Enterocin A-based antimicrobial film exerted strong antilisterial activity in sliced dry-cured ham immediately and after 6 months at 8 °C.

Teresa Aymerich*, Anna Jofré, Sara Bover-Cid

IRTA-Food Safety and Functionality Programme.

Finca Camps i Armet s/n. 17121 Monells. Girona. Spain

*Corresponding author: teresa.aymerich@irta.cat.

Declaration of interest: none

Credit author statement:

Teresa Aymerich: conceptualization, methodology, supervision, formal analysis, writing original draft preparation, funding.

Anna Jofré: investigation

Sara Bover-Cid: visualization, writing-review-editing

Abstract

To minimize the survival of *Listeria monocytogenes* on ready-to-eat (RTE)-products, active antimicrobial packaging based on polyvinyl alcohol films with Enterocin A or ethyl-lauroyl-arginate (LAE) have been designed and its antimicrobial activity assessed in vacuum-packed sliced dry-cured ham stored under refrigeration.

The Enterocin A-based antimicrobial film exerted a strong antilisterial activity, causing an immediate reduction of *L. monocytogenes* counts of 1 log units compared with the control without antimicrobial. Besides, Enterocin A film enhanced (4-fold higher) the die-off rate along the 6 months of storage at 8 °C. The antilisterial effect of Enterocin A film applied on dry-cured ham complies with the performance criteria requirement of Alternative 1 of the US Listeria rule regarding the control of *L. monocytogenes*. Films made with LAE did not exert an immediate bactericidal effect but slightly increased the die-off rate of the pathogen and reduced its counts during the shelf life compared to the control batch.

Keywords: active packaging, Enterocin A, bacteriocin, LAE, dry-cured ham, antilisterial.

1. Introduction

Listeria monocytogenes has become one of the major food safety concerns, particularly in ready-to-eat (RTE) food. Although the reported morbidity, as the total number of cases, is not high compared to other foodborne pathogens, *L. monocytogenes* has one of the highest reported mortality rates, 17.6% in European Union 2019 (EFSA-ECDC, 2021). Dry-cured ham is not considered a high-risk product for *L. monocytogenes* because it does not support the growth of the pathogen, mainly due to its low final water activity (FSIS, 2005; Serra-Castelló et al., 2020). Nevertheless, the pathogen could recontaminate the product from the food-processing environment, e.g., during the preparation of convenient formats of commercialization as RTE sliced and packaged product. Indeed, the ubiquity of the pathogen on food industries environment and presence on the product at low levels, usually < 100 CFU/g, has been reported (Nolan et al., 1992, Gómez et al., 2012; Martin et al., 2014). This level of contamination does not represent a problem for the accomplishment of the EU criterion of *L. monocytogenes* (< 100 CFU/g during the product shelf life, European Commission, 2005). However, to assure the accomplishment of the more conservative "zero tolerance" policies for RTE foods (not detected in 25 g) as applied for the United States Department of Agriculture - Food Safety Inspection Service (USDA-FSIS) (FSIS, 2015), some post-processing intervention technologies to decontaminate the final product could be needed if exportation to those countries is considered.

Active packaging with natural and Generally Recognized as Safe (GRAS) chemical additives could represent one of the alternatives to control *L. monocytogenes*, guaranteeing the accomplishment of zero tolerance requirements. Antimicrobial packaging has the advantage that the antimicrobial substances are not directly added to the food product, thus the antimicrobials can be stabilized and gradually released on the surface of the product, maintaining an adequate concentration along the shelf life, where they are mostly needed (Coma, 2008). Some factors such as the chemical

nature of the antimicrobial and the food, storage and distribution conditions, application mode, interactions between the antimicrobial and the film polymer, diffusion from the packaging material to the food and/or inactivation by interaction with the food matrix determine the antimicrobial effectiveness to prolong the shelf life or enhance safety (Appendini and Hotchkiss, 2002; Aymerich et al., 2008; Bastarrachea et al., 2010; Suppakul et al., 2003). Considering all these factors, the efficacy of specific strategies aiming at controlling *L. monocytogenes* in RTE food need to be validated (FSIS, 2015) in a product-specific oriented approach (Hereu et al, 2012). The objective of this study was to assess the potential of biodegradable active films prepared with Enterocin A, a type IIa bacteriocin from lactic acid bacteria, recognized as a potent antilisterial compound (Aymerich et al., 1996; Eijsink et al., 1998) and ethyl-lauroyl-arginate, (LAE), a GRAS (notice nº 000164) synthetic surfactant and EU authorised additive (code E-243) with wide antimicrobial spectrum, as effective intervention strategies to accomplish zero tolerance of *L. monocytogenes* in a RTE meat product such as packaged sliced dry cured ham.

