

## Effect of production process and high-pressure processing on viability of *Listeria innocua* in traditional Italian dry-cured coppa

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### Abstract

In this study the effect of the application of High Pressure Treatment (HPP) combined with four different manufacturing processes on the inactivation of *Listeria innocua*, used as a surrogate for *L. monocytogenes*, in artificially contaminated coppa samples was evaluated in order to verify the most suitable strategy to meet the *Listeria* inactivation requirements needed for the exportation of dry-cured meat in 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 *Listeria innocua* using a mixture of 5 strains. Surface contamination of the fresh anatomical cuts was carried out by immersion into inoculum containing *Listeria* spp. The conditions of the HPP treatment were: pressure 593 MPa, time 290 seconds, water treatment temperature 14°C. *Listeria innocua* was enumerated on surface and deep samples post contamination, resting, ripening and HPP treatment. The results of this study show how the reduction of the microbial load on coppa during the production process did not vary among three companies ( $P > 0.05$ ) ranging from 3.73 to 4.30 log CFU/g, while it was significantly different ( $P < 0.01$ ) for the fourth company (0.92 log CFU/g). HPP treatment resulted in a significant ( $P < 0.01$ ) deep decrease of *L.*

*innocua* count with values ranging between 1.63-3.54 log CFU/g with no significant differences between companies. Regarding superficial contamination, HPP treatment resulted significant ( $P < 0.01$ ) only in Coppa produced by two companies. The results highlight that there were processes less effective to inhibit the pathogen; in particular for company D an increase of *L. innocua* count was shown during processing and HPP alone cannot be able to in reaching the *Listeria* inactivation requirements needed for exportation of dry-cured meat in the U.S. According to the data reported in this paper, HPP treatment increases the ability of the manufacturing process of coppa in reducing *Listeria* count with the objective of a lethality treatment.

### Introduction

*Listeria monocytogenes* is a foodborne pathogen that causes listeriosis, a relatively rare but potentially fatal illness. Healthy individuals are usually not susceptible to *L. monocytogenes*, unlike those with compromised immune system such as elderly, pregnant women and newborns, in which mild to severe consequences can be observed (Jordan and McAuliffe, 2018). In 2018 there were 2,549 confirmed human cases of listeriosis in the EU, corresponding to a notification rate of 0.47 cases per 100,000 population. In the last years (period 2014–2018) there has been a statistically significant increasing trend of confirmed listeriosis cases in the EU/EEA, with a high case fatality (15.6%), which makes listeriosis one of the most serious foodborne diseases under EU surveillance (EFSA and ECDC, 2019). In the United States it has an incidence of 0.3 cases per 100,000 population (Tack *et al.*, 2019).

*L. monocytogenes*, being ubiquitous in the environment, can contaminate food-processing plants, survive for long periods due to its capability to resist to various stresses, such as exposure to sanitizers, pH and temperature, and form biofilm. It mainly represents a problem for the Ready-To-Eat (RTE) food industry, as there is no lethal treatment between production and consumption (Gray *et al.*, 2018; Jordan and McAuliffe, 2018). Over the 2016–2018 period, samples from pig meat were by far the main matrix tested for *L. monocytogenes* in the EU, 59.9% in 2018, out of the 24,814 RTE pig meat products tested, 1.3% was positive for *L. monocytogenes*, 1.4% at production level and 0.8% at retail level (EFSA and ECDC, 2019). Several authors evaluated the presence of *L. monocytogenes* in RTE pig meat products such as dry-cured

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ham (Garriga *et al.*, 2004; Meriardi *et al.*, 2015), dry-cured pork loins (Morales-Partera *et al.*, 2017) and others dry-cured pig meat products including coppa, bacon and smoked speck (Meloni *et al.*, 2009).

Coppa is a typical Italian cured pork meat product consisting of a whole piece of meat obtained from the neck muscles of heavy pigs, traditionally made in the provinces of Parma and Piacenza (Emilia Romagna Region, Northern Italy). Characteristics and product processing information can be found in the PDO specifications

([http://www.salumidoppiacentini.com/coppa-dp/index.jsp?doc?IdC=160&IdS=168&tipo\\_ccato=0&tipo\\_padre=0&nav=1&css=generico\\_dop.css&menu=1](http://www.salumidoppiacentini.com/coppa-dp/index.jsp?doc?IdC=160&IdS=168&tipo_ccato=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 (Zanardi *et al.*, 2000; Busconi *et al.*, 2014). The production process includes, after deboning, half-slicing, and trimming the anatomical cut, one or two salting procedures, using a mixture of salt, additives, and spices. Meat is massaged manually or mechanically, by a meat tumbling machine, in order to distribute the salting mixture evenly. Salting is generally followed by storage at low temperatures for a few days on steel trays (cold rest). After cold resting the meat is wrapped and tied and then exposed to higher temperatures and lower relative humidity, in order to reduce moisture. The last step consists in ripening,

which takes several weeks, at a lower temperature and higher relative humidity than drying, to reach the desired characteristics of the product. The production processes applied in this work are summarized in Table 1.

