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1	Modeling and designing a Listeria monocytogenes control strategy for dry-cured
2	ham taking advantage of water activity and storage temperature
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4	Cristina Serra-Castelló¹, Anna Jofré¹, Margarita Garriga, Sara Bover-Cid*
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6	IRTA. Food Safety Program. Finca Camps i Armet s/n. 17121 Monells (Spain)
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24	Declarations of interest: none
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28	¹ These authors contributed equally to this work.
29	*Corresponding author.
30	Tel.: +34 972 630052 extension 1488; E-mail address: sara.bovercid@irta.cat (S. Bover-Cid)

31	Abstract
32	Dry-cured ham is a shelf stable product that can be contaminated with Listeria monocytogenes
33	due to post-processing operations, compromising the compliance of zero tolerance policies (e.g.
34	US Listeria rule). The present study quantifies the behavior of L. monocytogenes in sliced
35	Spanish dry-cured ham of different water activity (a_{w}) during storage at different temperatures.
36	Inactivation kinetics were estimated by fitting primary models to the experimental data. The
37	effect of temperature and \boldsymbol{a}_{w} on kinetic parameters was characterized through secondary
38	polynomial models. L. monocytogenes viability decreased in all the assayed conditions,
39	confirming that dry-cured ham is not only listeriostatic but listericidal. The fastest and highest
40	reductions were observed at 25 °C, with 1 Log reduction after 6 and 9 days in Iberian and
41	Serrano ham respectively. The work provides scientifically-based data and models to design a
42	low-cost control measure based on a corrective storage as a post-lethality treatment to enhance

45 **Keywords**

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46 Listeria monocytogenes; RTE meat products; modeling; non-thermal inactivation; post-lethality

the accomplishment of zero tolerance requirements.

47 treatment

1. Introduction

- 50 Dry-cured ham is a raw ready-to-eat (RTE) meat product highly appreciated worldwide for its
- 51 particular sensory characteristics. In 2017, the production reached the 299,000 tonnes in Spain,
- more than 15% being intended for export, which represents a 70% increase of the tonnes
- exported in 2012 (ANICE, 2019). The traditional EU markets have been mainly France,
- 54 Germany, Portugal, Italy and the United Kingdom. Major emerging markets like Mexico, USA,
- Australia, South Korea, Chile, Japan, Argentina and New Zealand are foreseen of a great
- importance for the Spanish meat sector (ANICE, 2019). Dry-cured ham is considered a shelf-
- 57 stable RTE product due to its low water activity (a_w) resulting from the salting and drying
- process of manufacture that renders a product with a high salt content up to 15% of the dry
- 59 matter (Costa-Corredor, Serra, Arnau, & Gou, 2009; FSIS, 2010). Besides, the manufacturing
- process of dry-cured ham includes several steps, such as salting, post-salting, curing and
- drying/aging, with a duration depending on the type of dry-cured ham (from 7 months in the
- case of Serrano type, up to 18 to 48 month for Iberian type). The processing conditions have
- been proved to be lethal for *Listeria monocytogenes*, reducing the levels of the pathogen when
- 64 inoculated in meat raw material by 4 Log units (Reynolds, Harrison, Rose-Morrow, & Lyon,
- 65 2001) in US type of dry-cured ham to 6 Log units in Spanish type dry-cured ham (Medina,
- 66 2017).
- 67 However, it has also been demonstrated that when marketed as convenient packaged formats
- 68 (e.g. boneless blocks, diced, sliced), post-processing manipulation exposes the product to cross-
- 69 contamination with pathogens, L. monocytogenes being of particular concern due to its
- vbiquitous nature and persistence in processing areas (Martín, Perich, Gómez, Yangüela,
- 71 Rodríguez, Garriga, et al., 2014; Talon, Lebert, Lebert, Leroy, Garriga, Aymerich, et al., 2007).
- 72 The contamination during post-processing operations is highly dependent on the production
- plant, with a prevalence reported between 3.6% and 18.4% (Prencipe, Rizzi, Acciari, Iannetti,
- Giovannini, Serraino, et al., 2012). The overall occurrence of L. monocytogenes in retail dry-
- cured ham varies from not detected (Cabedo, Picart-Barrot, & Teixidó-Canelles, 2008;
- 76 Giovannini, Migliorati, Prencipe, Calderone, Zuccolo, & Cozzolino, 2007) to a prevalence of
- ca. 2% (Jemmi, Pak, & Salman, 2002; Prencipe, et al., 2012), 4% (Giovannini, Migliorati,
- 78 Prencipe, Calderone, Zuccolo, & Cozzolino, 2007) and up to 12% (Uyttendaele, De Troy, &
- 79 Debevere, 1999).
- 80 Food safety criteria regulations regarding *L. monocytogenes* in RTE products differ between
- 81 countries. For EU member states, Regulation (EC) 2073/2005 establishes a maximum of 100
- 82 CFU/g of L. monocytogenes during the shelf-life of the product provided it is not intended for
- infants or special medical purposes or it does not favor the growth of the pathogen to more than
- 84 100 CFU/g at the end of shelf-life. This regulation states that RTE foods with a_w equal or below