2. Material and methods

2.1. Dry-cured ham and its physico-chemical characterisation

A block of deboned vacuum-packed Spanish dry-cured ham was purchased directly from the producer and stored under refrigeration until use. Water activity was measured with an Aqualab™ equipment (series 3, Decagon Devices Inc., Pullman, WA, USA). pH was assessed by direct measurement with a penetration probe (52-32, Crison Instruments SA, Alella, Spain) connected to a portable pH-Meter (pH25, CRISON Instruments). Protein, fat, and moisture content were determined according to the AOAC official method 2007.04 (Anderson, 2007), using a near-infrared spectrophotometer system (FoodScan™Lab device, FOSS Analytic, Hillerod, Denmark). The salt content was measured according to ISO 1841-2:1996 method by

analyzing the chloride content with a potentiometric titrator 785 DMP Titrino (Metrohm AG, Herisau, Switzerland). Analyses were performed in triplicate.

2.2. Antimicrobials and antimicrobial packaging (film) preparation

The antimicrobial compound, Enterocin A was obtained from an overnight culture of *Enterococcus faecium* CTC492 (Aymerich et al. 1996). The centrifuged supernatant (8,000 x *g* 10 min) was purified through an ionic interchange resin (CM Sephadex™ C-25, GE Healthcare Bio Sciences AB, Uppsala, Sweden) (Abriouel et al., 2003). The bacteriocin was eluted from the column with 0.4 M NaCl in 10 mM phosphate buffer. The active fraction was dialyzed at refrigerated temperature (4 ºC) with 3 complete buffer changes (10 ml volume, cut off membrane, 500 Da MWCO, Float-A-lyzer G2, Spectrum Labs, Indiana, USA) and lyophilised for 24 h (CHRIST ALPHA 1-4 with LDC-1M controller, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode amb Harz, Germany). The purified dry extract of the bacteriocin Enterocin A was applied to the film matrix (see below) to obtain a maximum of 9 µg/g (13,000 AU/g) equivalent to 2.25 µg/cm^2 (3,250 AU/cm^2) of the antimicrobial onto the food matrix if 100% migration is considered. For LAE, a commercial preparation, Mirenat® DC (Vedeqsa, Grupo Lamirsa, Barcelona, Spain) containing 8.5% of the active substance was used. An estimated maximum final concentration of 160 µg/g (equivalent to 66.6 µg/cm^2 on the product if 100% migration) was considered, representing the maximum permitted concentration of the antimicrobial in Europe for heat-treated meat products (i.e., 160 mg/kg). In the USA, a maximum of 200 mg/Kg is permitted.

Three different types of polyvinyl alcohol-based films were prepared: a control (without antimicrobials), an antimicrobial active film containing Enterocin A (Enterocin A-film) and an antimicrobial active film containing LAE (LAE-film). The films were prepared from a suspension matrix of 13% w/v powdered polyvinyl alcohol (PVOH, Sigma Aldrich, St. Louis, MO, USA) in distilled water. To melt the polymer, the solution was autoclaved at 120°C for 30 min. The melted biodegradable plastic was cooled to 50 °C

and then, each antimicrobial (i.e., Enterocin A or LAE) was added at the concentration stated above and mixed with the matrix. The films were extended by casting (20 x 20 cm) with a thin layer chromatography coater (CAMAG, Switzerland) and air-dried for 20 h under the flow of a Biosecurity cabinet (BIO-II-A, Telstar, Terrassa, Spain). The film thickness was determined with an electronic digital micrometer (0 - 150 mm digital caliper, Mitutoyo, Japan) with 0.01 mm resolution. Three thickness measurements were randomly taken on each test sample. The films were maintained in food-grade hygienic sealed polyamide/polyethylene (PA/PE) plastic bags (Sacoliva, Barcelona, Spain) at room temperature until use.

The antimicrobial activity of the films against *L. monocytogenes* was verified *in vitro* accordingly to the agar spot test (Tagg et al., 1976). A piece of a 2 x 2 cm of the active film was placed on a seeded layer of an overnight culture of the indicator microorganism (*L. monocytogenes* strain pool, Table 1) in TSAYE soft made of tryptic soy broth (Merck, Darmstadt, Germany) with 6 g/L of bacteriological Agar (Merck) and 0.6% Bacto™ yeast extract (BD, Becton, Dickinson and Company, Erembodegem, Belgium). The plates were incubated at 37 °C and the inhibition halo was verified at 24h and 48h. The assay was performed immediately after film preparation, before the challenge test (after a week of storage at room temperature) and at each sampling time during the storage in order to assess the stability of the active films.

2.3. Inoculum, sample preparation and storage

Four different *L. monocytogenes* strains previously isolated from dry-cured ham (Table 1, Ortiz et al, 2010) and stored at - 80 °C with 20 % glycerol, were consecutively grown in tryptic soy broth (Merck) with 0.6% Bacto™ yeast extract (Becton, Dickinson and Company) for 24 h 30 °C and for 4 days at 8 °C to pre-adapt cells to the refrigeration temperature (EURLLm, 2021) until the stationary growth phase was reached. The

147 strains were mixed at equal concentrations in a cocktail used to inoculated dry-cured
148 ham as generally recommended for challenge tests studies (NACMCF, 2010).

