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Modeling the behavior of *Listeria innocua* in Italian salami during the production and High-Pressure validation of processes for exportation to the U.S.

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Abstract

The study evaluated 51 Italian commercial salami processes in association with High-Pressure Processing (HPP) to assess *Listeria innocua* lethality. We used data to model the decrease of *Listeria monocytogenes* according to process parameters. At the end of the process, the total *L. innocua* reduction always resulted in > 1 Log10 CFU/g (1.04-5.68). In the univariate analysis, we observed a significant association between the decrease of *L. innocua* count, a_w at the end of the acidification/drying process, a_w decrease during seasoning, duration of the seasoning, trimming and caliber. HPP further reduced the *L. innocua* count by 0.48-3.47 Log10 CFU/g. The model represents a useful tool for enterprises and Authorities to evaluate the efficacy of processes to reduce *L. monocytogenes*, predicting its load at the end of the process and the need for a process modification or for the addition of a final lethal process. The model of HPP treatment predicts treatment efficacy based on pH and a_w of the product.

Keywords: fermented sausages, Italian salami, *Listeria innocua, Listeria monocytogenes*, challenge test, linear regression model.

1. Introduction

Listeria monocytogenes is the causative agent of foodborne listeriosis; in healthy individuals, infection is usually mild, but it is possible to observe severe consequences in fetuses, infants, immunocompromised patients, elderly, and pregnant women with a high mortality rate (Tolvanen et al., 2008).

L. monocytogenes frequently contaminates meat and meat products, including fermented sausages. Such contamination occurs via raw materials, ingredients, and processing equipment. Furthermore, it can also be related to the post-processing phase (Hwang et al., 2009; Lin et al., 2006; Prencipe et al., 2012). *L. monocytogenes* is often isolated from fermented meat products due to its capability to survive their adverse conditions (Degenhardt & Sant'Anna, 2007), and the presence of *L. monocytogenes* in ready-to-eat (RTE) products, like salami, poses a risk to the consumer. Several studies have reported the presence of *L. monocytogenes* in fermented sausages worldwide, with prevalence reaching up to 60% (Bohaychuk et al., 2006; De Cesare, Mioni, & Manfreda, 2007; Doménech, Jimenez -Belenguer, Amoros, Ferrus, & Escriche, 2015; Ferreira et al., 2007; Glass & Doyle, 1989; Gounadaki, Skandamis, Drosinos, & Nychas, 2008; Martin, Garriga, & Aymerich, 2011; Meloni, 2015; Meloni et al., 2012, 2014).

The European Union food safety Regulations admit the presence of *L. monocytogenes* in RTE products that do not sustain its growth, to a concentration not exceeding 100 CFU/g throughout the defined shelf life. Regardless of whether or not they support the growth of the pathogen, the U.S. legislation applies a zero-tolerance approach for RTE products. According to the guidance document of the U.S. Department of Agriculture Food Safety and Inspection Service (FSIS & USDA, 2017), establishments producing dry-cured meat products should implement a process addressing lethality of *L. monocytogenes*, to ensure the safety of products for consumption. In this context, lethality is defined as the process or combination of processes, ensuring a specific and significant reduction in the number of pathogens in products.

Although literature frequently reported the presence of *L. monocytogenes* in dry and semi-dry fermented sausages, these products are considered at low risk for foodborne listeriosis due to their intrinsic properties (Barmpalia-Davis, Geornaras, Kendall, & Sofos, 2008; Simpson et al., 2008). On the other hand, fermented sausages were traced as a possible source of a listeriosis outbreak that involved 36 individuals in Philadelphia with four cases of death (Schwartz et al., 1989), while in 2010, two cases were linked to salami consumption in Ontario (http://outbreakdatabase.com/details/siena-foods-salame-2010/?organism=Listeria+monocytogenes&vehicle=sausage). Besides, the economic impact due to the recall of meat products contaminated with *L. monocytogenes*, despite their ability to cause illnesses, should be considered.

Several studies evaluated the fate of *L. monocytogenes* in dry sausage manufacturing processes, but their results vary considerably. Although most studies observed a reduction of *L. monocytogenes* contamination, others demonstrated the ability of *L. monocytogenes* to survive when the initial load of *L. monocytogenes* in raw materials was high (Nightingale, Thippareddi, Phebus, Marsden, & Nutsch, 2006); moreover, in some cases, growth was shown during processing (Campanini, Pedrazzoni, Barbuti, & Baldini, 1993; Nissen & Holck, 1998). Literature indicates that *L. monocytogenes* can contaminate fermented sausages and may not be completely eliminated during processing. Consequently, to comply with the zero-tolerance policy requested by the U.S. Federal Authorities, additional treatments must be necessary, including the implementation of post-processing operations (Hereu, Bover-Cid, Garriga, & Aymerich, 2012). High-Pressure Processing (HPP) can be used on several products to improve their microbiological characteristics without a significant modification of their organoleptic features (Hayman, Baxter, O'Riordan, & Stewart, 2004).

The variability of process procedures and parameters in salami production highlights the need for process validation research to reduce the prevalence of *L. monocytogenes* contamination and the risk

for consumers (Nightingale, Thippareddi, Phebus, Marsden, & Nutsch, 2006). The use of predictive models explaining the effect of a wide range of food variables can help producers to plan their formulae or process to achieve the goal of controlling *L. monocytogenes* contamination (Novelli et al., 2017). The objective of this study was, through the evaluation of 51 Italian commercial salami production processes, also in association with HPP, to assess the reduction of *Listeria innocua* during manufacturing; we used processing data, and *L. innocua* count to develop a model that describes the evolution of *Listeria* population according to process parameters.

