Use of antimicrobial biodegradable packaging to control *Listeria monocytogenes* during storage of cooked ham

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Abstract

The antimicrobial effect against *L. monocytogenes* of biodegradable films (alginate, zein and polyvinyl alcohol) containing enterocins was investigated. Survival of the pathogen was studied by means of challenge tests performed at 6ºC during 8 and 29 days, for air-packed and vacuum-packed sliced cooked ham, respectively. Air packaging was tested with two concentrations of enterocins (200 and 2000 AU/cm^2). Control air-packed cooked ham showed an increase of *L. monocytogenes* from 10^4 to 10^7 CFU/g after 8 days. By contrast, packaging with antimicrobial films effectively slowed down the pathogen's growth, leading to final counts lower than in control lots. Air-packaging with alginate films containing 2000 AU/cm^2 of enterocins effectively controlled *L. monocytogenes* for 8 days. An increase of only 1 log unit was observed in zein and polyvinyl alcohol lots at the same enterocin concentration. Vacuum packaging with films containing enterocins (2000 AU/cm^2) also delayed the growth of the pathogen. No increase from inoculated levels was observed during 15 days in antimicrobial alginate films. After 29 days of storage, the lowest counts were obtained in samples packed with zein and alginate films containing enterocins, as well as with zein control films. The most effective treatment for controlling *L. monocytogenes* during 6ºC storage was vacuum-packaging of sliced cooked ham with alginate films containing 2000 AU/cm^2 of enterocins. From the results obtained it can concluded that antimicrobial packaging can improve the safety of sliced cooked ham by delaying and reducing the growth of *L. monocytogenes*.

Keywords: biodegradable films, enterocins, air/ vacuum packaging, *L. monocytogenes*

1. Introduction

Ready-to-eat (RTE) products are completely processed prior to final packaging and are consumed without further cooking, therefore there is a possibility of the occurrence of food-borne illness if further contamination by pathogenic bacteria occurs (USDA, 2005). Cooked ham is an example of RTE meat product submitted to a thermal treatment enough to eliminate pathogens, but is subsequently exposed to the environment during peeling, slicing, and repackaging operations.

Listeria monocytogenes is a widely distributed pathogen that may be present in raw meat and other ingredients, and thus can be continuously introduced into the processing environment. The pathogen is able to survive for long periods of time in the environment, on foods, and in processing plants (USDA, 2003). Even after cleaning, a prevalence of *L. monocytogenes* of 10% was detected in surface samples of the investigated equipment of small Spanish processing plants of traditional fermented products (Garriga, Fadda, Aymerich & Hugas, 2004). *L. monocytogenes* can be destroyed after a heat treatment, however due to its prevalence on surface facilities there may be cross-contamination of the food product after the heat treatment and subsequent growth (Buncic, Paunovic, Radisic, 1990; Uyttendaele, Rajkovic, Benos, François, Devlieghere & Debevere, 2004).

The ability of *L. monocytogenes* to grow at refrigeration temperatures provides easy survival and proliferation of the organism in RTE meat products (Glass and Doyle, 1989; Duffy, Vanderlinde & Grau, 1994; Barakat and Harris, 1999). Additionally, cooked ham provides an adequate pH, a_w, and nutrients for the growth of the pathogen. That is why special efforts to prevent contamination of meat products with *L. monocytogenes* must be conducted at all levels of production.

Bacteriocins produced by lactic acid bacteria are cationic, hydrophobic, and amphiphilic peptides, with antibacterial activity against many Gram-positive bacteria (Ray, 1992; Chen and Hoover, 2003). Class IIa bacteriocins, described as being active against *Listeria*, are one of the most interesting groups of antimicrobial peptides used in food preservation (Drider, Fimland, Héchard, McMullen & Prévost, 2006). Among class IIa bacteriocins, enterocins have proved to be effective for controlling *L. monocytogenes* growth in meat products (Aymerich, Garriga, Ylla, Vallier, Monfort & Hugas, 2000; Vignolo et al., 2000; Ananou et al., 2005). Bacteriocins can be

applied to meat products in several ways: starter cultures, active packaging, in the meat batter and/or sprayed on the meat surface (Hugas, Garriga, Aymerich & Monfort, 2002).