Contamination of dry-cured meat products by *L. monocytogenes* may result from superficial contamination of the fresh anatomical cuts, both during slaughtering and production and/or from cross-contamination in case of manipulation by contaminated operators or contact with contaminated equipment or surfaces.

The current EU regulation for *L. monocytogenes*, Regulation (EC) No. 2073/2005 (European Commission, 2005), admits different levels of presence depending on whether or not the RTE product supports the growth of *L. monocytogenes* (products with  $\text{pH} \leq 4,4$  or  $a_w \leq 0,92$ , products with  $\text{pH} \leq 5,0$  and  $a_w \leq 0,94$  and products with a shelf-life of less than five days are considered non supporting its growth). In products supporting its growth EU regulation requires the absence (in 5x25g samples) before the food has left the immediate control of the food business operator, and levels  $<100$  CFU/g (in 5x25g samples) for products already placed on the market during their shelf life; in RTE products that do not sustain its growth it is supposed to be  $<100$  CFU/g (in 5x25g samples) at production and throughout the defined shelf-life. Differently in the U.S. the regulation in place applies a *zero tolerance* approach for RTE products, regardless of whether or not they support the growth of the pathogen (Food Safety Inspection Service, 2015). The U.S. Department of Agriculture Food Safety and Inspection Service (USDA/FSIS) requires the establishments producing dry-cured meat products to include a process addressing lethality of *L. monocytogenes* to consider their products safe for consumption, defining lethality as a process or combination of processes that ensures a specific, significant reduction in the number pathogens in the product.

High hydrostatic pressure (HPP) is a non-thermal food preservation technology used to extend the shelf life and to enhance

the microbiological safety without altering the organoleptic and nutritional characteristics of treated products. It is an emerging technology with applications to a wide variety of food, mainly used as a final sanitization measure after production and/or packaging procedures (Hugas *et al.*, 2002). HPP has been successfully applied for the treatment of a wide variety of food including meat-based products (cooked and dry ham, etc.), fish, pre-cooked dishes and fruit, vegetables and juices (Sandra *et al.*, 2004; Rastogi *et al.*, 2007; Zhang and Mittal, 2008; Van Hekken *et al.*, 2013). Several treated RTE dry-cured meat products such as ham and salami are currently available on the market in Europe, U.S.A., Japan, and Canada (Tao *et al.*, 2014).

In this study it was evaluated the effect of the application of HPP combined with different manufacturing processes on the inactivation of *Listeria innocua*, used as a surrogate for *L. monocytogenes* (Hu and Gurtler, 2017), in artificially contaminated *coppa* samples, in order to verify the ability of the combined processes to meet the requirements needed for exportation to the U.S.

## Materials and Methods

### Inoculum composition

The *L. innocua* inoculum culture was prepared using a mixture of 5 strains: IZSLER 111373/1 and IZSLER 111373/2 isolated from industrial site (superficial swab collected in pork meat transformation plant), IZSLER 257529/1 isolated from fresh pork sausages, IZSLER 257529/2 isolates from fresh swine meat and the reference strain ATCC 33090, 100  $\mu\text{L}$  of a stock culture (stored in 20% glycerol at  $-80^\circ\text{C}$ ) of each strain were transferred to 10 ml Brain Heart Infusion (BHI) broth and incubated for 24 h at  $30^\circ\text{C}$ . Subsequently, an aliquot of 100  $\mu\text{L}$  was transferred to 1000 mL BHI broth and incubated at  $12^\circ\text{C}$  for 72 h (Merialdi *et al.*, 2015).

Just before contamination, the 5

subcultures of *L. innocua* were mixed in equal volume and the resulting 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 artisanal delicatessen factories located in Northern Italy herein named A, B, C and D. Raw meats, with a weight ranging from 2.5 to 3 kg, underwent experimental contaminations with *L. innocua*. The anatomical cuts were contaminated by immersion for 10 minutes into the inoculum containing *L. innocua*, then dried for 30 minutes at room temperature.