2. Materials and methods

2.1 Salami production and inoculation

Data on ingredients and processing parameters of 51 different Italian salami were collected from thirteen manufacturers as previously described by Bonilauri et al. (2019); briefly, thirteen enterprises producing fermented sausages for the European market and exportation to the U.S. were involved in the study, and they were requested to supply the laboratory with the ingredient mix (sausage mix) to be inoculated and stuffed. We performed a total of 51 different challenge tests, and table 1 shows the details of ingredients and process parameters. The sausage mix was stored, refrigerated, and transported to the laboratory within 24 hours after production.

The sausage mix was inoculated with a blend of five strains of *Listeria innocua* isolates used as the surrogate of *L. monocytogenes* (Hu & Gurtler, 2017; Lebow et al., 2017; Merialdi, Ramini, Ravanetti, Gherri, & Bonilauri, 2015). More specifically, the laboratory used the following isolates: IZSLER 111373/1 and IZSLER 111373/2 isolated from environmental swabs collected in a pork meat transformation plant, IZSLER 257529/1 isolated from fresh pork sausages, IZSLER 257529/2 isolated from fresh pork meat and a collection strain ATCC 33090. The bacterial cultures were prepared following what indicated by Bonilauri et al. (2019). The enumeration of *L. innocua* count

was performed by serial decimal dilution and inoculation on Agar Listeria Ottaviani & Agosti (Biolife, Milan, Italy) plates, according to 11290-2 (ISO, 2017a). Following the study of Bonilauri and Colleagues (2019), we prepared a multi-strain cocktail; then, we stuffed the mix, and sausages were acidified/dried and seasoned according to the instructions of each enterprise. At the end of seasoning, 5 inoculated sausages were vacuum packed and used for HPP at 600 MPa for 300'' (Bover-Cid, Belletti, Aymerich, & Garriga, 2015; Merialdi, Ramini, Ravanetti, Gherri & Bonilauri, 2015; Rubio, Possas, Rincón, García-Gímeno & Martínez, 2018) by commercial apparatus. At the moment of HPP, the temperature of fermented sausages and treatment water was 4 and 14°C, respectively.

2.2 Sampling, physicochemical and microbiological examination

Five samples (of 25 g each) for each sausage mix were tested for the presence/absence of *Listeria* spp. according to according to ISO 11290-1 (ISO, 2017b) to evaluate the natural contamination of meat.

To determine pH and a_w values and to evaluate *L. innocua* count, for each challenge test, we collected and analyzed after the inoculation with *L. innocua* strains a total of 3 samples per sampling time namely; before stuffing (1), at the end of the acidification step (2) and at the end of seasoning (3), and, after HPP treatment, a total of 5 samples. Physicochemical analyses (pH and a_w) were performed as stated by Bonilauri and Colleagues (2019). *L. innocua* count was performed by serial dilution and direct surface plating onto Agar Listeria Ottaviani & Agosti (Biolife, Milan, Italy) plates according to ISO 11290-2 (ISO, 2017a). Whenever the count showed a result equal to < 10 CFU/g of *L. innocua,* we analyzed samples to detect *L. innocua* (presence/absence) 25g of each. *L. innocua* counts were expressed in CFU/g and converted into Log10 CFU/g. For the statistical elaboration of negative *Listeria* counts, a presence/absence test was performed and the result was reported respectively as 5 CFU/g (Log10 = 0.70) and 0.04 CFU/g (Log10 = -1.40) in case of presence or absence of the pathogen.

2.3 Data collection and the linear regression model

144 Data (mean of the 3 or 5 samples) on *L. innocua* count, pH, and a_w values were collected and used
145 for the linear regression model as detailed in the study of Bonilauri and Colleagues (2019) and as
146 outlined in Figure 1. Briefly, standard deviation (SD) of the *L. innocua* count obtained from the 3 or
147 5 samples of each challenge test was calculated; we considered the SD acceptable when <0.5 Log10
148 CFU/g. We calculated the Δ_a difference as well as Δs difference between *L. innocua* concentrations
149 between the different process steps as extensively described in the study of Bonilauri and
150 Colleagues (2019) and as summarized in Figure 1.

151 We used a linear regression model to describe the *L. innocua* count decrease observed during the
152 production process. In such a model, we reported the dependent and the independent variables in
153 Figure 1. Then, we used the variables that in the univariate model had a p-value <0.10 in a
154 multivariate stepwise linear regression with backward elimination. We considered second- and
155 third-order models (polynomial regression), and the polynomial regression model was kept only in
156 case of a significant increase in the coefficient of determination (R2). We tested a pair of
157 independent variables to detect co-linearity, while interactions were tested in those cases where two
158 or more factors were significant. Intercooled STATA 7.0 (Statacorp) was used to calculate the
159 coefficient of determination (R2) and significance of the model (F-statistic) ($p<0.05$).