Active packaging is a further potential application in which bacteriocins can be incorporated into packaging destined to be in contact with food (Deegan, Cotter, Hill & Ross, 2006). Antimicrobial packaging can extend the shelf life and safety of foods by preventing the growth of both pathogenic and spoilage microorganisms as a result of the extension of their lag phase and/or by the reduction of their growth rate (Han, 2000; Quintavalla and Vicini, 2002). Antimicrobial films have been developed for the delivery of lantibiotic bacteriocins like nisin and lacticin 3147 (Coma, Sebti, Pardon, Deschamps & Pichavant, 2001; Siragusa, Cutter & Willett, 1999), uncharacterized bacteriocins and class IIa bacteriocins such as pediocin (Ming, Weber, Ayres & Sandine, 1997; Mauriello, Ercolini, La Storia, Casaburi & Villani, 2004). However, other known class IIa bacteriocins have not yet been used in this manner (Drider et al., 2006).

The objective of this work was to control the growth of *L. monocytogenes* on sliced cooked ham packed with biodegradable films containing enterocins during refrigerated storage.

2. Materials and methods

2.1. Bacteriocin production

Enterococcus faecium CTC492, isolated from a meat product and producer of enterocins A and B (Aymerich, Holo, Havarstein, Hugas, Garriga & Nes, 1996; Casaus, Nilsen, Cintas, Nes, Hernandez & Holo, 1997), was grown in modified MRS broth. The composition of standard MRS was modified as follows: reduction of glucose to 0.5%, increase of Tween 80 (Sigma-Aldrich, Saint Louis, MO, U.S.A) to 0.75%, and no addition of beef extract. Enterocins A and B were obtained from a 2 litre culture grown for 15 h at 30ºC. The cells were removed by centrifugation at 10000 g for 10 min at 4ºC, and 300 g/l of ammonium sulphate (Sigma-Aldrich) were added. The protein precipitate was pelleted by centrifugation at 10000 g for 30 min and dissolved in 50 mM phosphate buffer, pH 6. An additional heat treatment of 10 min at 100ºC was applied. The obtained bacteriocin was stored at -80ºC.

2.2. Bacteriocin assay

The indicator strains, *L. monocytogenes* CTC1010, CTC1011, and CTC1034 were separately grown overnight in Triptic Soy Broth with 0.6% yeast extract (TSBYE; Merck, Darmstadt, Germany) at 30ºC.

Bacteriocin activity was quantified by the agar spot test (Tagg, Dajani, & Wannamaker, 1976). The media (BSB) composed by, in g/l, 20 beef extract, 20 glucose, and 15 agar, was used to support soft TSBYE (TSBYE with 7.5 g/l agar) seeded with 20 µl of the overnight mixture of *L. monocytogenes*. Enterocin samples were serially diluted twofold with 50 mM phosphate buffer, pH 6. A 10 µl sample of each dilution was spotted onto soft TSBYE lawn. The plates were incubated overnight at 30ºC. An arbitrary unit (AU) was defined as the highest dilution showing growth inhibition of the indicator lawn, and bacteriocin activity was expressed as AU/ml.

In vitro antimicrobial activity of control films and enterocin containing films against *L. monocytogenes* was determined by placing 2x2 cm squares of antimicrobial films on the agar surface (BSB media and TSBYE soft agar as described above). Agar plates were incubated at 30ºC overnight and bacteriocin activity was observed as a zone of inhibition of the indicator strains around the films.

2.3. Film manufacturing

Film forming solutions were obtained as suggested by Del Nobile, Buonocore, Altieri, Battaglia & Nicolais (2003) and Buonocore, Conte & Del Nobile (2005), with some modifications. Alginate solutions (A) were obtained by stirring for 2 hours at 80ºC a 5% (w/v) alginic acid (Sigma-Aldrich) solution in distilled water. Glycerol (5% v/v) was added as plasticizer, and the solution was stirred at ambient temperature for 30 min. Zein solutions (Z), 31% (w/v) zein from maize (Sigma-Aldrich) in ethanol, were dissolved stirring the solution for 2 hours at 80ºC. Glycerol (5% v/v) was added as plasticizer, and the solution was stirred at ambient temperature for 30 min. Polyvinyl alcohol solutions (P), 13% (w/v) polyvinyl alcohol (Sigma-Aldrich) in distilled water, were dissolved by keeping the solution for 20 min in an autoclave at 121ºC. After measuring the volume of the film blend, the active solution was obtained by adding the appropriate dilution from the stock solution of enterocins (409,600 AU/ml) to obtain a concentration of 200 or 2000 AU/cm^2. The solution was stirred at ambient temperature until completely dissolved.