The detailed processing procedures applied are detailed in Table 1. The protocols included one (company A and D) or two (company B and C) salting procedures with salting mixtures being supplied by the four companies. In all the protocols, meat samples underwent one or more steps in meat tumbling machine in order to get a homogenous distribution of the salting mixture. *Coppa* samples were processed according to the producer's specifications (Table 1) undergoing a resting phase (9 to 32 days at  $1-8^\circ\text{C}$ ), a drying phase (3 to 7 days at  $12-27^\circ\text{C}$ ), and a ripening phase (44 to 69 days at  $14-21^\circ\text{C}$ ).

After salting, *coppa* samples were singularly packed in synthetic casing and, at the end of the maturation period, they were separately transferred to nylon-polyethylene bags and vacuum sealed.

### HPP treatment

For each contamination study, 5 vacuum-packed Coppa samples were exposed to HPP treatment and 5 samples acted as control. For the HPP treatment the following settings were applied: pressure 593 MPa, time 290 seconds, water treatment temperature  $14^\circ\text{C}$ , product temperature at the time of treatment  $4^\circ\text{C}$ . 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  $14^\circ\text{C}$ , grew until  $32^\circ\text{C}$  during the treatment, and

**Table 1. Experimental scheme including the number of analyzed test units for each processing step, sampling characteristics and scheduled analyses.**

	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	7/27°C to 14°C	6/22°C to 16°C	3/12-27°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

immediately returned to 14°C after the end of pressure stress.

### Sampling procedure

After the inoculation with *L. innocua* strains, for each challenge test, a total of 3 samples for each sampling time, inoculum (T0), post resting (T1), and a total of 5 samples post ripening (Tend) and post HPP treatment (THPP) were collected and analyzed for the determination of pH and  $a_w$  values and the evaluation of *L. innocua* count. The analyses carried out for this study were made both on the surface and in depth of *coppa* samples. Superficial samples consisted of three squares of approximately 3x3 cm length and about 0.3 cm thickness to get a final weight of 25 g, excised from apical, central and terminal positions of each *coppa*. For deep samples, *coppa* was immersed for 60 seconds in boiling water, then a 25 g sample was extracted from the depth of *coppa* (Bonilauri *et al.*, 2004).

### Physicochemical analysis

Water activity ( $a_w$ ) was measured with AquaLab series 4 Model TE instrument, in accordance with ISO 21807:2004 (ISO, 2004). pH was evaluated through Mettler Toledo LE427 glass electrode probe connected to pHenomenal PC5000 L (VWR) pH/conductivity meter. Weight loss values (expressed as percentage of the initial weight) were determined throughout the production process, on three samples for each contamination study.

### Microbiological analysis

Before inoculation anatomical cuts were

controlled for the absence of *Listeria* spp. following ISO 11290-1:1996/Amd 1:2004 (ISO, 2004) protocol intended for *L. monocytogenes* detection, excepting that suspected colonies were confirmed by biochemical miniaturized tests (API Listeria kit; BioMérieux, France). For *L. innocua* enumeration, samples were diluted 1/10 in Buffered Peptone Water (homemade) and homogenized in stomacher for 60 s. Ten-fold serial dilutions were plated onto ALOA agar (Biolife, Milan, Italy) and incubated at 37°C for 48 h. Suspected colonies were confirmed by biochemical miniaturized tests (API Listeria kit; BioMérieux, France). In samples below the quantification limit (10 CFU/g), the qualitative analysis was carried out as described before. Results of *L. innocua* counts were expressed in CFU/g and converted into Log<sub>10</sub> CFU/g.

### Data analysis

For comparison between control and treated samples, if the pathogen resulted detectable but not quantifiable in enumeration analysis (under the limit of quantification: LOQ=10 CFU/g), it was assigned the value of 9 CFU/g (corresponding to log<sub>10</sub> 9 = 0.95 log CFU/g) (EFSA, 2010).

To compare the level of the pathogen observed during processing steps and post HPP treatment two way ANOVA test, was used; level 1 was Company productive process (A, B, C, D) and level 2 consisted in productive phases; (T0) inoculum, (T1) post resting, (Tend) post ripening, and (THPP) post HPP treatment. 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 carried out using the computer software program STATA 7.0 (STATA Corporation, College Station, TX, USA). Significance was established at P<0.05.

