.D.</td>
</tr>
<tr>
<td>Production process - $\Delta$(T0-Tend)</td>
<td>5.04</td>
<td>N.D.</td>
<td>4.86</td>
<td>N.D.</td>
<td>4.69</td>
<td>N.D.</td>
<td>1.58</td>
<td>N.D.</td>
</tr>
<tr>
<td>HPP - $\Delta$(Tend-THPP)</td>
<td>0.61</td>
<td>4.13</td>
<td>2.53</td>
<td>1.49</td>
<td>0.88</td>
<td>2.13</td>
<td>4.01</td>
<td>4.09</td>
</tr>
<tr>
<td>TOTAL - $\Delta$(T0-THPP)</td>
<td>5.65</td>
<td>N.D.</td>
<td>7.39</td>
<td>N.D.</td>
<td>5.57</td>
<td>N.D.</td>
<td>5.59</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D.: Not Determined.

### References


ISO 21807:2004. Microbiology of food and...
animal feeding stuffs — Determination of water activity.