120 The films were manufactured by casting 3 ml of the prepared solutions onto Rodac dishes and
121 were dried under a biological safety cabinet. After drying, alginate films were reticulated by
122 immersion in a 2% (w/v) calcium chloride solution. The thickness of the films obtained was
123 measured by means of a Digimatic Micrometer (Mitutoyo, Japan). The value of the film
124 thickness was obtained by averaging 10 measurements. The films obtained had an average
125 thickness of 120 µm.
126
127 *2.4. Cooked ham manufacturing*
128 Cooked ham was prepared with pork shoulder and the following additives (g/Kg; SKW
129 Biosystems, Rubí, Spain): water, 115; sodium chloride, 20.7; dextrose, 5.8; sodium
130 tripolyphosphate, 5.8; carragenate, 2.3; $NaNO_2$, 0.1; and L-ascorbate, 0.6. Pork shoulder meat
131 was minced in a cutter (Teqmaq, Spain) to a particle size of 4 mm. Ingredients were
132 homogenized in a mixer (model 35P, Tecnotrip S.A., Terrassa, Spain) for 30 min. The meat
133 batter was stuffed into impermeable plastic casings (Prolan SV 150) using a stuffing machine
134 (model H15, Tecnotrip). The product was cooked in an oven at 75ºC until internal temperature
135 reached 72ºC (total cooking time 2.6 h). Whole pieces of cooked ham were stored at 1ºC before
136 slicing.
137
138 *2.5. Sample preparation and storage*
139 Cooked ham was sliced at 7 mm thickness after aseptic removal of plastic casings. Slices were
140 inoculated with 10^4 CFU/g of an overnight culture of a 3-strain cocktail of *L. monocytogenes*
141 (CTC1010, CTC1011, CTC1034). Each slice was inoculated on both sides (25µl of the
142 appropriate dilution), the inoculum was spread uniformly with a sterile bent glass rod. Slices
143 were placed between two films and packed, both in air and under vacuum, in polyamide-
144 polyethylene bags (Sacoliva, Castellar del Vallès, Spain). Nine independent lots were air-
145 packed using: control films (AC, ZC, PC), films with 200 AU/cm^2 of enterocins added (AE200,
146 ZE200, and PE200), and films with 2000 AU/cm^2 (AE2000, ZE2000, and PE2000). Air-packed
147 samples were stored for 8 days at 6ºC. Six independent lots were vacuum-packed using: control
148 films (AC, ZC, PC), and films with 2000 AU/cm^2 of enterocins added (AE, ZE, and PE).
149 Vacuum-packed samples were stored for 29 days at 6ºC.

150 *2.6. Microbiological analysis*

151 During storage at 6ºC of air-packed cooked ham sampling was performed at days 0, 1, 3, 4, 6,
152 and 8. Vacuum-packed cooked ham was sampled at days 0, 1, 3, 4, 6, 8, 15, 22, and 29 of
153 storage at 6ºC.

154 At each selected time, 20 g of cooked ham were 10-fold diluted in sterile peptone water (AES
155 Laboratoires, Combourg, France). The solution was homogenized for 1 min in a Masticator (IUL
156 Instruments, Barcelona, Spain). After appropriate dilutions, enumeration of *Listeria* was
157 performed by spread plating on Palcam agar (Merck, Darmstadt, Germany) with supplement
158 SR0150 (Merck) incubated at 30 °C for 72 h. Lactic Acid Bacteria (LAB) and
159 *Enterobacteriaceae* enumeration was done by plating in MRS (Merck) incubated in anaerobiosis
160 at 30ºC for 72 h and VRBD (Merck) at 30ºC for 24 h, respectively. Three different samples were
161 analysed at each sampling time.

162

163 *2.7. Statistical analysis*

164 Data were analysed using the GLM procedure from the SAS statistical package (SAS © System
165 for Windows, Release 8.2, SAS Institute, Cary, NC, USA).

166 The model included enterocin concentration (0, 200, and 2000 AU/cm^2), film type (A, Z, and P),
167 packaging (air and vacuum), storage time, and their interaction as fixed effects. Differences
168 between effects were assessed by the Tukey test ($p<0.05$).

169

170 **3. Results**

171 Double interactions between the studied effects (enterocin concentration, film type, storage time
172 and air/vacuum packaging) were significant ($p<0.05$), therefore all the effects were evaluated
173 separately. Tables 1 and 2 show the significances of enterocin concentration, film type and
174 air/vacuum packaging effects. Time effect was significant in all cases ($p<0.05$).

175 *In vitro* antimicrobial activity of enterocin films on TSBYE lawn seeded with *L. monocytogenes,*
176 showed clear inhibition zones produced by films containing both 200 and 2000 AU/cm^2 of
177 enterocins. Control films showed no antimicrobial activity against *L. monocytogenes*.

