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

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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 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.

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-dp/index.jsp?IdC=160&IdS=168&tipo_cliccato=0&tipo_padre=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. (FSIS, 2017). Establishments most often achieve the target by cooking, but they can use other lethality treatments such as fermentation, drying, salt curing, alternative processing technologies or a combination of these (FSIS, 2017); the same requirement is due for exportation of meat products to the U.S. (Italian Ministry of Health, 2015). High hydrostatic pressure (HPP) is a non-thermal food preservation technology applied to enhance the microbiological safety and to extend the shelf life of the treated food while keeping the organoleptic and nutritional characteristics unaltered. HPP has been considered to be the main emergent preservation technology with more prospects for its application in the meat industry (Hugas et al., 2002); it is mainly applicable to protect the food product against food-borne pathogens such as *Salmonella* spp., and *L. monocytogenes*. Fish processing and HPP treatment (2018) led to a reduc...
used as a final sanitation 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 swamp 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 dripping at room temperature for 30 minutes.

The four production processes were carried out in IZS Ler 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 synthetic 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

pH was measured with AcquaLab, series 476-20 to 539-46, a w was measured with AcquaLab, series 482-765 to 538-782.

Table 1. Main characteristics of the three production processes reproduced in this study.

|                             | Company A | Company B | Company C | Company D |
|-----------------------------|-----------|-----------|-----------|-----------|
| Anatomic cut weight (Kg)    | 2.7       | 2.5       | 3         | 2.5/3     |
| Number of salting           | 1         | 2         | 2         | 1         |
| Resting length (days)/temperatures | 14/3-5°C | 32/3-5°C | 27/1-4°C | 9/6-8°C   |
| Drying length (days)/temperatures | 5/20°C   | 72/1°C to 14°C | 6/22°C to 16°C | 3/2-2°C   |
| Ripening length (days)/temperatures | 51/15°C | 40/14-18°C | 24/17-21°C | 69/14-16°C | 44/14°C to 16°C |

Table 2. Experimental scheme including the number of analyzed test units for each processing step, sampling characteristics and scheduled analyses.

| Sampling time | Processing step     | Test units | Type of Sampling | Analysis                        |
|---------------|---------------------|------------|------------------|---------------------------------|
| 7D            | Post-contamination  | 3          | Superficial      | Salmonella spp. enumeration, pH, a w |
| 7T            | Post-resting        | 3          | Superficial – In deep | Salmonella spp. enumeration, pH, a w |
| Tend          | Post-ripening       | 5          | Superficial – In deep | Salmonella spp. enumeration, pH, a w |
| THPP          | Post-HPP treatment  | 5          | Superficial – In deep | Salmonella spp. enumeration    |
### 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 (\( a_w \) ranging from 0.892 to 0.925, pH ranging from 5.61 to 6.13 on the surface and 9.95 to 10.22, 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 Cliccato=0&tipo_padre=0&css=general&menu=1) Artificial contamination gained at a superficial initial concentration ranging from 6.52 to 7.47 log CFU/g of Salmonella spp. (Table 4). A contamination of the inoculated bacteria from the surface to the depth of the anatomical cuts was shown, probably facilitated by the use of the meat tumbling machine in concomitance with salting. In particular at the end of cold progressing phases deep contamination was first examined and reached values comprised among 4.10 to 4.84 log CFU/g of Salmonella spp. 

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), in accordance with several other dry-cured meat products in which Salmonella spp. decrease resulted equal to 3.28 and 5.5 log in pork loins (Morales-Partera et al., 2017) and ham after 69 days of curing (Reynold et al., 2001) respectively, the observed differences were mainly due to different product characteristics and different production processes.

In detail, the superficial overall reduction resulted of 1.58 to 5.04 log CFU/g during the production process, being 4.66-5.04 log CFU/g for Company A, B and C and significantly lower (1.58 CFU/g 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 by using the computer software program STATA 7.0 (STATA Corporation, College Station, TX, USA). Significance was established at p <0.05.