85	0.92 automatically are considered to belong to the category of RTE food unable to support the
86	growth of L. monocytogenes (European Commission, 2005). This aw value is usually used by
87	manufacturers as the acceptable limit for the commercial production of dry-cured ham. A
88	similar tolerance approach is applied by Canadian regulation (Health Canada, 2011) and that of
89	Australia and New Zealand (Australian Government, 2017). In contrast, in the US Listeria rule
90	(FSIS, 2015), a zero-tolerance policy is imposed, which means that RTE products must not be
91	released if they contain L. monocytogenes or have been in contact with a food contact surface
92	contaminated with the pathogen. To meet this requirement, the establishment producing RTE
93	foods exposed to L. monocytogenes contamination can apply control alternatives, based on
94	antimicrobial agents or processes (AMA/P) to suppress pathogen growth and/or post-lethality
95	treatments (PLT) to eliminate or reduce L. monocytogenes (FSIS, 2015).
96	Although, to the authors knowledge, no listeriosis case or outbreak has been associated with
97	dry-cured ham, the pressure derived from zero-tolerance policies of the public health authorities
98	of some countries as well as commercial demands, poses a challenge for the dry-cured meat
99	industry due to the technical difficulties for the control and eradication of L. monocytogenes. To
100	fulfil legal and/or commercial requirements, dry-cured ham producers should design risk
101	minimization strategies to avoid sources of recontamination and/or apply validated PLT before
102	commercial expedition. For dry-cured ham, thermal based post-lethality treatments are not
103	suitable due to the negative impact on the organoleptic properties. Emerging non-thermal
104	alternatives, such as high pressure processing have been proposed, though they show limited
105	effect due to the piezoprotection caused by the low aw of the product (Bover-Cid, Belletti,
106	Aymerich, & Garriga, 2015; Hereu, Bover-Cid, Garriga, & Aymerich, 2012) . Moreover, the
107	economical investment needed to implement high pressure processing are not affordable for
108	many producers. Therefore, feasible alternative strategies based on the physicochemical
109	properties of the product itself should be investigated.
110	In this framework, the present study aimed to evaluate through a modeling approach the
111	behavior of L . monocytogenes in dry-cured ham, as a function of product a_w and storage
112	temperature. The final objective was to design a feasible control measure contributing to ensure
113	the accomplishment of zero-tolerance policies and commercial requirements. The study was
114	carried out in two Spanish dry-cured ham types as the most typical and appreciated by
115	consumer, Iberian ham and Serrano ham, showing differences in raw material (Iberian vs white
116	pigs, respectively) and the process conditions, including length (up to 600 days vs 210 days,
117	respectively) leading to end-products with different quality and prize (Lorido et al. 2015)

2. Material and methods

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2.1. Product characteristics

- 122 Two different types of dry-cured ham were studied: Serrano and Iberian. Three batches for each
- type with different weight loss (high, medium, low), corresponding to a_w values of 0.87, 0.89
- and 0.91 (Serrano type) and 0.85, 0.88 and 0.91 (Iberian type) in central sections of the ham
- piece, were used to study the impact of different values of a_w on the *L. monocytogenes* growth.
- Samples of hams were obtained directly from the producer, in vacuum-packed boneless blocks
- format and stored under refrigeration (<2 °C) until being used. Special attention was paid to
- obtain sections with the target a_w, which was measured at 25 °C using an Aqualab® equipment
- 129 (Decagon Devices, Pullman, WA, USA).

- 2.2. L. monocytogenes strains and inoculum preparation
- 132 A cocktail of equal concentration of four *L. monocytogenes* strains with different genotype and
- serotype (Table 1), isolated from pork meat industrial environment (Medina, 2017; Ortiz,
- López, Villatoro, López, Carlos Davila, & Martínez-Suárez, 2010) was used. The strains were
- kindly provided by Dr. M. Medina (INIA, Spain). Stock cultures of each strain were kept at -80
- °C in Brain Heart Infusion (BHI) broth (Beckton Dickinson, Sparks, Md., USA). A culture of
- each strain was separately grown in Tryptic Soy Broth with 0.6% Yeast Extract (TSBYE,
- Difco) following two consecutive incubation steps: firstly 18 h at 37 °C and secondly 4 days at
- 8 °C to obtain cold-adapted early stationary phase cultures according to the recommendations of
- the technical guidance document for conducting shelf-life studies on *Listeria monocytogenes* in
- 141 RTE (EURL Lm, 2014). This physiological state (cold adaptation) mimics the chilled
- 142 conditions usually found in clean rooms for production of RTE products (e.g. conveyor belts,
- slicing machines and packaging equipment).

- 145 2.3. Challenge test: sample preparation, inoculation and storage conditions
- Boneless block hams (Serrano and Iberian, described in section 2.1) were aseptically sliced.
- Each slice (of ca. 20-30 g) was inoculated (1% v/w) with the 4-strain cocktail of L.
- 148 *monocytogenes* described above to achieve *ca.* 10⁶-10⁷ CFU/g by properly diluting the culture in
- saline solution (0.85% NaCl and 0.1% Bacto Peptone (Beckton Dickinson)). The inoculum was
- spread on the dry-cured ham slice and left to absorb for 2 min under a laminar flow cabinet. The
- slices were overlaid cut in two and each part was individually vacuum packaged (in a EV-15
- vacuum packer; Tecnotrip, Terrassa, Spain) in PA/PE bags (oxygen permeability of 50
- cm³/m²/24 h and a low water vapor permeability of 2.8 g/m²/24 h; Sistemvac, Estudi Graf S.A.,
- Girona, Spain). Samples of each type of dry-cured ham were randomly distributed in 4 groups
- to be stored at 2, 8, 15 and 25 °C for a maximum of 6 months. These temperatures cover the
- reasonably foreseeable range for the storage and commercially display dry-cured ham, which
- has a maximum shelf-life of 6 months under refrigeration. The a_w value of the samples was not
- significantly different after the inoculation. A total of 390 samples were prepared.

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- 160 2.4. *Monitoring* L. monocytogenes *behaviour*
- To monitor *L. monocytogenes* survival, samples from 24 experimental conditions (2 types of
- dry-cured ham, 3 a_w and 4 storage temperatures) were periodically analyzed to get a total of 12
- to 19 data points distributed all along the storage period. This resulted in 201 and 189 samplings
- 164 for Serrano and Iberian ham, respectively. Each sample was homogenized 1/10 in saline
- solution in a bag Blender Smasher® (bioMérieux, Marcy-l'Etoile, France) for 1 minute and 10-
- fold serially diluted in saline solution. L. monocytogenes was enumerated on Chromogenic
- Listeria Agar (CLA; Oxoid Ltd., Basingstoke, Hampshire, UK) and incubated at 37 °C for 48 h.
- For samples with expected concentration of *L. monocytogenes* below the quantification limit of
- 4 CFU/g (resulting from plating 4 ml of homogenate in a 14 cm diameter plate), the
- presence/absence of the pathogen was investigated by enrichment of 25-30 g-samples in 225 ml
- of TSBYE and incubated 48 h at 37 °C. After enrichment, the presence of L. monocytogenes
- was confirmed by plating on CLA (Sara Bover-Cid, Serra-Castelló, Dalgaard, Garriga, & Jofré,
- 173 2019). For modeling purposes, samples below the detection of plate count with positive after
- enrichment were assumed to be 1 cell in 30 g (i.e. -1.5 Log cfu/g). Negative results (i.e. not
- detected in 25-30g) were not recorded in any analyzed sample.