178 LAB and *Enterobacteriaceae* counts were below the detection limit (<10 CFU/g) at time of
179 *Listeria* inoculation (day 0) and throughout storage (data not shown).

Air packaging

Counts of *L. monocytogenes* in air-packed cooked ham stored at 6ºC are shown in Figure 1. All samples packed with control films (alginate, AC; zein, ZC; and polyvinyl alcohol, PC) showed a significant increase of *L. monocytogenes* during storage, from inoculated levels (10^4 CFU/g) to 10^7 CFU/g in 8 days. The addition of enterocins to the films led, in general, to lower counts ($p<0.05$) of the pathogen during the entire storage period, compared with control samples. At the end of storage (day 8) all samples packed with enterocin containing films gave lower counts of *L. monocytogenes* than control lots ($p<0.05$).

Figure 1 shows how the antilisterial effectiveness of enterocin containing films (AE, ZE, PE) was dependent on the added concentration. In AC and ZC samples, an increase of *L. monocytogenes* population was detected from day 1, while no growth was detected until day 3 and 4 in AE200 and ZE200, respectively (Figs. 1A & 1B). Throughout storage lower counts were observed in AE200 and ZE200 than in control lots ($p<0.05$), the differences being 1 and 0.7 log cycles, respectively, at day 8. No differences between polyvinyl control and PE200 were observed until the end of storage, where it showed counts 0.6 log cycles lower than PC (Fig. 1C). AE200 gave the lowest levels of *L. monocytogenes* until day 4, thereafter no further differences ($p>0.05$) were observed between different film types containing 200 AU/cm^2 (table 1).

A higher concentration of enterocins added to alginate films (2000 AU/cm^2) produced an immediate bactericidal effect (1.8 log reduction). A subsequent recovery of the pathogen was observed, reaching the initial levels at the end of storage. From day 3 until the end of storage AE2000 counts were 3 logarithms lower than AC ones (Fig. 1A). In ZE2000 and PE2000 no growth of *L. monocytogenes* was detected until day 4 and 6, respectively (Figs. 1B & 1C). At the end of storage *L. monocytogenes* had increased 1.3 and 0.9 log CFU/g with respect to initial counts in ZE2000 and PE2000, respectively. When compared with ZC and PC, final counts of ZE2000 and PE2000 were 1.5 and 2.1 log CFU/g lower, respectively. Among films containing 2000 AU/cm^2, alginate films gave lower counts than zein and polyvinyl alcohol ones throughout storage.

Vacuum packaging

Figure 2 shows the behaviour of *L. monocytogenes* population in vacuum-packed cooked ham stored for 29 days at 6ºC. Among control lots, *L. monocytogenes* started to grow at day 3 in AC and PC, while no growth was detected until day 6 in ZC. Although higher counts ($p<0.05$) were detected in AC than in PC from day 4 until 8, no further differences ($p>0.05$) were observed, showing both final counts of 10^8 CFU/g. Besides, ZC samples gave lower *L. monocytogenes* counts than other control lots ($p<0.05$) from day 8 until the end of storage. At the end of storage (day 29) ZC counts were 1.6 and 1.7 logarithms lower than AC and PC, respectively.

Table 1 shows the significances of concentration effect (presence or not of enterocins in the films) on *L. monocytogenes* counts. The addition of 2000 AU/cm^2 to zein and polyvinyl alcohol films (ZE, PE) led to lower *L. monocytogenes* counts ($p<0.05$) with respect to control lots until days 15 and 22 of storage, respectively. The most important differences on *L. monocytogenes* levels between control and enterocin containing films were observed in alginate lots, with differences in a range of 1 to 4.6 log units throughout storage (29 days).

No increase from inoculated levels of *L. monocytogenes* was observed in cooked ham vacuum-packed with AE films until day 15 (Fig 2A). From day 15 it started to grow reaching maximum counts (6.81 log CFU/g) at day 22, values that were maintained until day 29. At the end of storage, *L. monocytogenes* counts were 1.7 log cycles lower in AE than AC. In ZE and PE lots a growth of 0.79 and 1.42 log units from initial levels, respectively, was detected at day 15 (Figs. 2B & 2C). Maximum growth was observed at day 22 (6.42 log CFU/g) in ZE and at day 29 (8.8 log CFU/g) in PE.