160

161 ## 3. Results

162 ### 3.1 Salami productions experiment

163 We observed no association between the decrease of *L. innocua* count after the acidification/drying
164 phase and other independent variables; t1 and t2, maximum, minimum, and mean temperature during
165 acidification/drying and seasoning phase, caliber, and trimming. The concentration of NaCl, nitrate,
166 and nitrite used by the different enterprises was very similar, and thus, it was not possible to show an
167 association with *L. innocua* count.

Table 2 reports the results of the *L. innocua* count decrease after acidification/drying, seasoning, and HPP and the associated pH and a_w measurements (before stuffing, after acidification/drying, and after seasoning). Each type of salami has very different process parameters, and also, within the same type of salami, a high variability of process parameters between the different manufacturers has to be mentioned.

Before stuffing, a_w and pH values ranged respectively from 5.57 to 6.11 and from 0.963 to 0.979 (see Table 2). The acidification/drying phase had a variable length between 5 and 17 days and led the pH and a_w values, respectively, to 4.78 and 5.41, and 0.936 and 0.971 (see Table 2).

After seasoning, it varied from a minimum of 20 to a maximum of 90 days, and pH values showed a general increase that reached values ranging from 4.99 to 6.53, whereas a_w values were reduced by 0.880 to 0.936 (see Table 2).

The observed *L. innocua* counts always resulted in an SD <0.5 Log10 CFU/g, and therefore, in the text and tables, data are reported exclusively as mean. A decrease of *L. innocua* count could be observed during acidification/drying by 0.02-2.20 Log10 CFU/g (median 0.63), and, this decrease resulted statistically significant ($p<0.05$) in the univariate analysis reporting an association between the decrease of *L. innocua* count and the pH value after acidification/drying (pH2), ΔpH1-pH2, water activity at the end of the acidification/drying process (a_w 2), and trimming (mm). Even pH2 and Δ pH1-pH2 results were correlated, and thus, in the multivariate analysis, it was used Δ pH1-pH2 exclusively.

During acidification/drying phase, the observed *L. innocua* decrease could be outlined by a highly significant equation with about 50% of the variability that could be explicated by the model ($R2 = 0.48$; $p<0.00001$):

$\Delta_a = 10.107 + 1.564\ \Delta$ pH1-pH2 $-10.607\ a_w\ 2 - 0.052$ trimming (mm)

The White general test (White, 1980) is used for heteroscedastic errors in the regression by the model (White general test statistic: 11.10227 Chi-sq(9) *P*-value = 0.27), and in the independence test, residuals resulted uncorrelated with all the previous inputs.

In Figure 2, the *L. innocua* decay (Δa) observed at the end of the acidification/drying process was reported by a graph of residuals against data fitted into the multivariate model.

Considering polynomial regression or the interaction between variables, no significant increase of the model was observed.

During seasoning, it was observed a *L. innocua* reduction, varying from 0.02 to 3.4 Log10 CFU/g (median 0.53); only in three cases (see tests 6, 23, 43 in Table 2) the *L. innocua* count slightly increased during seasoning, but this apparent growth did not reach 0.5 Log 10CFU/g and, therefore, these three cases were not included in the regression analysis (EURL Lm, 2014).

In the univariate analysis, the decrease of *L. innocua* count (Δ_s) resulted significantly ($p<0.05$) associated with water activity at the end of the acidification/drying process (a_w 2), Δ a_w 2- a_w3, duration of the seasoning in days (ts), trimming (mm), and caliber (mm). A_w 2 and Δ a_w 2- a_w 3, seasoning in days (ts) and caliber (mm) results autocorrelated, so only the Δ a_w 2- a_w 3 and caliber (mm) were used in the multivariate analysis.

This highly significant equation here reported describes the reduction of *L. innocua* during the seasoning phase even if it only explains about 30% of the variability ($R2 = 0.32$; $p<0.0007$):

$\Delta_s = 22.87\ \Delta\ a_w\ 2\text{-}\ a_w\ 3 + 0.008$ caliber (mm) $-10.607\ a_w\ 2 - 0.078$ trimming(mm) $- 0.092$.

Again, the White general test (White, 1980) is used for heteroscedastic errors in the regression by the model (White general test statistic: 15.89082 Chi-sq(9) *P*-value = .07), and in the independence test, residuals resulted not correlated with all the past inputs. In Figure 3, the graph of residuals against fitted values of the multivariate model reports the *L. innocua* decay (Δs) at the end of the seasoning

process. However, when polynomial regression or interaction between variables was considered, the model did not show a significant increase.

3.2 HPP of Salami at the end of the seasoning phase

We observed a further decrease of *L. innocua* count after HPP (0.48-3.47 Log10 CFU/g). The efficacy of HPP resulted associated with pH2, pH3, a_w 2, a_w 3, caliber (mm), and duration of acidification/drying phase (days) in the univariate analysis, while only a_w 2, a_w 3 and pH2 remained significant in multivariate analysis. In this way, the reduction of *L. innocua* after HPP process could be described by the model with almost 36% of the variability resulted by the following highly significant equation (R2 = 0.357; p=0.0001):

$$\Delta h = 2.069\ pH2 + 27.188\ a_w 2 + 24.431\ a_w 3 - 57.246$$

In the model, the test for heteroscedasticity of the regression (White, 1980) (White general test statistic: 9.416029 Chi-sq(9) *P*-value = .40) was passed; as before, residuals resulted not correlated with all the past inputs in the independence test.