### 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.

| pH Deep | Company A | Company B | Company C | Company D |
|---------|-----------|-----------|-----------|-----------|
| a_w     | pH Deep   | a_w       | pH Deep   | a_w       | pH Deep   | a_w       | pH Deep   | a_w       | pH Deep   | a_w       | pH Deep   |
| T0      | 6.90 (0.06) | 0.97 (0.01) | N.D.      | 6.09 (0.14) | 0.95 (0.01) | N.D.      | 6.60 (0.12) | 0.94 (0.002) | N.D.      | 6.55 (0.12) | 0.95 (0.02) | N.D.      |
| T1      | 6.86 (0.10) | 0.99 (0.09) | 6.16 (0.16) | 0.95 (0.01) | 6.19 (0.16) | 0.93 (0.04) | 6.19 (0.16) | 0.94 (0.014) | 5.82 (0.15) | 0.97 (0.02) | 5.48 (0.10) | 0.95 (0.04) |
| Tend    | 6.06 (0.18) | 0.92 (0.07) | 5.64 (0.13) | 0.94 (0.01) | 6.06 (0.25) | 0.92 (0.06) | 5.12 (0.16) | 0.92 (0.046) | 5.05 (0.19) | 0.94 (0.015) | 5.18 (0.12) | 0.93 (0.045) |

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.

| Company A | Company B | Company C | Company D |
|-----------|-----------|-----------|-----------|
| S Sup     | S Deep    | S Sup     | S Deep    | S Sup     | S Deep    | S Sup     | S Deep    |
| T0        | 6.60 (0.22) | N.D.      | 7.35 (0.18) | N.D.      | 6.52 (0.25) | N.D.      | 7.47 (0.02) | N.D.      |
| T1        | 5.38 (0.27) | 4.89 (0.30) | 5.32 (0.33) | 4.84 (0.12) | 4.85 (0.27) | 4.10 (0.44) | 4.81 (0.87) | 4.71 (0.97) |
| Tend      | 1.56 (0.56) | 2.61 (0.20) | 2.49 (0.27) | 1.95 (0.70) | 1.86 (1.22) | 1.60 (0.37) | 5.89 (1.05) | 3.06 (1.20) |
| THPP      | 0.95 (0.50) | -1.52 (0.00) | -0.04 (1.36) | 0.11 (0.46) | 0.95 (0.00) | -0.53 (1.35) | 1.88 (0.60) | -1.03 (1.11) |

N.D.: Not Determined; ND assumed value: 0.95 when in all replicates pathogen was detectable but not countable <10 CFU/g, -1.52 when in all replicates pathogen is not detected, -0.04 when in 3 out of 5 replicates pathogen was detectable but not countable and the last two were not detected, -0.53 when in 2 out of 5 replicates pathogen was detectable but not countable and the last three were not detected, -0.46 when in 4 out of 5 replicates pathogen was detectable but not countable and the last one was not detected, -1.03 when in 1 out of 5 replicates pathogen was detectable but not countable and in 4 replicates were not detected; when assumed value is used no statistical comparison was possible. Different capital letter significant differences between results in different rows. * * means differences between superficial and deep contamination in rows. Differences between Companies were not significant (see text).
specifically the United States, HPP treatment used as a final sanitation 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.

| Sampling Step              | S(ACTOR) | S(Deep) | S(ACTOR) | S(Deep) | S(ACTOR) | S(Deep) | S(ACTOR) | S(Deep) |
|----------------------------|----------|---------|----------|---------|----------|---------|----------|---------|
| Resting - Δ(T0-T1)         | 1.22     | N.D.    | 2.03     | N.D.    | 1.67     | N.D.    | 2.66     | N.D.    |
| Drying and Ripening - Δ(T1-Tend) | 3.82 | N.D.    | 2.83     | N.D.    | 3.02     | N.D.    | -1.08    | N.D.    |
| Production process - Δ(T0-Tend) | 5.04 | N.D.    | 4.86     | N.D.    | 4.69     | N.D.    | 1.58     | N.D.    |
| HPP - Δ(Tend-THPP)         | 0.61     | 4.13    | 2.53     | 1.49    | 0.88     | 2.13    | 4.01     | 4.09    |
| TOTAL - Δ(T0-THPP)         | 5.65     | N.D.    | 7.39     | N.D.    | 5.57     | N.D.    | 5.59     | N.D.    |

N.D.: Not Determined

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