The samples packed with ZC, AE and ZE films showed the lowest *L. monocytogenes* levels at the end of storage at 6ºC. After 8 days of storage (end of air packaging storage), vacuum packaging of cooked ham led to lower counts of *L. monocytogenes* ($p<0.05$) compared with air packaging in all lots, except in AC (table 2). Differences that were 1.7 log CFU/g lower in ZC and 1 log CFU/g lower in PC, ZC, AE2000, ZE2000 and PE2000.

4. Discussion

With respect to the hygienic conditions of the product, it should be noted that in both challenge tests, the absence of LAB and *Enterobacteriaceae* throughout storage would indicate a proper

heat processing and good manufacturing practices during post-processing (peeling, slicing, and packaging) of cooked ham.

L. monocytogenes is a pathogen able to grow on cooked ham surface at refrigeration temperature of 7ºC (Aymerich et al., 2000; Uyttendaele et al., 2004). The use of a growth inhibitor would greatly reduce the risk of pathogen development in cooked ham if post-processing contamination occurs. Bacteriocins added directly or via antimicrobial packaging have proved to be efficient against meat surface contamination with *L. monocytogenes* (Ming et al., 1997; Franklin, Cooksey & Getty 2004; Aymerich, Jofré, Garriga & Hugas, 2005).

Before application of antimicrobial films to food products, it is important to assess the antimicrobial film stability, as well as bacteriocin release, it being necessary to exert the antimicrobial activity (Scannell, Hill, Ross, Marx, Hartmeier & Arendt, 2000). Alginate, zein, and polyvinyl alcohol were chosen as carrier polymers for enterocins because of its good film-forming properties (Lai and Padua, 1997; Hemeda, Hemeda & Said, 2003; Rhim, 2004), and because they have proved to be able to effectively entrap and release antimicrobial substances such as nisin and lysozyme (Natrajan and Sheldon, 2000; Bamore, Luthra, Mueller; Pressley & Beckwith; 2003; Buonocore, Sinigaglia, Corbo, Bevilacqua, La Notte & Del Nobile, 2004). Preliminary experiments conducted in TSBYE broth (non-published data) indicated that the addition of enterocins to those polymers gave films with a bactericidal effect against *L. monocytogenes.* Although enterocins were released into TSBYE broth, the films were still active after 30 days of storage at 6ºC.

Monitoring of *L. monocytogenes* in air-packed sliced cooked ham was performed for 8 days because of the short shelf life of this product when packed in the presence of air.

Antimicrobial packaging with enterocin containing films proved to be effective in delaying the growth of *L. monocytogenes*. The lower enterocin concentration (200 AU/cm^2) was, though, insufficient to prevent the growth of the pathogen. A concentration of 2000 AU/cm^2 gave better results, being antimicrobial packaging with alginate films (AE2000) the only treatment able to control *L. monocytogenes* throughout storage at 6ºC of air-packed sliced cooked ham.

In foods refrigerated at temperatures of 5-6ºC, *L. monocytogenes* is known to have a lag phase of 1 to 3 days (Bell, 2002), as was also observed in the present study. In that sense, an

extension of its lag phase was obtained by packaging with antimicrobial films containing 2000 AU/cm^2, being the most effective vacuum-packaging with AE.

The efficiency of antimicrobial packaging is based on the fact that the release rate of the antimicrobial agent from the film to food matches with the growth kinetics of the target microorganism (Han, 2003). AE was the only lot that gave lower counts of the pathogen than its control throughout the storage period, suggesting a more balanced ratio between the release rate of enterocin and the growth rate of *L. monocytogenes* in alginate films.

Vacuum packaging with PE films proved to be unable to control *L. monocytogenes* during a long storage period of cooked ham at 6ºC as it led to the same final levels than the control lot. Bacteriocins have a particular mechanism of action that can inhibit as many cells as molecules available in the medium (Moll, Konings & Driessen, 1999). Thus, the lack of antilisterial effect detected by the end of storage of PE, could indicate that there were not enough molecules of enterocins to inhibit *L. monocytogenes*. In that sense, Han (2003) suggested that the antimicrobial agent would be depleted before the expected storage period and, therefore, the packaging system would lose its antimicrobial activity when the migration rate was faster than the growth rate of the target microorganism.

The antilisterial effect observed when vacuum-packaging with zein control films was also observed by Lungu and Johnson (2005). They reported a bactericidal effect on *Listeria* of zein films (ZEG) prepared, as in the present study, with ethanol and glycerol (EG) as solvents. They attributed the antilisterial effect of zein films to the solvents, as samples treated with EG alone gave the same bactericidal effect as samples in contact with ZEG films. *In vitro assays* of ZC films on TSBYE agar seeded with *L. monocytogenes* showed no inhibition zone. Therefore, it seems that vacuum-packaging would facilitate the contact of the pathogen with the solvents.