The graph of residuals against fitted values of the multivariate model, which describes the *L. innocua* decay (Δs) observed at the end of the s acidification/drying process, is reported in Figure 4. No significant increase of the model was observed by polynomial regression or interaction between variables. This relation means that the higher are pH and a_w after the acidification/drying and seasoning phases, respectively, the higher will be the reduction of *L. innocua* after HPP.

3.4 Global model

The reduction of *L. innocua* globally observed during the validation experiment (acidification/drying, seasoning and HPP treatment) could be described by the combination of the three models:

Dt = Da + Ds + Dh = 2.069 pH2 +1.564 Δ pH1-pH2 +16.5808 a_w 2 + 24.431 a_w 3 + 22.8704 Δ a_w 2- a_w 3 - 0.1292 trimming(mm) + 0.00793 caliber (mm) -47.2304, in Figure 5, it is possible to observe the graph of residuals against fitted values of the global model that describes approximately 65% of the total variability (R2=0.648). Finally, *L. innocua* count reduction always resulted > 1 Log10 CFU/g, varying from 1.04 to of 5.68.

4. Discussion

Data on the reduction of *L. innocua* during the processing of fifty-one challenge studies and after HPP were collected: we used the overall data to define a model that could be utilized and could be useful to predict the fate of *L. monocytogenes* in other types of salami or other enterprises.

In our study, we noted an overall reduction of *L. innocua* count during processing (acidification/drying plus seasoning) in all the challenge tests performed, ranging from 0.34-4.32 Log10 CFU/g. These results are roughly in agreement with previously reported studies on fermented sausages for example: in Soudjouk style fermented sausage a 0-1.86 Log CFU/g reduction of *L. monocytogenes* during production was reported (Hwang et al., 2009); Porto-Fett et al., 2010 reported a 1.1 to 2.2 reduction during fermentation and drying of Genoa salami; and Johnson et al. (Johnson, Doyle, Cassens, & Schoeni, 1988) reported a 1.2-1.8 reduction in salami; in Chouriço de Vinho Garcìa Dìez & Patarata (2013) it was observed a reduction of about 2 Log CFU/g, higher when *Lactobacillus sakei* was present as a starter culture. In Italian salami (Cacciatore and Felino), a < 1-log reduction was observed during the production process (Mataragas et al., 2015). Some authors (Degenhardt & Sant'Anna, 2007) observed a slightly higher reduction of *L. monocytogenes* in Italian sausages (2.57-3.81 log at final pH 5.10-5.16 and a_w at 0.883-0.897) and, in some cases, a growth during processing was shown (Campanini, Pedrazzoni, Barbuti, & Baldini, 1993; Nissen & Holck, 1998).

In traditional dry-cured fermented sausages, salt concentration, pH, and a_w are the main parameters controlling pathogenic microorganisms (Messier, Smith, & Tittiger, 1989) and also *L. monocytogenes* (Lindqvist & Lindblad, 2009). Additional hurdles that should be considered are nitrite, and another preservative addiction, the temperature of fermentation, length of the fermentation and drying phases, smoking, starter addition; also an influence of polyphosphate addition was reported (Gonzales-Barron et al., 2015); many of these last variables are more or less related to the pH and a_w reduction during processing.

In our study, we did not show any association between the concentration of NaCl, nitrate, and nitrite in the sausage mix, and the decrease of *L. innocua* count to be quite similar in the production processes of different types of salami. All the investigated enterprises used starter cultures. The duration and temperature of the acidification/drying process, as well as the temperature of the seasoning process, resulted not to be related to the reduction of *L. innocua* in the multivariate analysis. The reduction of *L. innocua* during processing turned out to be related to the pH value at the end of acidification/drying (pH2 or ΔpH1-pH2), to water activity at the end of the seasoning process (a_w3 or Δ a_w2- a_w 3), to the caliber of salami, and the duration of the seasoning in days (ts) which autocorrelated. In none of the challenge tests performed during acidification/drying phase, pH and a_w reached the no-growth limits indicated by EU Regulation 2073/2005 (Regulation (EC) 2073/2005/EC): pH≤4.4 or a_w≤ 0.92 or pH ≤5.0 and ≤0.94; despite this, in all the tests carried out, a reduction of *L. innocua* count was observed in the acidification/drying phase (0.02-2.20 Log10 CFU/g median 0.63). A further reduction was observed during seasoning (0.02-3.4 Log10 CFU/g median 0.53).

These results are generally in agreement with the results reported in the literature. In Linguiçola, a Portuguese traditional dry fermented sausage, at least three hurdles determined the evolution of *L. monocytogenes* population (low a_w, low pH and nitrite at an input level of about 150 ppm), and other factors contributed to its control like a more prolonged ripening and maceration period, a_w at the end of smoking (Gonzales-Barron et al., 2015). Hwang et al. (2009) reported that the decrease of *L.*

286 *monocytogenes* was significantly related to pH and a_w during the production of Soudjouk style
287 fermented sausage and that *L. monocytogenes* decrease occurred after pH was lowered to pH 5.1; a
288 significant lethality was observed at $a_w < 0.92$. Garriga et al. (2005) highlighted the importance of the
289 decrease of pH during the first 7 days of ripening for the prevention of *L. monocytogenes*
290 multiplication, suggesting the use of starter cultures in Mediterranean fermented sausages; also
291 Chikthimmah, Guyer, & Knabel (2001) reported that fermentation alone at pH 4.7 allowed a 2.4-log
292 reduction of *L. monocytogenes* load.