- 177 2.5. Primary model fitting
- Four different inactivation primary models (Table 2), including the Weibull, Log-linear, Log-
- 179 linear with tail and Log-linear with shoulder models (as described in Hereu, Dalgaard, Garriga,
- Aymerich, Bover-Cid, 2012) were used. For modeling purposes, to avoid small differences in
- initial concentrations, models were fitted to the *L. monocytogenes* inactivation data, expressed
- in terms of Log (N/N_0) (Martino & Marks, 2007) as a function of time (days) for each of the 24
- 183 combinations of conditions (type of ham, a_w and storage temperature). In addition, the Log N/N₀
- at time zero (the initial inactivation) was fixed to 0 for parsimony purposes. All primary models
- were fitted using R with the nls2 and nls packages of R software (R Core Team, 2019).
- Besides visual evaluation of the fitted curves, the standard error of the coefficients, the residual
- sum of squares (RSS) and the adjusted coefficient of determination (R²_{adj}) were calculated as
- measures for goodness of fit. The primary model with a better goodness of fit, e.g. lower RSS
- and higher R²_{adj} was chosen.

- 191 2.6. Secondary model fitting
- Polynomial models were developed to quantitatify the effect of the independent variables (a_w
- and storage temperature) on the primary kinetic parameters.. Different transformations,
- including square root, inverse, Ln and Log, of the primary kinetic parameters were assessed.
- 195 Estimation of the model parameters was carried out with R software (R Core Team, 2019)

196	applying stepwise backward linear regression to obtain equations with only the significant
197	parameters. The standard error of the coefficients, RSS and R^2_{adj} were calculated as measures
198	for goodness of fit.
199	Besides the two-step modeling approach described above, the global one-step regression was
200	applied for the fine tuning of the model parameters of L. monocytogenes inactivation on Serrano
201	and Iberian type hams. For this, the secondary models for δ and p were integrated into the
202	primary Weibull model and the combined model was fitted to the entire set of inactivation data
203	points by one-step global non-lineal regression approach (Jewell, 2012; Martino & Marks,

204 2007).

The goodness of fit the one-step global models were assessed in terms of standard error of the coefficients, RSS and R²_{adj} and by using graphs of observed and fitted values. The F-test was applied to assess the need of two different models for each product type compared to the suitability of a single model for both types of dry-cured ham.

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2.7. Model predictive performance

- 211 Inactivation data recorded for *L. monocytogenes* on dry-cured Serrano and Iberian hams
- collected from scientific literature (Bover-Cid, Jofré, & Garriga, 2016; Hereu, Bover-Cid,
- Garriga, & Aymerich, 2012; Morales, Calzada, & Nuñez, 2006) were compared with
- 214 predictions obtained by the models developed in the present study. To compare the observed
- and predicted inactivation during storage, the Acceptable Simulation Zone (ASZ) approach was
- used. Simulations were considered acceptable when at least 70% of the observed Log (N/N₀)
- values were inside the corresponding acceptable zone, e.g \pm 0.5 (Møller, Ilg, Aabo, Christensen,
- 218 Dalgaard, & Hansen, 2013).

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3. Results and discussion

- 3.1. Description of the behavior of L. monocytogenes on sliced dry-cured ham
- 223 The survival of *L. monocytogenes* under the 24 experimental conditions assayed is shown in
- Figure 1. The viability of *L. monocytogenes* was compromised in all the 24 conditions assayed,
- showing in most of the cases a significant reduction of the counts during the storage of sliced
- and vacuum packed dry-cured ham.
- Therefore, the results indicated that under these conditions dry-cured ham is not only
- 228 listeriostatic but listericidal. The magnitude of the lethal effect varied significantly according to
- the product characteristics and storage temperature. Thus, Iberian type ham favored an earlier
- and more pronounced inactivation of L. monocytogenes, compared with Serrano type, even if a_w
- was similar. The greater inactivation of *L. monocytogenes* in Iberian type can hardly be
- explained by the slightly lower pH (5.7 in Iberian versus 5.9 in Serrano), and probably other