149 The dry-cured ham was aseptically sliced at 2 mm thickness. Slices were cut in pieces
150 of 4 x 4 cm (ca. 4 g) for the challenge test with the active films containing Enterocin A
151 (lab-scale extract) and in pieces of 9 x 9 cm (*ca.* 15 g) for challenge with active films
152 containing LAE (commercial product) and the control film (without antimicrobial).

153 To quantify the lethal effects (inactivation) of the active films on *L. monocytogenes*,
154 high inoculum level was used as recommended by the National Advisory Committee on
155 Microbiological Criteria for Food guidelines (NACMCF 2010). Therefore, the surface of
156 the dry-cured ham slices was inoculated with 1 % v/w of the *L. monocytogenes* cocktail
157 (described above) to reach *ca.* 6 Log CFU/g. The inoculum was distributed over the
158 surface of the product with a sterile *Digraslki* spreader until absorbed. Afterwards,
159 samples were covered with active antimicrobial film of 5 x 5 cm for Enterocin A-films
160 and 10 x 10 cm for LAE-films and control-films, respectively. Finally, the samples were
161 vacuum-packed (EV-15-2-CD, Tecnotrip, Terrassa, Spain) in PA/PE (Sacoliva) plastic
162 bags and stored at 8 °C (7.86 °C ± 0.36). Along the storage, temperature was recorded
163 by a wireless temperature sensor connected to the Evisense-labguard® system
164 (BioMérieux, France).

165 Samples of each treatment together with the control batch were analysed in duplicate
166 at 10 different times: 0, 5, 9, 16, 30, 49, 69, 86, 114, 141 and 177 days, during
167 refrigerated storage. The experiment was performed in two independent batches.

168 ***2.4. Microbiological analysis***

169 Each sample was 10-fold diluted and homogenized in a Masticator blender (IUL, S.A.,
170 Barcelona, Spain) for 60 s. Afterwards, 10-fold serial dilutions were performed in
171 physiological saline water with 0.85% NaCl (Merck, Mollet del Vallés, Spain) and 0.1%
172 Bacto peptone (BD). Enumeration of *L. monocytogenes* was performed on

Chromogenic Listeria Agar (CLA, Oxoid Ltd., Basingstoke, UK) after incubation at 37 ºC for 48 - 72 h. To increase limit of detection to 10 CFU/g, 1 ml of the 10-fold diluted sample homogenate was spread on CLA plates of 14 cm diameter. When counts were below the plate detection limit, the presence of the pathogen was investigated by sample enrichment in TSBYE at 37ºC for 48 h and streaking the enriched broth on CLA. Detection of *L. monocytogenes* in the enriched sample was recorded as 0.9 log CFU/g for fitting purposes.

2.5. Estimation of inactivation kinetic parameters

Inactivation kinetic models were fitted to the log count data to estimate the inactivation kinetic parameters. In particular, log-linear model was used for data from control and LAE active film batches, while log-linear with tail was used for the Enterocin A active film batch. The goodness of fit indexes RSS, RMSE and R^2_{adj} were recorded. Model fitting was implemented using the packages nls2, and nls tools and the functions nls2, nls and confint2 of R software (http://www.R-project.org/).

3. Results and discussion

The antilistericidal activity of Enterocin A active films, with an average thickness of 0.5 ± 0.1 mm, was confirmed *in vitro*, either after film preparation, before the challenge test and during the whole period of the product storage study by the presence of an inhibition growth halo around the film on the *L. monocytogenes* grown plate. This result indicates that the added antimicrobial concentration in the active film was stable enough to support a release of the antimicrobial to the surface all over the storage period, which is a necessary for an efficient antimicrobial packaging (Quintavalla and Vicini, 2002). The LAE films, with an average thickness of 0.5 ± 0.2 mm did not present an halo of inhibition around the film, similarly to what was reported for nisin films in a cellulose polymer by dos Santos Pires et al. (2008) and Scannell et al. (1997). The formation of a zone of inhibition depends on the diffusion capacity of the antimicrobial

compound from the film into the culture media and the indicator microorganism growth rate. Similarly, in the current study, the antimicrobial film could have exerted its action by migration or by direct contact of the active substance to the matrix. Due to the importance of the matrix effect, a product-specific approach is needed to validate the antilisterial activity in real food.