The results obtained in vacuum packaging until day 8 confirm greater effectiveness of antimicrobial films when packed under vacuum compared with air packaging. In order to exert an antimicrobial effect, active films must be maintained in direct contact with the food (Quintavalla and Vicini, 2002; Ercolini, La Storia, Villani & Mauriello, 2006). Thus, the tightening effect exerted by vacuum packaging would improve its efficiency. Moreover, the release of hydrosoluble components from polymer films in which they are incorporated is dependent on the simultaneous entry of water (Vasquez, San Roma, Peniche & Cohen, 1997). Therefore, the

slight exudation observed in vacuum-packed ham would facilitate film hydration and thus enterocin diffusion.

This study leads to the general conclusion that antimicrobial packaging can improve the safety of sliced cooked ham by delaying and reducing the growth of *L. monocytogenes*. However, additional hurdles combined with antimicrobial films will be further investigated in order to obtain growth inhibition and destruction of *L. monocytogenes* during long storage periods of the product.

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426

427 **Figure captions**

428

429 **Figure 1.** Growth of *L. monocytogenes* in sliced cooked ham air-packed with alginate (A), zein
430 (B), and polyvinyl alcohol (C) films containing 200 AU/cm2 of enterocins (△), 2000 AU/cm2 of
431 enterocins (○), and control (●) stored at 6ºC.

432

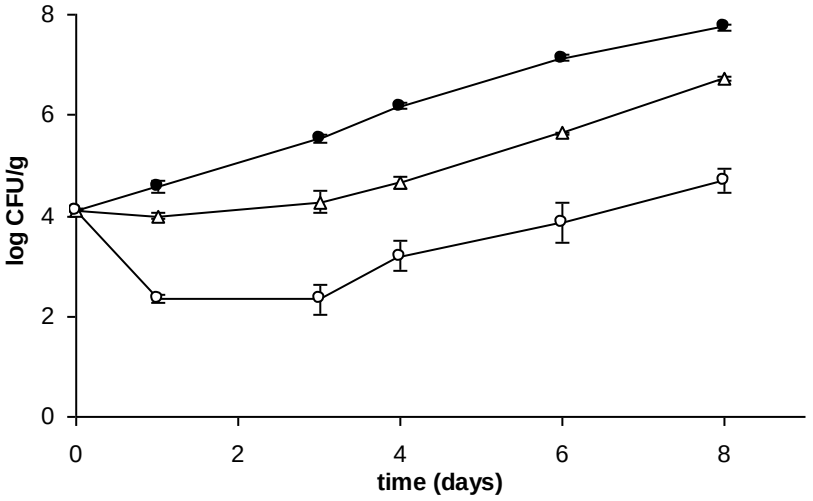
433 **Figure 2.** Growth of *L. monocytogenes* in sliced cooked ham vacuum-packed with alginate (A),
434 zein (B), and polyvinyl alcohol (C) 2000 AU/cm2 of enterocins (○), and control (●) stored at 6ºC.

435

436 **Figure 1.** Growth of *L. monocytogenes* in sliced cooked ham air-packed with alginate (A), zein
437 (B), and polyvinyl alcohol (C) films containing 200 AU/cm^2 of enterocins (△), 2000 AU/cm^2 of
438 enterocins (○), and control (●) stored at 6ºC.