293 Based on an analysis of studies in literature carried out by Mataragas et al. (2015) relating the
294 inactivation rate of *L. monocytogenes* in fermented sausages, to the temperature of fermentation, pH
295 decrease in the fermentation phase and a_w decrease; temperatures above 20°C, especially in the first
296 48 hours of fermentation, turned out to be necessary for rapid inactivation of *L. monocytogenes*. The
297 same authors reported that the relation of pH and a_w with *L. monocytogenes* decrease appears to be
298 temperature-related and that temperature is related to *L. monocytogenes* reduction when pH and a_w
299 values are in the range that prevents *L. monocytogenes* growth. In our study the decrease of *L. innocua*
300 count resulted not related to the temperature of fermentation: we should consider that pH values at
301 the end of fermentation never resulted lower than the no-growth limit for *L. monocytogenes* (4.4)
302 resulting in 12 tests < 5.0 (4.78 – 4.98) and in 39 tests >5.00. A fundamental aspect that should be
303 mentioned is the relation of higher trimming and higher *L. innocua* load reduction; it is known that
304 physical status can influence bacterial behavior in food: in our study, we observed better acidification
305 in Milano type salami with their typical thin grain.

306 Italian salami has a higher pH (Garriga et al., 2005) in comparison to northern European sausages,
307 which are characterized by a sharper and faster pH reduction (Holck et al., 2011). In Mediterranean
308 sausages, the a_w reduction and the increase in aging can be more critical than in reducing *L.*
309 *monocytogenes* contamination (Mataragas et al., 2015; Nightingale, Thippareddi, Phebus, Marsden,
310 & Nutsch, 2006). In our study, the a_w reached values between 0.88-0.952 in different types of salami

311 at the end of seasoning with a_w >0.92 in only three challenge tests (median of 51 challenge tests 0.92).
312 The importance of a_w in controlling *L. monocytogenes* was reported in almost all studies on fermented
313 sausages (Degenhardt & Sant'Anna, 2007; Gonzales-Barron et al., 2015; Mataragas et al., 2015;
314 Nightingale, Thippareddi, Phebus, Marsden, & Nutsch, 2006; Novelli et al., 2017; Porto-Fett et al.,
315 2010; Roccato et al., 2017).

316 HPP was able to reduce further the *L. innocua* count by 0.48-3.47 Log10 CFU/g in all the challenge
317 tests with efficacy that resulted directly associated with a_w3 (a_w at the end of seasoning) and pH2 (pH
318 at the end of acidification/drying). The few references available on meat products, and dried
319 fermented sausages showed similar results: in cooked ham Jofré et al. (2008) showed a 3.5 Log CFU/g
320 reduction through treatment at 600 MPa for 5 minutes and a 3.4 log (400 MPa for 10 minutes)
321 reduction was observed by Marcos et al. (2008). Bover-Cid et al. (2015) showed a reduction of *L.*
322 *monocytogenes* in dry-cured ham at 600MPa for 5 minutes ranging from 2.24 log to 6.82 Log CFU/g
323 depending on the a_w and fat content of the ham.

324 Regarding fermented, dried products, the following studies reported a 1.6 – 5.0 Log CFU/g reduction
325 at 600MPa for 5 min in Genoa salami (Porto-Fett et al., 2010), a 1.79-3.15 Log CFU/g reduction in
326 Spanish chorizo at 600 MPa for 5-10 minutes (Rubio, Possas, Rincón, García-Gímeno, & Martínez,
327 2018), and a 0.9 Log CFU/g reduction in slightly fermented sausages at 400MPa for 10 minutes
328 (Garriga et al., 2005).

329 Several studies have shown that *L. monocytogenes* baroresistance increases when meat products
330 present a low a_w. Findings demonstrated that water activity could influence the efficacy of HPP
331 treatment and that low water activity protects microorganisms from environmental stresses. Bover-
332 Cid et al. (2015) showed that a_w affected the efficacy of HPP in *L. monocytogenes* inactivation in dry-
333 cured ham at different pressures; for example, a difference of more than 4 Log CFU/g of inactivation
334 was registered with an a_w of 0.960 vs an a_w of 0.860 at 600 MPa. An increase of a_w from 0.79 to 0.92
335 raised the reduction of *L. monocytogenes* from 0.07 to 2.17 Log in Spanish Chorizo sausage at 475

MPa (Rubio, Possas, Rincón, García-Gímeno, & Martínez, 2018); similarly, an increase of a_w from 0.88 to about 0.92 decreased *L. monocytogenes* significantly in Genoa salami (Porto-Fett et al., 2010).

To our knowledge, no data are available on the influence of pH on the baroresistance of *L. monocytogenes* in fermented meat products, but in other food low pH resulted in a noticeable synergistic effect with pressure on the inactivation of *L. monocytogenes* (Dogan & Erkmen, 2004; Gao, Ju, & Wu-Ding, 2007; Xu, Hyeon-Yong, & Ahn, 2009). The combination of low pH values with HHP processing resulted in a higher efficacy of this technology for reducing *L. monocytogenes* levels (Possas, Pérez-Rodríguez, Valero, & García-Gimeno, 2017); this relation should be further investigated in subsequent studies to improve the accuracy models on the survival of *L. monocytogenes* in fermented meat products undergoing HPP.