In this study, the antilisterial activity of the active films was assayed on sliced dry-cured ham with the following physico-chemical characteristics:16.7% (standard deviation, SD =5.07) fat, 28.0% (SD= 3.1) protein, 45.3% (SD= 3.0) moisture, 5.7% (SD= 0.02) salt, a water activity of 0.890 (SD= 0.005) and pH 5.93 (SD= 0.1). These values, mainly water activity, make dry-cured ham a product that does not support the growth of *L. monocytogenes,* thus being classified into category 1.3 according to the EU microbiological criteria (European Commission, 2005). Nevertheless, without any further control intervention *L. monocytogenes* can survive on the product (Figure 1, control batch). Indeed, Hereu et al. (2012) reported that the pathogen did not suffer any significant reduction on a dry-cured ham of a water activity of 0.92, and only 1 log reduction after 60 days of storage at 8 °C in products with a water activity of 0.88. The water activity of dry-cured ham in the present study was between these two values and the time for 1 log reduction was 88 days. The Food Safety and Inspection Service (FSIS,2015) will consider a water activity of ≤0.85 at the time the product is packed to be a post-lethality treatment and to be an antimicrobial treatment if the establishment provides supporting documentation that *L. monocytogenes* is reduced by at least 1 log before the product leaves the establishment. Thus, to enhance the die-off, some intervention technologies, such as the ones proposed in this study, could be needed.

Figure 1 shows *L. monocytogenes* counts in dry-cured ham with the three types of films during the storage at 8ºC. The estimated inactivation kinetics parameters are summarised in Table 2. The active packaging with Enterocin A-films exerted an immediate bactericidal effect over the pool of *L. monocytogenes* strains artificially

226 inoculated onto the dry-cured ham, reducing the counts of *L. monocytogenes* by 1.5 log
227 units in just the 5 days. After about 2 weeks of storage, a 2-log reduction was achieved.
228 The Enterocin A-active film was able to further reduce *L. monocytogenes* counts all
229 over the storage period, with the die-off rate (k_{max}, days^{-1}) being 4.2-fold higher when
230 compared to the control-film batch without antimicrobial. According to the model fit, a
231 tail occurred after 92 days of storage (with a CI_{95} between 71-114 days). However, at
232 the end of the storage study, after *ca.* 6 months, *L. monocytogenes* was below the
233 quantification limit but detected in the 4 g sample unit, which represented a
234 concentration 4 log lower when compared to the final concentration observed in the
235 control batch (ca. 5 log CFU/g). Therefore, the results of the challenge test in dry-cured
236 ham confirmed the *in-situ* efficacy of the antimicrobial active film. The application of
237 Enterocin A-antimicrobial active films could facilitate the accomplishment of the zero-
238 tolerance policy. If used in dry-cured ham the product could fall into Alternative 1
239 operating procedure considered within the US Listeria rule (which combines a post-
240 lethality kill step with an antimicrobial agent; 9 CFR 417.4 and 430.4; FSIS, 2015),
241 which would allow the producer to label the product with the claim “enhanced
242 protection against *L. monocytogenes*”. This is in contrast with the results of Hereu et al
243 (2012), which reported a more limited antimicrobial effect of nisin-added polyvinyl film
244 applied in dry-cured ham of two different values of water activity (0.92 and 0.88).
245 Contrary to nisin (E234, GRAS recognized notice nº 000065), Enterocin A is not an
246 authorized food additive and is not yet recognized as GRAS. However, its more
247 powerful antilisterial activity when used in active packaging compared to nisin, points
248 out its potential as reliable antimicrobial to be considered for antimicrobial packaging.

249 Enterocin A, nisin and some other bacteriocins produced by lactic acid bacteria have
250 already been assayed for its antilisterial effect when added in antimicrobial packaging
251 intended for other type of meat products, especially those products that support the
252 growth of the pathogen. The Enterocin A active polyvinyl alcohol films were tested on

253 cooked ham and fermented sausages (Marcos et al., 2007; Marcos et al., 2013).
254 Enterocin 416K1 from *Enterococcus casseliflavus* IM416K1, entrapped in an organic
255 hybrid coating applied to LDPE (low-density polyethylene) were assayed on
256 contaminated frankfurters and fresh soft cheeses (Iseppi et al., 2008). Bacteriocins
257 from *L. curvatus* CRL705 active films were tested on wiener sausages (Blanco Massani
258 et al. 2008, Blanco Massani et al. 2013 and Blanco Massani et al. 2014), while
259 bacteriocin 32Y from *L. curvatus* 32Y, coated in polythene (PE) film, on pork steak and
260 ground beef (Mauriello et al., 2004) and sakacin A from *L. sakei* DSMZ 6333, included
261 in active pulllulan films, on turkey breast (Trinetta et al., 2010). Montiel et al (2019)
262 applied enterocin directly to the surface of dry-cured ham and observed a reduction of
263 2-log of *L. monocytogenes* after 14 days of storage at 7ºC when compared to control
264 ham. This lethal effect was of the same order as the effect reported in the present
265 study using active films. Therefore, this is the first time that an effective antimicrobial
266 film based on Enterocin A is proved for dry-cured ham.