439

440 **A**



441

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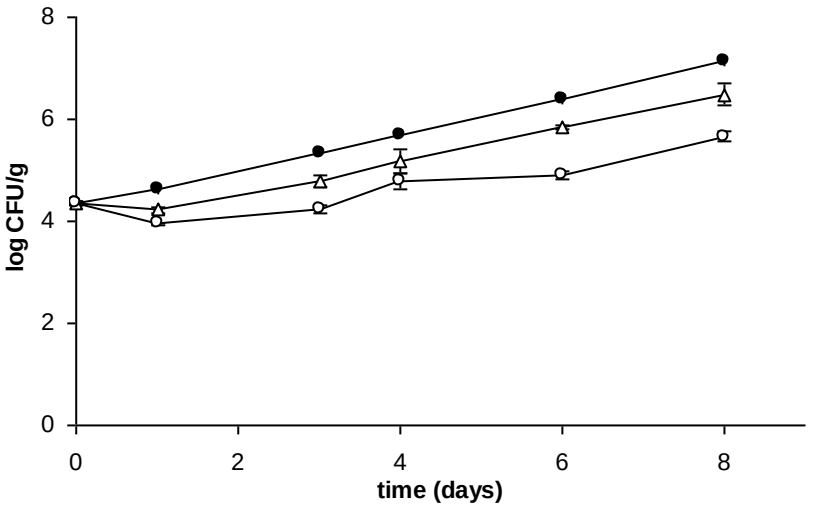
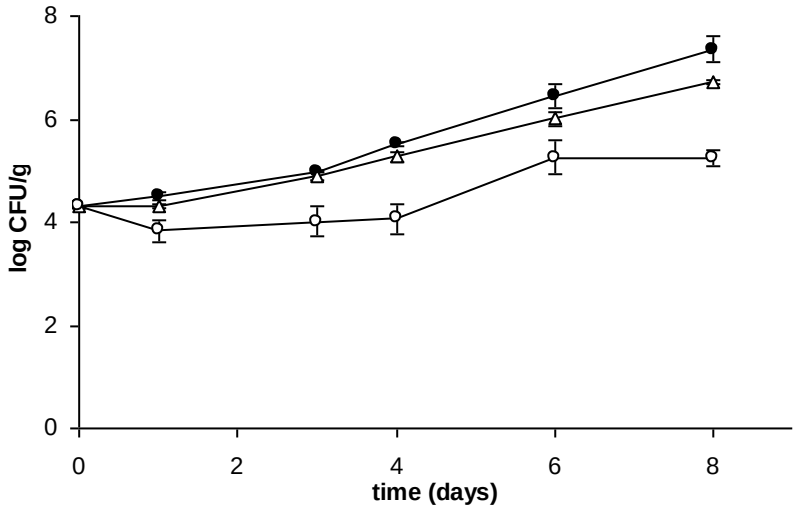


443

444

445 **C**



446

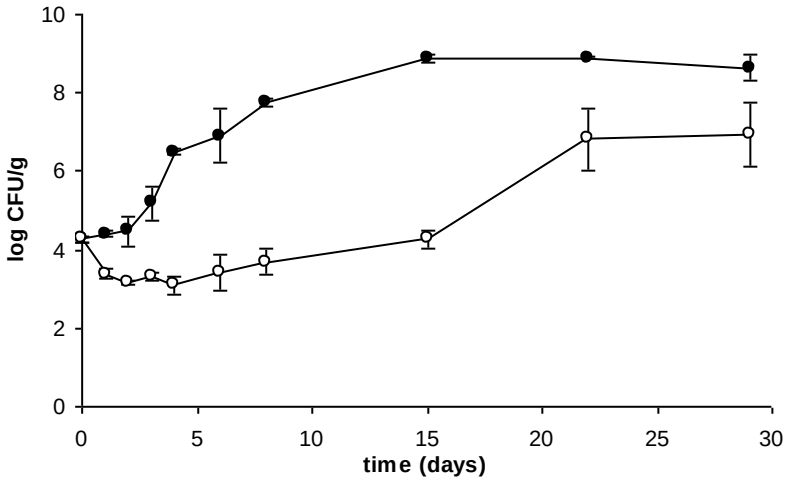
447

448 **Figure 2.** Growth *of L. monocytogenes* in sliced cooked ham vacuum-packed with alginate (A),

449 zein (B), and polyvinyl alcohol (C) 2000 AU/cm^2 of enterocins (○), and control (●) stored at 6ºC.