## Results

Number of salting, resting, lengths and temperatures of resting, drying and ripening phases were different for the four production processes, as reported in Table 1, resulting in dry-cured *coppa* with different physicochemical characteristics (pH and  $a_w$  values are summarized in Table 2): before resting, the pH and  $a_w$  values ranged from 5.93 to 6.40 and from 0.994 to 0.997 respectively; the resting phase had a variable length between 9 and 32 days and led the pH and  $a_w$  values from 5.58 to 6.20 and from 0.933 to 0.972 respectively on surface samples and from 5.62 to 6.03 and from 0.972 to 0.979 on deep samples. After ripening, which also had different durations across the four companies (44 to 69 days), observed pH values ranged from 5.66 to 6.61 on surface and 5.61 to 6.13 in depth, whereas  $a_w$  values were reduced by 0.892 to 0.922 and 0.916 to 0.925 on surface and in depth respectively (Table 2).

The initial concentration, obtained through artificial contamination, ranged from 7.11 to 7.60 log CFU/g of *L. innocua* into superficial samples (Table 3).

**Table 2. Results of chemophysical 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.**

	Company A				Company B				Company C				Company D			
	pH <sub>Sup</sub>	$a_w$ <sub>Sup</sub>	pH <sub>Deep</sub>	$a_w$ <sub>Deep</sub>	pH <sub>Sup</sub>	$a_w$ <sub>Sup</sub>	pH <sub>Deep</sub>	$a_w$ <sub>Deep</sub>	pH <sub>Sup</sub>	$a_w$ <sub>Sup</sub>	pH <sub>Deep</sub>	$a_w$ <sub>Deep</sub>	pH <sub>Sup</sub>	$a_w$ <sub>Sup</sub>	pH <sub>Deep</sub>	$a_w$ <sub>Deep</sub>
T0	5.95 (0.07)	0.997 (0.001)	N.D.	N.D.	6.40 (0.12)	0.997 (0.001)	N.D.	N.D.	5.99 (0.25)	0.994 (0.002)	N.D.	N.D.	5.93 (0.12)	0.995 (0.002)	N.D.	N.D.
T1	6.04 (0.10)	0.959 (0.009)	6.03 (0.16)	0.979 (0.003)	6.20 (0.18)	0.933 (0.008)	5.92 (0.12)	0.973 (0.005)	5.86 (0.13)	0.964 (0.038)	5.62 (0.15)	0.972 (0.002)	5.58 (0.05)	0.972 (0.001)	5.75 (0.09)	0.976 (0.005)
Tend	5.66 (0.18)	0.892 (0.017)	5.61 (0.13)	0.924 (0.005)	6.61 (0.23)	0.922 (0.005)	6.13 (0.16)	0.925 (0.006)	6.05 (0.19)	0.904 (0.019)	5.80 (0.05)	0.916 (0.010)	6.20 (0.05)	0.914 (0.002)	5.99 (0.08)	0.924 (0.007)

**Table 3. Mean value log CFU/g (standard deviation) of *L. innocua*. (L) enumeration analyses carried out in superficial (Sup) and deep (Deep) Samples.**

	Company A		Company B		Company C		Company D	
	L <sub>Sup</sub>	L <sub>Deep</sub>	L <sub>Sup</sub>	L <sub>Deep</sub>	L <sub>Sup</sub>	L <sub>Deep</sub>	L <sub>Sup</sub>	L <sub>Deep</sub>
T0	7.11 <sup>A</sup> (0.19)	N.D.	7.34 <sup>A</sup> (0.24)	N.D.	7.54 <sup>A</sup> (0.20)	N.D.	7.60 <sup>A</sup> (0.12)	N.D.
T1	6.65 <sup>Ax</sup> (0.27)	5.30 <sup>Ay</sup> (0.56)	5.95 <sup>Bx</sup> (0.23)	4.23 <sup>Ay</sup> (0.29)	6.02 <sup>B,x</sup> (0.39)	4.51 <sup>Ay</sup> (2.02)	5.78 <sup>Bx</sup> (0.30)	2.31 <sup>Ay</sup> (0.58)
Tend	3.85 <sup>Bx</sup> (0.51)	4.37 <sup>Ax</sup> (0.80)	4.34 <sup>Cx</sup> (0.51)	4.20 <sup>Ax</sup> (0.58)	4.65 <sup>C,x</sup> (0.56)	3.94 <sup>Ax</sup> (0.25)	7.62 <sup>Cx</sup> (0.24)	4.54 <sup>By</sup> (1.52)
THPP	3.38 <sup>Bx</sup> (0.45)	1.80 <sup>B,y</sup> (0.91)	3.60 <sup>Dx</sup> (0.30)	1.71 <sup>By</sup> (0.26)	3.24 <sup>D,x</sup> (0.41)	2.31 <sup>By</sup> (0.51)	6.68 <sup>Cx</sup> (1.37)	1.00 <sup>*Cy</sup> (0.10)