267 The active antimicrobial packaging consisting of LAE-film, applied to a concentration
268 equivalent to the maximum EU dose authorized for cooked meat products (i.e., 160
269 mg/kg, European Commission, 2014) did not exert an immediately bactericidal effect
270 (Figure 1). The die-off rate (expressed by the k_{max}, Table 2) was slightly higher (1.65-
271 fold) when compared to the control batch. However, the treatment could not be
272 considered cost-effective in terms of *L. monocytogenes* reduction because more than
273 50 days of storage at 8 ºC would be necessary to cause 1 log reduction before
274 releasing the product to the market to make this intervention compatible with
275 Alternative 1 of US *Listeria* rule. The lower efficacy of LAE- films in the product-specific
276 approach when compared to Enterocin A-films is in accordance with the results
277 obtained in *in vitro* assays.

278 On RTE-products where *L. monocytogenes* is able to growth, EVOH29-films containing
279 10% LAE where able to reduce 4 log the growth of *L. monocytogenes* on infant formula

milk after 6 days at 4°C and exerted a bacteriostatic effect, inhibiting *L. monocytogenes* growth, in RTE-surimi stick after 10 days 4ºC (Muriel-Galet et al., 2012; Muriel-Galet et al., 2015). Besides, bactericidal effects to *L. innocua* have been observed when LAE was applied at higher concentrations (*c.a.* 6-fold, compared to this study) as coating of polylactic acid films together with chitosan, and not filled into the polymer. The coating composed by chitosan 0.388 mg/cm^2 and LAE 0.388 mg /cm^2 was able to reduce 2.4 log the counts of *L. innocua* on RTE-dely turkey meat while a coating with chitosan 1.94 mg/cm^2 and LAE 0.388 mg/cm^2 was able to reduce counts by 4.6 log, thus while chitosan enhanced the action of high LAE concentrations, coating facilitated the contact with the food surface (Guo et al. 2014). In cooked ham slices vacuum stored at 4 °C wrapped with pullulan film containing LAE (2% w/v of the film solution), *L. monocytogenes* Scott A was strongly inactivated from 5.6 log CFU/cm^2 to undetectable levels after 24h. The levels of *L. monocytogenes* were kept below the detection limit for 2 weeks followed by a slight increase of the pathogen concentration at the end of 28 days of storage (Pattanayaiying et al. 2015). LAE is a synthetic surfactant that has been reported to disrupt the membrane lipid bilayer, alter metabolic processes and hampers the cellular cycle without cellular lysis (Bakal and Díaz, 2005). The bactericidal effect of pullulan film with 2% LAE applied to cooked ham was higher than that observed in this study with PVOH film with 8.5% LAE applied on dry cured ham, possibly due, apart from product effect to the higher migration rate from film to product, facilitating higher interaction with food.

4. Conclusions

Sliced dry-cured ham is not considered a product of high-risk regarding *L. monocytogenes* because it does not support its growth. Nevertheless, the potential of the pathogen to contaminate and to survive in dry-cured ham during the shelf life makes Enterocin A active films an interesting technology to efficiently reduce *L. monocytogenes* levels and to facilitate the compliance with “zero tolerance” policies.

Acknowledgments

The work was financially supported by the Spanish project, *Listeria* cero. A research and innovation plan of INIA and the Spanish inter-professionals of the pork chain, INTERPORC and ASICI, to reduce the presence of *Listeria*. Moreover, we thank the CERCA Programme/Generalitat de Catalunya for its financial institutional support. The authors thank Yolanda Beltran and Carmen Raya for her valuable technical support.

329 **Table 1**: Strains of *Listeria monocytogenes* used to inoculate sliced dry-cured ham.