233 non-determined intrinsic factors of the product may have contributed to these differences. In 234 both types of ham, the lower the a_w the higher the inactivation of the pathogen. 235 The impact of the temperature during storage of sliced dry-cured ham was also very noticeable. 236 At refrigeration temperatures (2 and 8 °C) the listericidal effect of the product was limited, 237 especially in higher a_w products (ca. only 1 Log reduction was achieved after 6 months of 238 storage). On the other hand, at higher temperatures, especially at 25 °C, the inactivation was 239 considerably more intense, achieving between 6 and 7 Log reductions of the level of the 240 pathogen within 2 and 3 months of storage. Reynolds et al. (2001) also reported higher 241 inactivation of L. monocytogenes during storage at room temperature of post-processing 242 inoculated dry-cured ham. 243 The loss of viability of L. monocytogenes in dry-cured ham under the tested storage conditions 244 can be explained by the metabolic exhaustion phenomenon associated with antimicrobial 245 hurdles. The characteristics of the product, pH and mainly a_w of the ham did not allow the 246 growth of the pathogen. In non-growth conditions of shelf-stable foods, the microorganisms 247 tend to die, and die more rapidly when the conditions of shelf-stability approach the limits of 248 growth, for example, as in this case, at room temperature (Leistner, 2000). These results point 249 out that proper storage conditions of dry-cured ham would favor inactivation of L. 250 monocytogenes contaminating the finished products before their release to retail, distribution, 251 export, etc. Thus, dry-cured ham manufacturers can take advantage of this phenomenon as an 252 opportunity to design a control measure into their production process, e.g. a validated post-253 lethality treatment, in order to minimize the risk of non-compliance of the zero-tolerance 254 requirements. 255 256 3.2. Inactivation kinetics of L. monocytogenes on dry-cured ham. Primary modeling 257 Four primary inactivation models (Log-linear, Log-linear with tail, Log-linear with shoulder and 258 Weibull) were fitted to inactivation data. The estimated kinetic parameters obtained using Log-259 linear based models together with the goodness of fit are summarized in supplementary material 260 (Table S1 for Serrano and Table S2 for Iberian dry-cured ham). The fitted kinetic parameter 261 values and measures of goodness of fit obtained for the Weibull model are reported in Table 3. 262 The graphical results of the Weibull model fit to inactivation of L. monocytogenes on sliced 263 vacuum-packed dry-cured ham, according to the type of ham, aw and storage temperature are shown in Figure 1. The Weibull model with two parameters (δ and p) allowed the fitting of 264 265 different inactivation shapes through the p parameter and resulted in the best fit of the 266 experimental data as indicated by the lower RSS and the higher R²_{adj} values in comparison with 267 the Log-linear based models. Therefore, the Weibull model was selected to describe the 268 inactivation kinetics of *L. monocytogenes* on dry-cured ham.

- The estimated δ parameter, e.g. the time needed for the first Log reduction, was systematically
- lower in Iberian than in Serrano ham, confirming that Iberian type favored an earlier
- inactivation of *L. monocytogenes*. In addition, the higher the storage temperature the lower the
- δ , pointing out that increasing up to room temperature favored the inactivation of the pathogen.
- On the other hand, the opposite effect was found for a_w , as the higher the a_w , the higher the δ . At
- 274 refrigeration temperatures (e.g. 2 and 8 °C), especially for products with high a_w (>0.91), the δ
- showed values higher than 100 days, indicating that refrigeration slowed down the metabolic
- 276 reactions preventing the metabolic exhaustion *L. monocytogenes* cells.
- 277 At the highest studied storage temperature (e.g. 25 °C) the shape of the inactivation curve (p)
- was highly dependent on the a_w of the product. In low a_w hams (0.85 and 0.87), L.
- 279 monocytogenes inactivation showed a concave shape (p<1), indicating a higher inactivation at
- 280 the beginning of the storage, and thus, the occurrence of a sort of tail of resistant cells. On the
- other hand, in products with the highest $a_w(0.91)$, L. monocytogenes fate showed a convex
- shape (p>1), indicating lower inactivation at the beginning of the storage, and being in
- concordance with the highest time to the first 1 Log reduction (δ) found in higher a_w products
- compared to lower a_w products.

- 286 3.3. Secondary models for L. monocytogenes inactivation on dry-cured ham
- Polynomial models were developed in order to quantify the impact of product a_w and storage
- 288 temperature on the inactivation kinetic values obtained from the selected primary model fitting
- 289 (e.g. the δ and p parameters of the Weibull model). Four different transformations were
- assessed, namely square root, inverse, Ln and Log.
- The square root transformation of δ value was chosen for both products, Serrano and Iberian
- ham, as resulted with the best fit indicated by the higher R^2_{adj} . The δ parameter of both types of
- 293 ham was dependent on product a_w and storage temperature. The F-test indicated that the
- 294 equations for δ obtained for Serrano and Iberian hams were statistically different, thus a unique
- 295 model for δ for both types of ham was not considered.
- 296 The best transformation of the p values was different depending on the type of ham. For Serrano
- 297 ham, the inverse transformation of p values provided the best fit. It is noticeable that the
- transformed p values of L. monocytogenes in Serrano ham were statistically dependent on a_w but
- 299 not on temperature, indicating the great effect of a_w on the shape of the inactivation curve. On
- the other hand, for Iberian ham the Log transformation fitted best the data and the resulting
- 301 polynomial models indicated that p values were statistically dependent on temperature but also
- on the interaction between temperature and a_w.
- The estimated parameters and the goodness of fit of the polynomial models developed for the
- inactivation of *L. monocytogenes* in dry-cured ham as a function of a_w and/or storage
- temperature are reported in Table 4.

In order to obtain refined model parameters, the equations obtained for the secondary models were combined with the selected primary model to use a single mathematical equation to fit the entire set of inactivation data though the one-step global fitting. The resulting readjusted values of the terms describing the inactivation of L. monocytogenes for the two types of dry-cured ham are shown in Table 4. The coefficients of equations of the global models clearly confirmed that different models were needed for Serrano and Iberian ham types because a combined model for the two types did not describe the experimental data appropriately. For each type of ham, statistical goodness of fit indices showed the one-step global models provided a better description of the inactivation data when compared to the classical two-step approach. This result was expected because the one-step global procedure fully considered the raw data, resulting in increased degrees of freedom and more accurate and robust parameter estimates (Jewell, 2012; Martino & Marks, 2007). 3.5. Evaluation of the developed models After model formulation and selection based on its statistical performance to accurately describe the experimental dataset, it is important to evaluate the model predictive performance in real food systems with independently acquired data from similar food matrices. To this purpose, the Acceptable Simulation Zone (ASZ) approach was used to compare the 63 inactivation values obtained from scientific articles dealing with L. monocytogenes in dry-cured ham with the respective predictions provided by the developed inactivation model (Table 5). Overall, the model tended to overestimate the inactivation of the pathogen by an average of 0.3 Log units, which can be considered satisfactory taken into account that it is a slight conservative (fail-safe) prediction. In addition, for Serrano ham, 72.9 % of the predictions were within the ASZ (Table 5), proving the good predictive performance of the developed models. Due to the lack of independent data from Iberian ham, the evaluation of the developed L. monocytogenes inactivation model could not be properly conducted for this type of product. However, the few available data regarding Log (N/N_0) values of L. monocytogenes in Iberian hams collected from literature were all within the ASZ (Table 5). 3.6. Application of developed models Within the alternatives recognized by the US Food Safety Inspection Service (FSIS) to control L. monocytogenes in RTE, the results of the present study constitute a scientific evidence that dry-cured ham can be considered an AMA/P, suppressing the growth of L. monocytogenes during the storage, thus making the product to fulfill the Alternative 2b requirements of the US Listeria rule (FSIS, 2015). It is worth to highlight that the listericidal effects observed in the present work during the storage of dry-cured ham could be exploited as PLT to achieve a level of control complying with Alternative 1 of US Listeria rule. For this, almost 1 Log reduction of