Usually, *L. monocytogenes* contamination in raw meat is approximately < 100 CFU (Farber & Peterkin, 1991). Whereas some processes are adequate to ensure the absence of *L. monocytogenes* others may permit the survival of *L. monocytogenes* in the final product, notably when raw materials are highly contaminated (Glass & Doyle, 1989; Johnson, Doyle, Cassens, & Schoeni, 1988) and additional treatment could be necessary. In this work, based on 51 challenge tests, we demonstrated the ability of Italian type salami production process with a final HPP treatment aimed at addressing lethality of *L. monocytogenes,* in order to validate products for export to the U.S.

Control of *L. monocytogenes* during the Italian Salami production process was attributed mainly to the decrease of pH during the fermentation and low a_w at the end of seasoning. Differences in fermentation and drying parameters in Italian style salami studied by different authors, together with strain resistance variation, may explain the different results reported in the literature (Nightingale, Thippareddi, Phebus, Marsden, & Nutsch, 2006). In this study, we performed 51 challenge tests by using the same 5 *L. innocua* strains, avoiding different strains behavior.

The highly significant equations of the model allow us to predict the Log CFU/g reduction of *L. monocytogenes* during the processing of Italian Salami based on the process parameters; the model

361 enables us to assess the further reduction of a commercial HPP process depending on the intrinsic
362 characteristics of the product. By evaluating raw materials to determine the initial level of
363 contamination, processors and Authorities may assess the ability of the process to control incoming
364 pathogens, to predict the *L. monocytogenes* load at the end of the process, and to evaluate the need
365 for a process modification or for the addition of a final lethal process.

366

367 ## 5. References

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Table 1. Process parameters and ingredients of the different types of Italian salami used for challenge tests.

Test	Salami type	Enterprise	Acidification/drying		Seasoning		Characteristics of different salami					
			Time (days)	Temperature °C[1]	Time (days)	Temperature °C[1]	NaCl %	Nitrate	Nitrite	Caliber (mm)	Trimming (mm)	Type of envelope
1	Low fat	A	7	18-22	31	12-15	2.3	150 ppm	50 ppm	80	5.0	synthetic
2	Milano	B	11	7-19	65	7	2.7	125 ppm	67 ppm	80	3.0	synthetic
3	Finocchiona	B	10	15-20	68	10	2.7	125 ppm	67 ppm	120	6.0	synthetic
4	Napoli	B	7	15-20	28	10	3.0	125 ppm	67 ppm	90	12.0	synthetic
5	Felino	C	5	14-22	40	13-14	2.5	70 ppm	120 ppm	60	5.0	synthetic
6	Felino	C	7	14-22	55	12-13	2.5	70 ppm	70 ppm	80	7.0	synthetic
7	Pepperoni	C	5	14-22	30	13-14	2.5	70 ppm	120 ppm	60	8.0	synthetic
8	Strolghino	C	5	14-22	21	13-14	2.5	70 ppm	120 ppm	38	8.0	synthetic
9	Felino large caliber	D	5	15-25	69	10-15	2.3	150ppm	75ppm	80	6.0	synthetic
10	Genoa	E	8	18-27	41	16-18	2.3	50 ppm	150 ppm	60	6.0	synthetic
11	Milano	F	5	16-24	70	9-11	2.5	150 ppm	0 ppm	110	3.5	synthetic
12	Milano	F	5	16-24	90	9-11	2.5	150 ppm	0 ppm	110	3.5	synthetic
13	Cacciatore	G	6	18-26	21	18-19	2.5	150 ppm	0 ppm	38	5.0	synthetic
14	Cacciatore	G	6	18-26	28	18-19	2.6	150 ppm	0 ppm	38	5.0	synthetic
15	Napoli	H	3	19-24	35	14-16	2.6	150 ppm	0 ppm	65	6.5	synthetic
16	Napoli	H	3	19-24	31	14-16	2.3	150 ppm	0 ppm	65	6.5	synthetic
17	Napoli	H	3	19-24	27	14-16	2.3	150 ppm	0 ppm	65	6.5	synthetic
19	Varzi	I	6	15-25	43	12-19	2.3	150 ppm	0 ppm	65	13.0	natural
18	Varzi	I	6	15-25	43	12-19	2.5	150 ppm	0 ppm	65	13.0	natural
20	Milano	J	7	18-22	63	10-14	2.5	150 ppm	0 ppm	100	3.5	synthetic
21	Cacciatore	B	6	12.5-21	28	10-16	2,4	150 ppm	70 ppm	45	8.0	synthetic
22	Pepperoni small caliber	B	6	12.5-21	28	10-16	2.4	150 ppm	70 ppm	45	8.0	synthetic
23	Flattened pepperoni	B	17	9-21	43	8-12.5	2.7	150 ppm	70 ppm	60	13.0	synthetic
24	Flattened pepperoni	B	17	9-21	43	13	2.7	150 ppm	70 ppm	60	13.0	synthetic
25	Flattened pepperoni	B	17	9-21	72	8-12.5	2.7	150 ppm	70 ppm	60	13.0	synthetic
26	Cacciatore	B	8	12.5-21	31	10-16	2.4	150 ppm	70 ppm	45	8.0	synthetic
27	Milano small caliber	B	8	12.5-21	35	10-16	2.4	150 ppm	70 ppm	45	4.5	synthetic
28	Hungarian small caliber	B	6	12.5-21	25	10-16	2.4	150 ppm	70 ppm	45	3.0	synthetic
29	Pepperoni small caliber	B	6	12.5-21	24	10-16	2.4	150 ppm	70 ppm	45	8.0	synthetic
30	Pepperoni small caliber	B	8	12.5-21	29	10-16	2.4	150 ppm	70 ppm	45	8.0	synthetic
31	Felino	G	8	14-24	57	14-15	2.5	0 ppm	0 ppm	44	7.0	synthetic
32	Felino	L	4	15-24	33	10.5-17	2.3	150 ppm	50 ppm	65	7.0	natural
33	Garlic	M	4	16-23	37	13-15	2.4	120 ppm	30 ppm	65	8.0	synthetic
34	Genoa	D	8	14-24	57	14-15	2.5	150 ppm	0 ppm	44	7.0	synthetic