Strain	Pulsotype	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

330

331

332

333

334

335

336

337

338

339

340

341

342

343

344

345

346

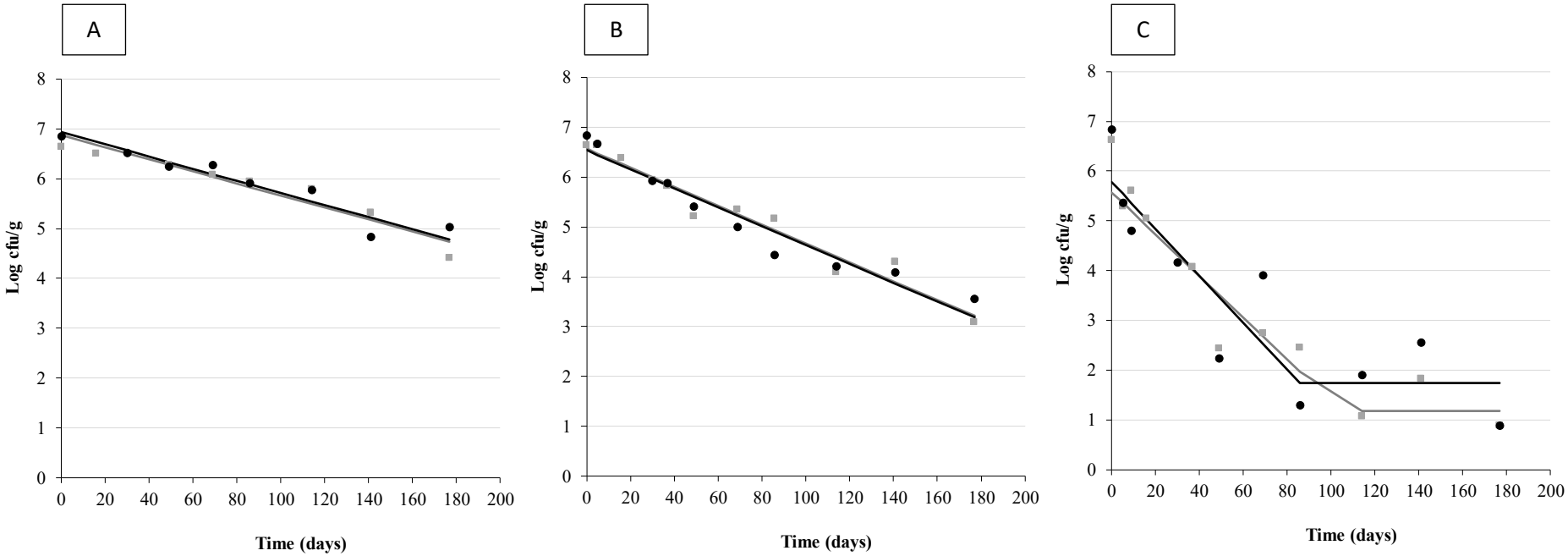
347

348

Table 2. Kinetic parameters[a] of the inactivation of *Listeria monocytogenes* in dry-cured ham without (control) and with antimicrobial active packaging during the storage at 8ºC.

				Goodness of fit			
	Kinetic parameter (units)	**estimate *(standard error)***	**CI_{95}**	**n**	**RSS**	**RMSE**	**R^2_{adj}**
Control	Log N_0 (Log CFU/g)	6.82 *(0.09)*	6.62 - 7.03	16	0.622	0.211	0.91
	k_{max} ($days^{-1}$)	0.026 *(0.002)*	0.022 - 0.031				
LAE	Log N_0 (Log CFU/g)	6.55 *(0.09)*	6.36 - 6.73	21	1.259	0.257	0.95
	k_{max} ($days^{-1}$)	0.043 *(0.002)*	0.039 - 0.048				
Enterocin_A	Log N_0 (Log CFU/g)	5.85 *(0.29)*	5.25 - 6.44				
	k_{max} ($days^{-1}$)	0.110 *(0.014)*	0.080 - 0.140	22	10.194	0.732	0.86
	t_{shift} (days)	92.5 *(10.3)*	70.9 - 114.1				

Figure 1: *Listeria monocytogenes* inactivation in dry-cured ham without antimicrobial (A), LAE (B) and Enterocin A (C) active packaging during the storage at 8ºC. Dots are the observed counts, while lines correspond to the fit of the inactivation kinetic model (i.e., Log-lineal for A and B, Log-lineal with tail for C). Different colors correspond to 2 batches.



366 **References**

367 Abriouel, H., Valdivia, E., Martinez-Bueno, M., Maqueda, M., Galvez, A., 2003. A
368 simple method for semi-preparative-scale production and recovery of enterocin AS-
369 48 derived from *Enterococcus faecalis* subsp. liquefaciens A-48-32. Journal of
370 Microbiological Methods 55, 599-605.
371 Anderson, S., 2007. Determination of fat, moisture, and protein in meat and meat
372 products by using the FOSS FoodScan near-infrared spectrophotometer with FOSS
373 artificial neural network calibration model and associated database: collaborative
374 study. Journal of AOAC International, 90 (4), 1073-1083.
375 Appendini, P., Hotchkiss, J.H., 2002. Review of antimicrobial food packaging.
376 Innovative Food Science & Emerging Technologies 3, 113-126.
377 Aymerich, T., Holo, H., Havarstein, L.S., Hugas, M., Garriga, M., Nes, I.F., 1996.
378 Biochemical and genetic characterization of enterocin A from *Enterococcus faecium*,
379 a new antilisterial bacteriocin in the pediocin family of bacteriocins. Applied and
380 Environmental Microbiology 62 (5), 1676-1682.
381 Aymerich, T., Picouet, P.A., Monfort, J.M., 2008. Decontamination technologies for
382 meat products. Meat Science 78, 114-129.
383 Bakal G. and Diaz,A., 2005. “The lowdown on lauric arginate: Food antimicrobial
384 hammers away at plasma membrane, disrupting a pathogen’s metabolic process,”
385 Food Quality. 12 (1), 54-61.
386 Bastarrachea, L., Dhawan, S., Sablani, S.S., Mah, J.H., Kang, D.H., Zhang, J., Tang,
387 J., 2010. Biodegradable Poly (butylene adipate-co-terephthalate) films incorporated
388 with Nisin: Characterization and effectiveness against *Listeria innocua*. Journal of
389 Food Science 75 (4), E215-E224.
390 Blanco Massani, M., Fernandez, M.R., Ariosti, A., Eisenberg, P., Vignolo, G., 2008.
391 Development and characterization of an active polyethylene film containing