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343	L. monocytogenes before dry-cured nam is released to the market should be validated. The
344	application of validated predictive models is an accepted option to validate PLT according to the
345	FSIS (FSIS, 2014). In this framework, the predictive models developed in this study allow to set
346	the time necessary to reduce 1 Log the level of L. monocytogenes at a given storage temperature
347	for different types of dry-cured ham as a function of their a _w . To this aim, Figure 2 shows the
348	1-Log iso-reduction plots enabling the easy identification of time/temperature combinations
349	suitable for a corrective storage (as the PLT) for each type of dry-cured ham and $a_{\rm w.}$ In the
350	lowest a _w products, the time required to achieve 1 Log reduction was of 9 and 6 days at 25 °C
351	for Serrano and Iberian hams, respectively.
352	Considering that the estimated shelf-life of dry-cured ham is about 6 months, the application of
353	such a short corrective storage time before product is released would be a feasible control
354	measure as PLT, in form of a quarantine period, to reduce L. monocytogenes levels in products
355	exposed to re-contamination after the drying process (e.g. during deboning, slicing, packaging)
356	and thus, to ensure the accomplishment of the zero-tolerance policies, by operating under
357	Alternative 1 of the Listeria rule (FSIS, 2015). This control measure could also be helpful for
358	companies within EU aiming to meet the commercial agreements of specific clients with zero
359	tolerance requirements to their providers.
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361	4. Conclusions
362	The physicochemical characteristics, mainly low a _w , make dry-cured ham not only listeriostatic
363	but listericidal and thus, compromising the viability of L. monocytogenes depending on the
364	product a _w and storage temperature.
365	In the framework of the design of risk minimization strategies, the quantified listericidal effect
366	of dry-cured ham can be used to establish a corrective storage, a feasible low-cost control
367	measure taking advantage of the product characteristics, as a PLT in products exposed to re-
368	contamination after the drying process (e.g. during deboning, slicing, packaging). This measure
369	could be implemented by the dry-cured ham producers to guarantee the fulfilment of restrictive
370	legal and commercial requirements regarding L. monocytogenes derived from zero tolerance
371	policies (such as the US Listeria rule).
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373	Acknowledgements
374	This work was supported by Listeria 0 project (INIA-PA 14/83 Lote 2) and by the CERCA
375	Programme/Generalitat de Catalunya. The authors thank Dr. Margarita Medina (INIA, Madrid)
376	for kindly providing the strains of <i>Listeria monocytogenes</i> .
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Figure	captions
	Figure

- **Figure 1.** Behavior of *L. monocytogenes* in Serrano and Iberian dry-cured hams with different
- 3 a_w and stored at 2, 8, 15 or 25 °C. Symbols represent the observed pathogen inactivation, Log
- (N/N_0) , and lines show the fit of the primary Weibull model.

- **Figure 2**. Predicted time for 1 Log reduction of *L. monocytogenes* according to the storage
- 7 temperature in Serrano (a) and Iberian (b) hams with different a_w.

Figure 1

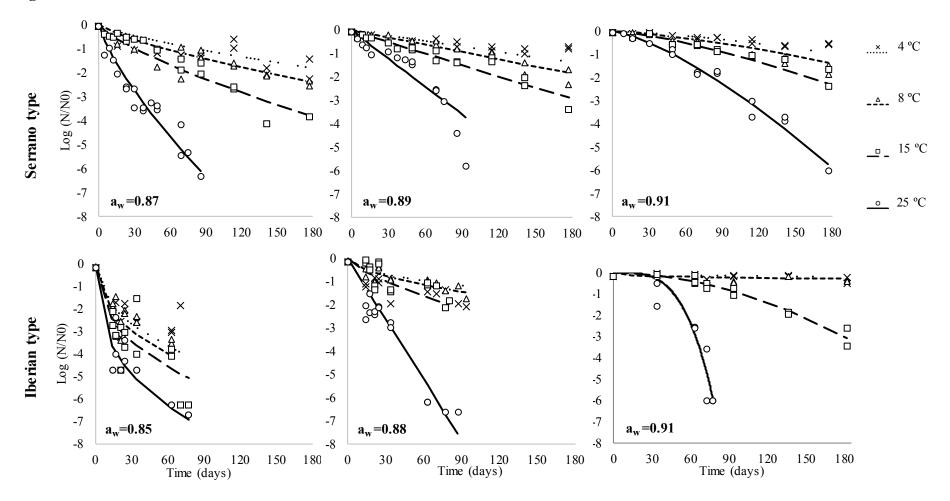
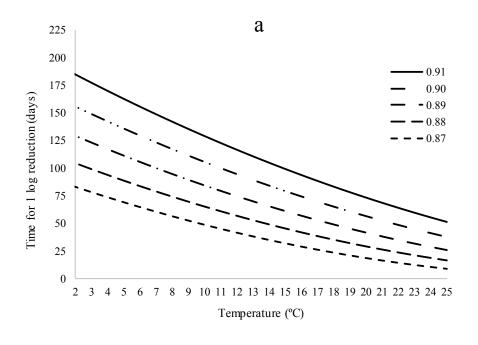


Figure 2



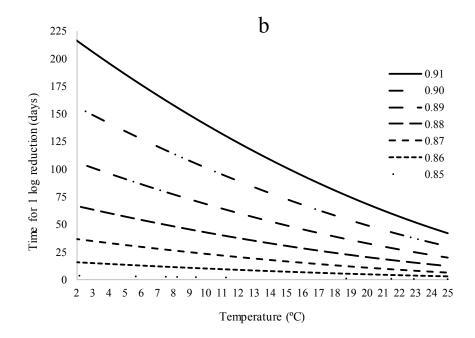


Table S1. Primary inactivation models (Log-linear, Log-linear with shoulder and Log-linear with tail) used to fit the *L. monocytogenes* inactivation data (Log N/N_0) as a function of time (days) on Serrano dry-cured ham.