35	Genoa	I	6	17-26	47	17	2.3	150 ppm	50 ppm	95	6.0	synthetic
36	Felino	I	9	6-22	38	12-13	2.4	0 ppm	0 ppm	65	8.0	synthetic
37	Felino	I	5	19-24	20	19	2.5	150 ppm	50 ppm	45	3.5	synthetic
38	Felino	N	5	26-18	42	14-16	2.4	150 ppm	0 ppm	75	7.0	synthetic
39	Felino	O	6	16-23	37	13-15	2.4	150 ppm	30 ppm	43	8.0	synthetic
40	Milano	O	6	8-19	20	12-13	2.6	150 ppm	30 ppm	43	3.0	synthetic
41	Pepperoni	O	6	8-19	26	12-13	2.6	150 ppm	30 ppm	43	5.0	synthetic
42	Pepperoni	O	6	8-19	31	12-13	2.6	150 ppm	30 ppm	50	5.0	synthetic
43	Pepperoni	O	6	8-19	29	12-13	2.6	150 ppm	30 ppm	50	3.0	synthetic
44	Milano	O	7	8-16	42	14-16	2.6	150 ppm	30 ppm	90	6.0	synthetic
45	Milano	O	5	16-24	69	12-14	2.5	150 ppm	30 ppm	100	3.0	synthetic
46	Pepperoni	O	7	8-19	26	12-13	2.6	150 ppm	30 ppm	43	5.0	synthetic
47	Pepperoni	O	5	8-19	24	12-13	2.6	150 ppm	30 ppm	43	5.0	synthetic
48	Pepperoni	O	5	8-19	34	12-13	2.6	150 ppm	30 ppm	50	5.0	synthetic
49	Pepperoni	O	5	8-19	37	12-13	2.6	150 ppm	30 ppm	50	5.0	synthetic
50	Felino	O	5	8-19	24	12-13	2.6	150 ppm	30 ppm	40	5.0	synthetic
51	Felino	O	5	8-19	31	12-13	2.6	150 ppm	30 ppm	50	5.0	synthetic

[1]= range of temperature: a variable temperature can be applied during the phase.

Table 2. *Listeria innocua* Log CFU reduction and pH, a_w values during manufacturing and after HPP treatment of Italian salami.