392 *Lactobacillus curvatus* CRL705 bacteriocins. Food Additives and Contaminants. Part
393 A-Chemistry Analysis. Control Exposure & Risk Assessment 25, 1424-1430.
394 Blanco Massani, M., Molina, V., Sanchez, M., Renaud, V., Eisenberg, P., Vignolo, G.,
395 2014. Active polymers containing *Lactobacillus curvatus* CRL705 bacteriocins:
396 Effectiveness assessment in Wieners. International Journal of Food Microbiology
397 178, 7-12.
398 Blanco Massani, M., Vignolo, G.M., Eisenberg, P., Morando, P.J., 2013. Adsorption of
399 the bacteriocins produced by *Lactobacillus curvatus* CRL705 on a multilayer-LLDPE
400 film for food-packaging applications. LWT-Food Science and Technology 53, 128-
401 138.
402 Coma, V., 2008. Bioactive packaging technologies for extended shelf life of meat-
403 based products. Meat Science 78, 90-103.
404 dos Santos Pires, A.C., Ferreira Soares, N.d.F., de Andrade, N.J., Mendes do Silva,
405 L.H., Camilloto, G.P., Bernardes, P.C., 2008. Development and evaluation of active
406 packaging for sliced mozzarella preservation. Packaging technology and Science
407 21, 375-383.
408 EFSA-ECDC (European Food Safety Authority and European Centre for Disease
409 Prevention and Control), 2021. The European Union One Health 2019 zoonoses
410 report. EFSA Journal 19(2): e06406.
411 Eijsink, V.G.H., Skeie, M., Middelhoven, P.H., Bruberg, M.B., Nes, I.F., 1998.
412 Comparative studies of class IIa bacteriocins of lactic acid bacteria. Applied and
413 Environmental Microbiology 64, 3275-3281.
414 *EURL Lm*. 2021. EURL Lm Guidance Document on challenge tests and durability
415 studies for assessing shelf-life of ready-to-eat-foods related to *Listeria*
416 *monocytogenes* in ready-to-eat foods. Version 4 of 1 July 2021. European
417 Reference laboratory for *Listeria monocytogenes*. ANSES. Maisons-Alfort. France.

418 European Commission, 2005. Commission Regulation (EC) No 2073/2005 of 15
419 November 2005 on microbiological criteria for foodstuffs. Official Journal of the
420 European Communities L 338, 1-26.
421 European Commission, 2014. Commission Regulation (EU) No 506/2014 of 15 May
422 2014 amending Annex II to Regulation (EC) No 1333/2008 of the European
423 Parliament and of the Council and the Annex to Commission Regulation (EU) No
424 231/2012 as regards ethyl lauroyl arginate as a preservative in certain heat-treated
425 meat products. Official Journal of the European Union L 145/35.
426 FSIS, 2005. Principles of Preservation of Shelf-Stable Dried Meat Products FDA/ FSIS.
427 FSIS, 2015. 9 CFR Part 430: Control of *Listeria monocytogenes* in ready-to-eat meat
428 and poultry products. Federal Register 80, 35178 -35188.
429 Gómez, D., Arino, A., Carraminana, J.J., Rota, C., Yanguela, J., 2012. Sponge versus
430 mini-roller for the surface microbiological control of *Listeria monocytogenes*, total
431 aerobic mesophiles and *Enterobacteriaceae* in the meat industry. Food Control 27,
432 242-247.
433 Guo, M., Jin, T.Z., Wang, L., Scullen, O.J., Sommers, C.H., 2014. Antimicrobial films
434 and coatings for inactivation of *Listeria innocua* on ready-to-eat deli turkey meat.
435 Food Control 40, 64-70.
436 Hereu, A., Bover-Cid, S., Garriga, M., Aymerich, T., 2012. High hydrostatic pressure
437 and biopreservation of dry-cured ham to meet the Food Safety Objectives for
438 *Listeria monocytogenes*. International Journal of Food Microbiology 154, 107-112.
439 Iseppi, R., Pilati, F., Marini, M., Toselli, M., de Niederhäusern, S., Guerrieri, E., Messi,
440 P., Sabia, C., Manicardi, G., Anacarso, I., Bondi, M., 2008. Anti-listerial activity of a
441 polymeric film coated with hybrid coatings doped with Enterocin 416K1 for use as
442 bioactive food packaging. International Journal of Food Microbiology 123, 281-287.
443 Marcos, B., Aymerich, T., Garriga, M., Arnau, J., 2013. Active packaging containing
444 nisin and high pressure processing as post-processing listericidal treatments for
445 convenience fermented sausages. Food Control 30, 325-330.