Primary inactivation model	Dry-cured ham a _w	Temperature (°C)	k_{max} (1/days)	$\frac{Log \ N_{res}}{(\text{Log (N/N}_0))}$	Shoulder (days)	RSS	R ² adj
Log-linear		(0)	(Ir awys)	(208 (11110))	(44) 5)		
C	0.87	2	0.02	-	-	2.414	0.546
	0.87	8	0.03	-	-	2.368	0.731
	0.87	15	0.05	-	-	1.400	0.947
	0.87	25	0.15	-	-	4.186	0.917
	0.89	2	0.01	-	-	0.342	0.732
	0.89	8	0.03	-	-	0.624	0.906
	0.89	15	0.04	-	-	1.246	0.903
	0.89	25	0.11	-	-	5.761	0.842
	0.91	2	0.01	-	-	0.147	0.783
	0.91	8	0.02	-	-	0.216	0.951
	0.91	15	0.02	-	-	0.535	0.902
	0.91	25	0.07	-	-	2.254	0.947
Log-linear with shoulde							
	0.87	2	0.03	-	0.0	3.663	0.311
	0.87	8	0.04	-	0.0	3.434	0.609
	0.87	15	0.05	-	0.0	1.406	0.947
	0.87	25	0.18	-	0.0	7.474	0.853
	0.89	2	0.01	-	0.0	0.529	0.584
	0.89	8	0.02	-	0.0	0.665	0.900
	0.89	15	0.04	-	0.0	1.289	0.899
	0.89	25	0.12	-	9.0	5.118	0.860
	0.91	2	0.01	-	0.0	0.156	0.771
	0.91	8	0.02	-	0.0	0.236	0.947
	0.91	15	0.02	-	0.0	0.546	0.900
	0.91	25	0.08	-	27.2	1.466	0.966
Log-linear with tail							
_	0.87	2	0.03	-2.31	-	3.663	0.311
	0.87	8	0.04	-2.28	-	3.153	0.641
	0.87	15	0.05	-4.45	-	1.406	0.947
	0.87	25	0.18	-5.81	-	7.530	0.851
	0.89	2	0.02	-4.00	-	0.200	0.843
	0.89	8	0.02	-2.01	-	0.665	0.900
	0.89	15	0.04	-2.88	-	1.289	0.899
	0.89	25	0.10	-4.00	-	5.925	0.837
	0.91	2	0.01	-0.58	_	0.120	0.823
	0.91	8	0.02	-2.33	_	0.236	0.947
	0.91	15	0.02	-1.88	-	0.546	0.900
	0.91	25	0.06	-5.28	_	2.957	0.930

Table S2. Primary inactivation models (Log-linear, Log-linear with shoulder and Log-linear with tail) used to fit the *L. monocytogenes* inactivation data (Log N/N_0) as a function of time (days) on Iberian dry-cured ham.

Primary inactivation model	Dry-cured ham a _w	Temperature (°C)	k_{max} (1/days)	$\frac{Log \ N_{res}}{(\text{Log (N/N}_0))}$	Shoulder (days)	RSS	R ² adj
Log-linear		(0)	(I/ aajs)	(208 (11110))	(44) 5)		
	0.85	2	0.1	-	-	16.364	0.409
	0.85	8	0.1	-	-	6.725	0.536
	0.85	15	0.1	-	-	18.536	0.696
	0.85	25	0.2	-	-	20.090	0.654
	0.88	2	0.0	-	-	2.192	0.567
	0.88	8	0.0	-	-	1.018	0.680
	0.88	15	0.0	-	-	2.807	0.602
	0.88	25	0.2	-	-	4.141	0.937
	0.91	2	0.0	-	-	0.290	0.400
	0.91	8	0.0	-	-	0.958	0.229
	0.91	15	0.0	-	-	1.652	0.901
	0.91	25	0.2	-	-	11.779	0.810
Log-linear with shoulde	er						
	0.85	2	0.1	-	0.0	23.695	0.144
	0.85	8	0.2	-	0.0	12.750	0.120
	0.85	15	0.2	-	0.0	30.483	0.499
	0.85	25	0.3	-	0.0	49.438	0.149
	0.88	2	0.1	-	0.0	4.063	0.198
	0.88	8	0.0	-	0.0	1.589	0.500
	0.88	15	0.1	-	0.0	2.948	0.582
	0.88	25	0.2	-	0.0	5.822	0.912
	0.91	2	0.0	-	6.1	0.295	0.390
	0.91	8	0.0	-	14.0	0.956	0.231
	0.91	15	0.1	-	50.6	0.683	0.959
	0.91	25	0.2	-	27.9	7.758	0.875
Log-linear with tail							
_	0.85	2	0.3	-2.89	-	15.477	0.441
	0.85	8	0.3	-2.77	-	3.828	0.736
	0.85	15	0.4	-4.83	-	24.718	0.594
	0.85	25	0.5	-5.40	-	17.837	0.693
	0.88	2	0.1	-4.58	-	4.063	0.198
	0.88	8	0.0	-3.41	-	1.589	0.500
	0.88	15	0.1	-4.43	-	2.948	0.582
	0.88	25	0.2	-16.98	-	5.822	0.912
	0.91	2	0.0	-0.20	-	0.318	0.342
	0.91	8	0.0	-0.32	-	0.761	0.388
	0.91	15	0.0	-2.52	-	3.142	0.812
	0.91	25	0.1	-11.50	_	14.311	0.770

Table 1. Listeria monocytogenes strains used in this worka.