Test	Salami type	Salami before stuffing		Salami after acidification/drying			Salami after seasoning			Process + HPP		
		pH1	a_w1	pH2	a_w2	$-\Delta_a$	pH3	a_w3	Δ_s	Δp	Δ HPP treatment	Total reduction
1	Low fat	6.00	0.969	5.12	0.971	0.25	5.11	0.951	0.31	0.56	3.01	3.57
2	Milano	5.75	0.970	5.24	0.966	0.49	5.47	0.930	0.47	0.96	3.00	3.96
3	Finocchiona	5.91	0.974	5.41	0.960	0.61	5.20	0.940	1.32	1.93	1.79	3.72
4	Napoli	5.79	0.968	5.25	0.957	0.78	5.38	0.933	0.43	1.21	2.69	3.90
5	Felino	5.57	0.970	5.27	0.962	0.30	5.74	0.920	0.80	1.10	3.46	4.56
6	Felino	6.11	0.965	5.29	0.963	0.84	5.42	0.916	-0.02	0.82	2.50	3.32
7	Pepperoni	5.65	0.966	5.28	0.971	0.37	5.56	0.880	1.54	1.91	1.43	3.34
8	Strolghino	5.87	0.970	5.10	0.963	0.77	5.83	0.922	0.10	0.87	2.55	3.42
9	Felino large caliber	5.99	0.973	5.06	0.970	1.51	5.62	0.910	1.53	3.04	1.17	4.21
10	Genoa	5.89	0.973	4.98	0.967	1.65	6.11	0.917	1.70	3.35	1.31	4.66
11	Milano	6.02	0.966	5.34	0.953	0.41	5.45	0.922	0.51	0.92	3.03	3.95
12	Milano	6.02	0.966	5.34	0.953	0.41	5.47	0.898	1.09	1.50	2.29	3.79
13	Cacciatore	5.73	0.971	5.38	0.948	0.64	5.40	0.910	0.22	0.86	2.65	3.51
14	Cacciatore	5.73	0.971	5.38	0.948	0.64	5.52	0.896	0.36	1.00	1.60	2.60
15	Napoli	6.10	0.974	5.11	0.947	1.33	5.58	0.898	0.73	2.06	1.81	3.87
16	Napoli	6.10	0.974	5.11	0.947	1.33	5.63	0.923	0.27	1.60	1.57	3.17
17	Napoli	6.10	0.974	5.11	0.947	1.33	5.44	0.932	0.09	1.42	3.47	4.89
18	Varzi	5.80	0.972	5.20	0.967	0.06	5.96	0.913	0.53	0.59	1.78	2.37
19	Varzi	5.80	0.972	5.20	0.967	0.06	5.96	0.913	0.53	0.59	2.20	2.79
20	Milano	5.65	0.967	4.78	0.953	1.32	5.16	0.905	0.97	2.29	1.03	3.32
21	Cacciatore	5.93	0.970	5.22	0.950	0.60	5.79	0.908	0.02	0.62	0.49	1.11
22	Pepperoni small caliber	5.89	0.970	4.86	0.950	0.32	5.33	0.910	0.13	0.45	0.59	1.04
23	Flattened pepperoni	5.74	0.967	4.97	0.956	0.58	5.46	0.921	-0.24	0.34	1.03	1.37
24	Flattened pepperoni	5.74	0.967	5.05	0.956	0.58	5.12	0.915	0.23	0.81	0.48	1.29
25	Flattened pepperoni	5.76	0.976	4.87	0.957	0.22	4.99	0.908	0.26	0.48	0.69	1.17
26	Cacciatore	5.69	0.970	5.21	0.960	0.40	5.40	0.917	1.26	1.66	1.13	2.79
27	Milano small caliber	5.71	0.970	5.15	0.960	0.17	4.99	0.917	2.14	2.31	1.35	3.66
28	Hungarian small caliber	5.95	0.970	5.31	0.960	0.51	5.26	0.911	2.22	2.73	0.73	3.46
29	Pepperoni small caliber	5.71	0.970	5.16	0.960	0.11	5.22	0.913	1.25	1.36	1.00	2.36
30	Pepperoni small caliber	5.66	0.977	5.18	0.957	0.02	5.52	0.920	0.71	0.73	1.46	2.19
31	Felino	5.92	0.975	5.09	0.953	1.08	6.17	0.921	1.20	2.28	1.73	4.01
32	Felino	5.76	0.975	5.28	0.969	0.04	5.34	0.920	0.58	0.62	3.20	3.82
33	Garlic	5.91	0.969	5.16	0.950	0.59	5.38	0.919	1.51	2.10	1.11	3.21
34	Genoa	5.90	0.979	5.26	0.959	0.53	6.33	0.920	0.57	1.10	2.52	3.62
35	Genoa	5.77	0.965	4.88	0.960	1.36	5.28	0.912	1.20	2.56	2.54	5.10

36	Felino	5.88	0.963	5.32	0.956	0.05	5.64	0.915	0.48	0.53	2.43	2.96
37	Felino	5.75	0.971	5.10	0.960	1.16	6.53	0.921	0.63	1.79	2.93	4.72
38	Felino	5.89	0.971	5.05	0.968	0.6	6.16	0.921	1.64	2.24	1.96	4.20
39	Felino	5.76	0.971	5.14	0.952	0.92	5.89	0.919	0.65	1.57	2.36	3.93
40	Milano	5.75	0.969	5.06	0.954	0.81	5.85	0.921	0.47	1.28	1.86	3.14
41	Pepperoni	5.82	0.970	4.91	0.944	1.85	5.76	0.918	0.72	2.57	1.06	3.63
42	Pepperoni	5.82	0.970	4.91	0.949	2.2	6.10	0.923	0.21	2.41	1.37	3.78
43	Pepperoni	5.75	0.969	4.97	0.955	1.43	5.75	0.921	-0.42	1.01	2.59	3.60
44	Milano	5.68	0.971	4.98	0.960	0.75	5.12	0.918	0.95	1.70	2.30	4.00
45	Milano	5.88	0.970	5.04	0.966	0.92	6.08	0.921	3.40	4.32	1.36	5.68
46	Pepperoni	5.72	0.971	4.97	0.952	0.68	5.37	0.920	0.49	1.17	0.99	2.16
47	Pepperoni	5.80	0.972	5.14	0.936	1.03	5.21	0.921	0.26	1.29	0.85	2.14
48	Pepperoni	5.80	0.972	5.10	0.941	1.040	5.12	0.906	0.07	1.11	0.62	1.73
49	Pepperoni	5.72	0.971	4.97	0.956	0.580	5.29	0.920	0.84	1.42	0.55	1.97
50	Felino	5.77	0.973	5.13	0.938	0.680	5.16	0.925	0.22	0.90	0.75	1.65
51	Felino	5.77	0.973	4.95	0.945	0.460	5.06	0.903	0.17	0.63	0.81	1.44

Data are expressed as mean of three or five (only for HPP) samples; SD was calculated and considered acceptable when <0.5 Log10 CFU/g.

-Δ_a: reduction of *Listeria innocua* after acidification/drying; Δ_s: reduction of *Listeria innocua* at the end of seasoning; Δ_s: reduction of *Listeria innocua* as sum of Δ_a and Δ_s; Δ HPP: reduction of *Listeria innocua* after HPP; Total Log reduction: reduction of *Listeria innocua* as sum of Δ_s and Δ HPP