446 Marcos, B., Aymerich, T., Monfort, J.M., Garriga, M., 2007. Use of antimicrobial
447 biodegradable packaging to control *Listeria monocytogenes* during storage of
448 cooked ham. International Journal of Food Microbiology 120, 152-158.
449 Martin, B., Perich, A., Gomez, D., Yangueela, J., Rodriguez, A., Garriga, M., Aymerich,
450 T., 2014. Diversity and distribution of *Listeria monocytogenes* in meat processing
451 plants. Food Microbiology 44, 119-127.
452 Mauriello, G., Ercolini, D., La Storia, A., Casaburi, A., Villani, F., 2004. Development of
453 polythene films for food packaging activated with an antilisterial bacteriocin from
454 *Lactobacillus curvatus* 32Y. Journal of Applied Microbiology 97, 314-322.
455 Montiel R., Quesille-Villalobos A., Alessandria V., Medina, M, Cocolin .S., Rantsiou, K.,
456 2019. Antilisterial effect and influence on *Listeria monocytogenes* gene expression
457 of enterocin or *Enterococcus faecalis* in sliced dry-cured ham stored at 7°C. Journal
458 of Food Protection 82 (9), 1598-1606.
459 Muriel-Galet, V., López-Carballo, G., Gavara, R., Hernández-Muñoz, P., 2012.
460 Antimicrobial food packaging film based on the release of LAE from EVOH.
461 International Journal of Food Microbiology 157, 239-244.
462 Muriel-Galet, V., Lopez-Carballo, G., Gavara, R., Hernandez-Munoz, P., 2015.
463 Antimicrobial effectiveness of lauroyl arginate incorporated into ethylene vinyl
464 alcohol copolymers to extend the shelf-life of chicken stock and surimi sticks. Food
465 and Bioprocess Technology 8, 208-217.
466 NACMCF (National Advisory Committee on Microbiological Criteria for Foods), 2010.
467 Parameters for determining inoculated pack/challenge study protocols. Journal of
468 Food Protection, 73(1), 140–202.Nolan, D.A., Chamblin, D.C., Troller, J.A., 1992.
469 Minimal water activity levels for growth and survival of *Listeria monocytogenes* and
470 *Listeria innocua*. International Journal of Food Microbiology 16, 323-335.Ortiz, S.,
471 Lopez, V., Villatoro, D., Lopez, P., Carlos Davila, J., Martinez-Suarez, J.V., 2010. A
472 3-Year Surveillance of the genetic diversity and persistence of *Listeria*

473 *monocytogenes* in an Iberian Pig Slaughterhouse and Processing Plant. Foodborne
474 Pathogens and Disease 7(10), 1177-1184.
475 Pattanayaiying, R., H-Kittikun, A., Cutter, C.N., 2014. Effect of lauric arginate, nisin Z,
476 and a combination against several food related bacteria. International Journal of
477 Food Microbiology 188, 135-146.
478 Pattanayaiying, R., H-Kittikun, A., Cutter, C.N., 2015. Incorporation of nisin Z and lauric
479 arginate into pullulan films to inhibit foodborne pathogens associated with fresh and
480 ready-to-eat muscle foods. International Journal of Food Microbiology 207, 77-82.
481 Quintavalla, S., Vicini, L., 2002. Antimicrobial food packaging in meat industry. Meat
482 Science 62, 373-380.
483 Scannell, A.G.M., Hill, C., Buckley, D.J., Arendt, E.K., 1997. Determination of the
484 influence of organic acids and nisin on shelf-life and microbiological safety aspects
485 of fresh pork sausage. Journal of Applied Microbiology 83, 407-412.
486 Serra-Castelló C, Jofré A, Garriga M, Bover-Cid S., 2020. Modeling and designing a
487 *Listeria monocytogenes* control strategy for dry-cured ham taking advantage of
488 water activity and storage temperature. Meat Science 165,108-131.
489 Suppakul, P., Miltz, J., Sonneveld, K., Bigger, S.W., 2003. Active packaging
490 technologies with emphasis on antimicrobial packaging and its applications. Journal
491 of Food Science 68, 408-420.
492 Tagg, J.R., Dajani, A.S., Wannamaker, L.W., 1976. Bacteriocins of Gram-positive
493 bacteria. Bacteriological Reviews 40, 722-756.
494 Trinetta, V., Floros, J.D., Cutter, C.N., 2010. Sakacin A-containing pullulan film: an
495 active packaging system to control epidemic clones of *L. monocytogenes* in ready-
496 to-eat foods. Journal of Food Safety 30, 366-381.