Strain	Genotype	Serotype
EF 051005/3/A	S2	1/2a
EF 151105/2/A	S4-2	1/2b
EF 010207/24/A	S12-1	1/2c
EF 270406/1/A	S7-2	4b

a: strains were isolated from pork meat industrial environment (Medina, 2017; Ortiz, López, Villatoro, López, Carlos Davila, & Martínez-Suárez, 2010)

Table 2. Primary inactivation models used to fit the *L. monocytogenes* inactivation data as a function of time.

Model	Equation ^a
Weibull	$Log(N/N_0) = -\left(\frac{t}{\delta}\right)^p$
Log-linear	$Log(N/N_0) = -\left(\frac{k_{max} \cdot t}{\ln{(10)}}\right)$
Log-linear with tail	$Log(N/N_0) = Log\left[(1 - 10^{Log(N_{res})}) \cdot e^{(-k_{max} \cdot t)} + 10^{Log(N_{res})} \right]$
Log-linear with shoulder	If $t \le shoulder$; $Log(N/N_0) = 0$ If $t > shoulder$; $Log(N/N_0) = -\left(\frac{k_{max} \cdot t}{\ln(10)}\right) + Log\left(\frac{e^{(k_{max} \cdot shoulder)}}{1 + \left[e^{(k_{max} \cdot shoulder)} - 1\right] \cdot e^{(-k_{max} \cdot t)}}\right)$

^a $Log (N/N_0)$: bacterial inactivation at specific time (t); $Log N_{res}$: inactivation tail (maximum inactivation); t: time (days); δ : time for the first Log reduction; p: shape of the inactivation curve; k_{max} : inactivation rate; shoulder: time before inactivation (initial resistance to stress).

Table 3. Estimated inactivation kinetic parameters resulting from fitting the primary Weibull model to the L. monocytogenes inactivation data obtained for dry-cured ham with different a_w and stored at different storage temperatures.

Product	Experimental conditions		Kinetic par	imeters		Goodness of fit ^a	
Dry-cured ham type	$\mathbf{a}_{\mathbf{w}}$	Temperature (°C)	δ (days) ^b	P b	n	RSS	R^2_{adj}
Serrano	0.87	2	34.9 ± 16.2	0.32 ± 0.13	16	0.123	0.677
		8	32.2 ± 10.2	0.48 ± 0.12	16	0.129	0.795
		15	47.5 ± 3.6	1.20 ± 0.08	19	0.062	0.947
		25	6.0 ± 1.1	0.65 ± 0.06	19	0.164	0.945
	0.89	2	>180	0.46 ± 0.08	16	0.013	0.860
		8	101.4 ± 5.2	1.28 ± 0.15	16	0.037	0.922
		15	64.2 ± 5.6	1.04 ± 0.12	19	0.075	0.900
		25	39.5 ± 3.4	1.93 ± 0.23	16	0.197	0.924
	0.91	2	>180	0.77 ± 0.16	16	0.010	0.798
		8	113.8 ± 3.9	1.14 ± 0.10	16	0.015	0.953
		15	105.3 ± 5.3	1.19 ± 0.14	16	0.035	0.911
		25	51.5 ± 3.4	1.41 ± 0.09	16	0.082	0.973
Iberian	0.85	2	2.0 ± 2.6	0.36 ± 0.16	16	12.675	0.542
		8	1.8 ± 1.3	0.30 ± 0.10	14	2.764	0.809
		15	2.0 ± 1.3	0.47 ± 0.10	18	14.073	0.769
		25	0.3 ± 0.2	0.33 ± 0.06	16	6.325	0.891
	0.88	2	15.8 ± 6.2	0.32 ± 0.10	16	1.291	0.745
		8	46.2 ± 6.5	0.46 ± 0.11	16	0.777	0.756
		15	38.3 ± 6.3	0.74 ± 0.20	17	2.690	0.559
		25	7.3 ± 1.2	0.79 ± 0.06	16	3.548	0.946
	0.91	2	_c	0.39 ± 0.31	16	0.363	0.249
		8	_c	0.32 ± 0.32	16	1.042	0.161
		15	100.8 ± 5.0	1.89 ± 0.18	16	0.777	0.954
		25	46.8 ± 5.2	3.55 ± 0.85	12	4.823	0.922

 $[\]overline{a}$ n: number of inactivation data, Log (N/N₀), included for fitting, RSS: residual sum of squares; R^2_{adj} : adjusted coefficient of determination.

^b Parameter estimate \pm standard error.

^c No inactivation was recorded. δ had an infinitive value.

Table 4. Estimated coefficients of the polynomial models resulting from the fitting to values of the primary inactivation kinetics.