N.D.: Not Determined; # in 2 out of 5 replicates value 0.95 was assumed since pathogens were detected but not countable; \* in 4 out of 5 replicates value 0.95 was assumed since pathogens were detected but not countable. In each column, different capital letters mean significant differences between *L. innocua* contamination evaluated at each sampling step. Significant (x,y) or not significant (x,x) differences between surface and deep contamination, separately evaluated for each step and each company.

Throughout the early phases of the four production processes, the progressive contamination of deeper parts of the anatomical cuts by inoculated bacteria took place, seemingly facilitated by the use of the meat tumbling machine in concomitance with salting. The first examinations were made at the end of the resting phase, obtaining values for deep contamination comprised between 2.31 and 5.30 log CFU/g (Table 3).

The overall superficial reduction resulted of 3.73 to 4.30 log CFU/g for companies A, B, C and 0.92 log CFU/g for company D. Table 4 reports details of *L. innocua* count reduction showing how the reduction of the microbial load on the surface of the coppa during the production process was not different among companies A, B and C ( $P>0.05$ ) ranging from 2.89 to 3.26 log CFU/g, while it was significantly different ( $P<0.01$ ) (-0,02 log CFU/g) for company D (Table 4). Similarly, the microbial reduction occurred in depth during drying and ripening process, resulting between 0.03 and 0.93 log CFU/g ( $P>0.05$ ) for companies A, B, C while an increase of *L. innocua* count (2.23 log CFU/g) was shown for company D ( $P<0.01$ ).

HPP treatment resulted in a significant ( $P<0.01$ ) deep decrease of *L. innocua* with values ranging between 1.63-3.54 log CFU/g (Table 4) with no significant differences between companies. Regarding superficial contamination HPP treatment resulted significant ( $P<0.01$ ) only in companies B and C.

## Discussion and Conclusions

This study reports data of 4 challenge tests performed on Italian dry-cured coppa, produced by following four different companies' procedures, and subsequently treated with HPP in order to verify the ability of the overall procedure to obtain a lethal process for *Listeria*.

*L. innocua* reduction during curing did

not show any significant difference between companies A, B, C ( $P>0.05$ ) with values ranging from 2.89 to 3.26 log CFU/g (Table 4); similar results have been shown by other authors in several dry-cured meat products. In particular a reduction of 4.0 log CFU/g in dry-cured ham after 69 days of curing was observed by Reynolds *et al.* (2001), and a 2.5 log CFU/g decrease was reported in dry-cured Serrano ham after 60 days of ripening (Montiel *et al.*, 2020), while Barbuti *et al.* (2009) reported after 108 days of ripening a decrease of *L. monocytogenes* of 4.5 CFU/g in Italian Parma ham. Differences with the values obtained in this study can be addressed mainly to different products characteristics and different production processes.

In the early phase of the production process, the decrease of *L. innocua* in companies A, B and C could primarily be related to the salting treatment to which the anatomical cuts were exposed as it was observed a higher reduction in the two processes that included double salting, while in the subsequent phases the loss of microbial vitality could mainly be related to the progressive reduction of the  $a_w$  values correlated with the weight loss of the product.

Differently, values obtained from company D, in terms of *L. innocua* reduction after resting (-1.84 log CFU/g) and at the end of the process (-0,02 log CFU/g), were significantly lower than the others, (see Table 4), probably because of shorter resting, drying and ripening phases. Several authors reported how a longer maturation period leads to higher reduction of pathogenic bacteria: it was demonstrated that a short ripening period in fermented sausages was associated with greater survival of *L. monocytogenes* (Gonzales-Barron *et al.*, 2015; Nightingale *et al.*, 2006) and that the length of the drying period may be particularly important in the control of *L. monocytogenes*. Reynolds *et al.* (2001) found that in dry-cured ham *L. monocytogenes* populations continued to decline to undetectable levels after the hams

completed the dry-aging process highlighting the importance of ageing in the control of *L. monocytogenes*.