450

451 **A**



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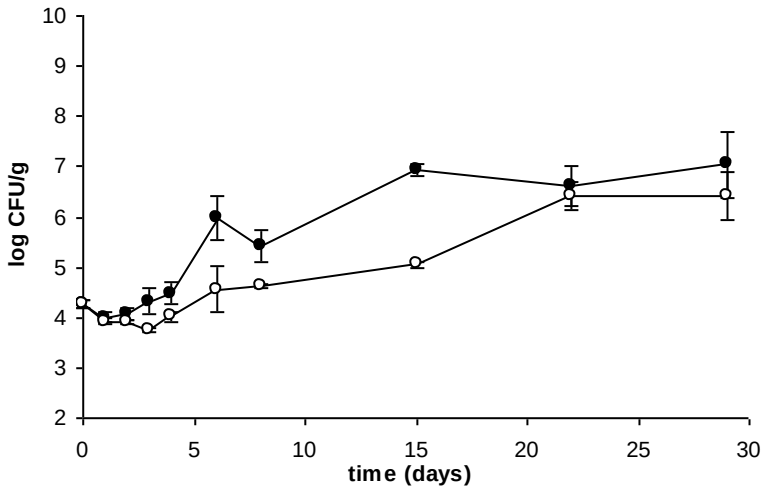
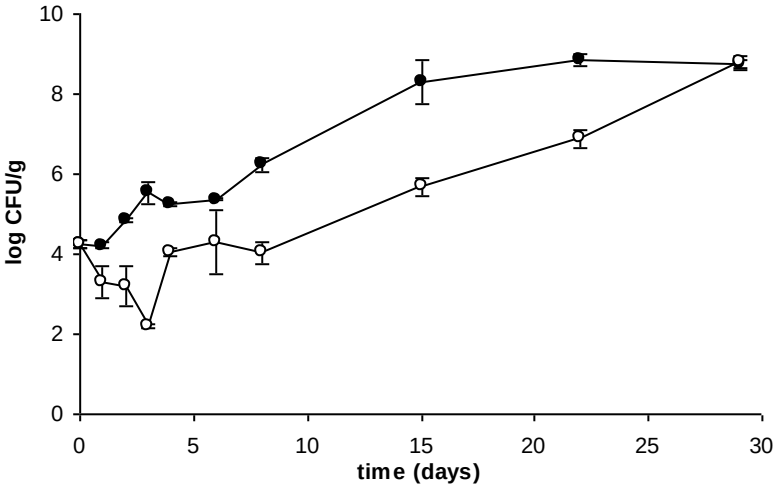
454 **B**



455

456

457 **C**



458

459

460 **Table captions**

461

462 **Table 1.** Table of significance for enterocin concentration (0, 200, 2000 AU/cm^2) and film type
463 (A: alginate, Z: zein, P: polyvinyl alcohol) effects for each day of storage and by air/vacuum
464 packaging. Bold characters identify not significant values ($p>0.05$).

465

466 **Table 2.** Table of significance for air/vacuum packaging effect for each day of storage, by film
467 type (A: alginate, Z: zein, P: polyvinyl alcohol) and enterocin concentration (0, 200, 2000
468 AU/cm^2). Bold characters identify not significant values ($p>0.05$).

469

470 **Table 1.** Table of significance for enterocin concentration (0, 200, 2000 AU/cm^2) and film type (A: alginate, Z: zein, P: polyvinyl alcohol) effects for

471 each day of storage and by air/vacuum packaging. Bold characters identify not significant values (p>0.05).

472

	Air						Vacuum				
	Concentration effect			Film type effect			Concentration effect			Film type effect	
storage (days)	A	Z	P	0	200	2000	A	Z	P	0	2000
1	<0.0001	<0.0001	<0.0018	**0.3803**	0.0011	<0.0001	0.0002	**0.2780**	0.0178	0.0019	0.0399
3	<0.0001	<0.0001	<0.0012	<0.0001	0.0060	<0.0001	0.0018	0.0209	<0.0001	0.0104	<0.0001
4	<0.0001	0.0016	0.0002	<0.0001	0.0073	0.0008	<0.0001	0.0312	<0.0001	<0.0001	0.0005
6	<0.0001	<0.0001	0.0031	0.0016	0.0041	0.0032	0.0017	0.0123	0.0783	0.0184	**0.1258**
8	<0.0001	<0.0001	<0.0001	0.0128	**0.0794**	0.0013	<0.0001	0.0146	0.0003	<0.0001	0.0102
15	-	-	-				<0.0001	<0.0001	0.0085	0.0009	0.0010
22	-	-	-				0.0102	**0.5688**	0.0002	0.0016	**0.5178**
29	-	-	-				0.0294	**0.2486**	**0.5876**	0.0049	0.0043

473 **Table 2.** Table of significance for air/vacuum packaging effect for each day of storage, by film
474 type (A: alginate, Z: zein, P: polyvinyl alcohol) and enterocin concentration (0, 200, 2000
475 AU/cm^2). Bold characters identify not significant values (p>0.05).

476

storage (days)	A		Z		P	
	0	2000	0	2000	0	2000
1	**0.0659**	0.0002	0.0018	**0.5766**	0.0061	**0.1134**
3	**0.2801**	0.0049	0.0028	0.0008	0.0194	0.0005
4	0.0077	**0.6530**	0.0007	0.0022	0.0024	**0.9459**
6	**0.5971**	**0.2643**	**0.4157**	**0.2623**	0.0013	**0.1212**
8	**0.8266**	0.0129	0.0009	0.0001	0.0036	0.0024

477