		Coefficients of the polynomial models ^a							Goodness of fitb		
Serrano dry-cured ham		a	b	c	d	e	f	g	P	RSS	R^2_{adj}
Secondary polynomial models	$\sqrt{\delta} = a + b \cdot a_{\rm w} + c \cdot a_{\rm w} \cdot T$	-132.60 ± 34.32	163.19 ± 38.57	-0.33± 0.08	-	-	-	-	3	42.782	0.746
	$1/p = e + f \cdot a_{w}$	-	-	-	-	28.66 ± 10.84	-30.84 ± 12.18	-	2	4.748	0.330
Global model	$\operatorname{Log}(N/N_0) = \operatorname{Log}(N/N_0)_{initial} - \left(\frac{t}{(a+b \cdot \mathbf{a}_{w} + c \cdot \mathbf{a}_{w} \cdot \mathbf{T})^2}\right)^{\frac{1}{e+f \cdot a_{w}}}$	-88.52 ± 5.22	112.83 ± 5.84	-0.31 ± 0.01	-	13.93 ± 1.71	-14.51 ± 1.90	-	5	24.778	0.919
Iberian dry	-cured ham										
Secondary polynomial models	$\sqrt{\delta} = a + b \cdot \mathbf{a_w}^2 + c \cdot \mathbf{T} + d \cdot \mathbf{a_w} \cdot T$	-90.99 ± 12.66	127.02 ± 16.31	3.96 ± 1.65	-4.66 ±1.88	-	-	-	4	15.162	0.913
	$Log p = e + f \cdot T + g \cdot a_w \cdot T$	-	-	-	-	-0.52 ± 0.08	-0.53 ± 0.10	0.63 ± 0.12	3	0.185	0.824
Global model	$\log (N/N_0) = \log (N/N_0)_{initial} - \left(\frac{t}{(a + b \cdot a_w^2 + c \cdot T + d \cdot a_w \cdot T)^2}\right)^{10^{(e + f \cdot T + t)}}$	-90.11± 7.44	127.42 ± 10.15	4.29 ± 0.65	-5.11 ± 0.76	-0.34 ± 0.07	-0.48 ± 0.05	0.56 ± 0.06	7	71.069	0.892

^a Parameter estimates ± standard error.

 $^{^{}b}P$: number of estimated parameters of the model; RSS: residual sum of squares; R^{2}_{adj} : adjusted coefficient of determination.

Table 5. Comparison of observed and predicted *L. monocytogenes* inactivation in Serrano and Iberian dry-cured hams.

Refa	Dry- cured ham	$a_{\rm w}$	Temperature (°C)	Time (days)	Observed inactivation $(Log(N/N_0))$	Predicted inactivation (Log(N/N ₀))	Observed-Predicted inactivation
[1]	Serrano	0.88	4	7	-0.71	-0.11	-0.6
		0.88	8	7	-0.59	-0.13	-0.5
		0.88	4	30	-1.24	-0.38	-0.9
		0.88	8	30	-1.38	-0.46	-0.9
		0.88	4	60	-1.35	-0.68	-0.7
		0.88	8	60	-1.25	-0.83	-0.4
[2]	Serrano	0.93	2	15	-0.05	0.00	-0.1
		0.93	2	15	0.01	0.00	0.0
		0.93	2	15	0.08	0.00	0.1
		0.93	2	15	-0.16	0.00	-0.2
		0.93	2	27	-0.04	-0.01	0.0
		0.93	2	27	-0.08	-0.01	-0.1
		0.93	2	41	-0.07	-0.02	-0.1
		0.93	2	43	-0.22	-0.02	-0.2
		0.93	2	55	-0.08	-0.03	-0.1
		0.93	2	70	-0.08	-0.06	0.0
		0.93	2	97	-0.25	-0.12	-0.1
		0.93	2	166	-0.22	-0.40	0.2
		0.93	2	166	-0.20	-0.40	0.2
		0.93	8	15	-0.28	0.00	-0.3
		0.93	8	15	-0.26	0.00	-0.3
		0.93	8	15	-0.14	0.00	-0.1
		0.93	8	15	-0.07	0.00	-0.1
		0.93	8	27	-0.16	-0.01	-0.2
		0.93	8	27	-0.27	-0.01	-0.3
		0.93	8	41	-0.26	-0.03	-0.2
		0.93	8	43	-0.12	-0.03	-0.1
		0.93	8	55	-0.37	-0.05	-0.3
		0.93	8	70	-0.15	-0.09	-0.1
		0.93	8	97	-0.67	-0.19	-0.5
		0.93	8	166	-1.20	-0.66	-0.5
		0.93	15	15	-0.23	-0.01	-0.2
		0.93	15	15	-0.46	-0.01	-0.5
		0.93	15	15	-0.23	-0.01	-0.2
		0.93	15	15	-0.20	-0.01	-0.2
		0.93	15	27	-0.46	-0.02	-0.4
		0.93	15	27	-0.23	-0.02	-0.2
		0.93	15	41	-0.15	-0.05	-0.1
		0.93	15	43	-0.26	-0.06	-0.2
		0.93	15	55	-0.57	-0.10	-0.5
		0.93	15	70	-0.88	-0.18	-0.7

		0.93	15	70	-0.92	-0.18	-0.7	
		0.93	15	98	-1.36	-0.38	-1.0	
		0.93	25	7	-0.18	0.00	-0.2	
		0.93	25	7	-0.22	0.00	-0.2	
		0.93	25	7	0.03	0.00	0.0	
		0.93	25	7	-0.21	0.00	-0.2	
		0.93	25	15	-0.29	-0.02	-0.3	
		0.93	25	15	-0.17	-0.02	-0.2	
		0.93	25	15	-0.21	-0.02	-0.2	
		0.93	25	15	-0.25	-0.02	-0.2	
		0.93	25	41	-0.92	-0.19	-0.7	
		0.93	25	43	-0.81	-0.21	-0.6	
		0.93	25	43	-0.94	-0.21	-0.7	
		0.93	25	55	-0.98	-0.36	-0.6	
[3]	Serrano	0.92	8	5	-0.52	0.00	-0.5	
		0.92	8	13	-0.65	-0.01	-0.6	
		0.92	8	32	-0.79	-0.06	-0.7	
		0.92	8	61	-1.04	-0.18	-0.9	
[3]	Iberian	0.88	8	5	-0.67	-0.27	-0.4	
		0.88	8	13	-0.84	-0.46	-0.4	
		0.88	8	32	-0.69	-0.79	0.1	
		0.88	8	61	-0.7	-1.15	0.5	
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^a References: [1] Morales et al. (2006); [2] Bover-Cid et al. (2016) [3] Hereu et al. (2012)

Highlights

- Dry-cured ham is not only listeriostatic, it may be listericidal
- Listericidal effect was quantified as a function of aw and storage temperature
- Iberian ham type favors an early inactivation of *L. monocytogenes*
- A low-cost control measure is proposed as a post-lethality treatment

Authors have no conflict of interests