In the present study, after HPP treatment at 593 MPa for 290 sec., a reduction of 0.47-1.41 log CFU/g and 1.63-3.54 log CFU/g on surface and in depth respectively; there are no data in literature on HPP of coppa to compare our results. In studies regarding HPP treatment of fermented dry meat products at time/pressure parameters similar to those applied in this study, a 1.6 – 5.0 Log CFU/g reduction at 600MPa for 5 min in Genoa salami (Porto-Fett *et al.*, 2010), a 1.79-3.15 Log CFU/g reduction in Spanish chorizo at 600 MPa for 5-10 minutes (Rubio *et al.*, 2018) and a 0.9 Log CFU/g reduction in slightly fermented sausages at 400MPa for 10 minutes (Garriga *et al.*, 2005) were reported.

Various authors have tested the inactivation of *L. monocytogenes* in other unfermented meat products by HPP (Garriga *et al.*, 2004; Hugas *et al.*, 2002; Meriardi *et al.*, 2015), many of them in dry-cured ham (Morales *et al.*, 2006; Bover-Cid *et al.*, 2011; Hereu *et al.*, 2012; Bover-Cid *et al.*, 2015); Hereu *et al.* (2012) have reported a reduction of *L. monocytogenes* from 1.82 to 3.85 log CFU/g in artificially contaminated sliced ham after treatment at 600 MPa for 5 min, Bover-Cid *et al.* (2011), through a HPP treatment at 613 MPa for 5 min., achieved the 2.39D proposed by Hoz *et al.* (2008) to meet the USA zero tolerance policy, Bover-Cid *et al.* (2015) showed a reduction of *L. monocytogenes* in dry-cured ham at 600MPa for 5 minutes ranging from 2.24 log to 6.82 Log CFU/g depending on the  $a_w$  and fat content of the ham.

In our study a general lower effect on *L. innocua* count reduction was observed probably due to variables as a result of intrinsic characteristics of the product (pH,  $a_w$ , fat, protein and solute content) that influence the efficacy of HPP treatment on *Listeria* spp.; also the different bacterial baroresistance of different strains used in different studies should be taken into account (Possas *et al.*, 2017).

**Table 4. Logarithmic unit reductions of *L. innocua* (L) in superficial and deep samples after each sampling step.**

	Company A		Company B		Company C		Company D	
	$L_{Sup}$	$L_{Deep}$	$L_{Sup}$	$L_{Deep}$	$L_{Sup}$	$L_{Deep}$	$L_{Sup}$	$L_{Deep}$
Resting - $\Delta(T0-T1)$	0.46	-	1.39	-	1.52	-	1.82	-
Drying and Ripening - $\Delta(T1-Tend)$	2.80	0.93	1.61	0.03	1.37	0.57	-1.84	-2.23
Production process - $\Delta(T0-Tend)$	3.26	-	3.00	-	2.89	-	-0.02	-
HPP - $\Delta(Tend-THPP)$	0.47	2.57	0.74	2.52	1.41	1.63	0.94	3.54
TOTAL - $\Delta(T0-THPP)$	3.73	-	3.74	-	4.30	-	0.92	-

-- values were not calculated since T0 was not determined.

However, the results of this study confirm that using a HPP treatment can be an effective supplemental intervention strategy for controlling *L. monocytogenes* contaminations in dry-cured meat products such as *coppa*. Nevertheless, being HPP effectiveness strongly dependent on product's characteristics, it remains necessary to set and validate HPP treatment conditions on each specific food product due to its uniqueness in composition and manufacturing process.

For products intended for exportation to countries with *zero tolerance* policy for *Listeria*, in particular the United States, HPP treatment used as a post-production process for sanitation, resulted to be a decisive factor to achieve the USDA/FSIS requirement. According to the data reported in this paper, processes of companies A, B, and C resulted able to reduce *L. innocua* contamination, and HPP treatment increases the ability in reducing *L. innocua* count with the objective of a lethality treatment. For company D an increase of *L. innocua* count during the process and HPP failed in reaching the superficial *Listeria* inactivation requirements needed for exportation of dry-cured meat in the U.S. Processors should assess the ability of their process to control incoming pathogens, to predict the *L. innocua* load at the end of the process, and to evaluate the need for a process modification and/or for the addition of a final lethal process.

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