coatings

MDPI

Article

Inactivation of *Listeria* in Foods Packed in Films Activated with Enterocin AS-48 plus Thymol Singly or in Combination with High-Hydrostatic Pressure Treatment

Irene Ortega Blázquez, María José Grande Burgos, Rubén Pérez-Pulido, Antonio Gálvez * and Rosario Lucas

Department of Health Sciences, University of Jaen, 23071 Jaén, Spain; iob00003@red.ujaen.es (I.O.B.); mjgrande@ujaen.es (M.J.G.B.); rppulido@ujaen.es (R.P.-P.); rlucas@ujaen.es (R.L.)

* Correspondence: agalvez@ujaen.es; Tel.: +34-953-212160

Academic Editor: Stefano Farris

Received: 16 October 2017; Accepted: 15 November 2017; Published: 18 November 2017

Abstract: The aim of the present study was to determine the efficacy of films activated with enterocin AS-48 plus thymol singly, or in combination with high-hydrostatic pressure (HHP) on the inactivation of *Listeria innocua* in sea bream fillets and in fruit puree stored under refrigeration for 10 days. *L. innocua* proliferated in control fish fillets during storage. The activated film reduced viable *Listeria* counts in fillets by 1.76 log cycles and prevented growth of survivors until mid-storage. Application of HHP treatment to fillets packed in films without antimicrobials reduced *Listeria* counts by 1.83 log cycles, but did not prevent the growth of survivors during storage. The combined treatment reduced viable counts by 1.88 log cycles and delayed growth of survivors during the whole storage period. *L. innocua* survived in puree during storage. The activated film reduced *Listeria* counts by 1.80 and 2.0 log cycles at days 0 and 3. After that point, *Listeria* were below the detection limit. No viable *Listeria* were detected in the purees after application of HHP treatment singly, or in combination with the activated film. Results from the study indicate that the efficacy of activated films against *Listeria* is markedly influenced by the food type.

Keywords: *Listeria*; activated film; bacteriocin; thymol; high-hydrostatic pressure; fish fillets; fruit puree

1. Introduction

Listeria monocytogenes is a psychrotrophic foodborne Gram-positive bacterium which is widely found in the environment, such as in plants, soil, animal, water, dirt, dust, and silage [1,2]. In the EU, there was a statistically significant increasing trend of listeriosis over 2008–2015, with a case fatality of 17.7% [3]. Ready-to-eat (RTE) fish and other fishery products showed the highest percentages of non-compliance at retail, followed by other foods of animal origin, such as meats and dairy products [3]. *L. monocytogenes* was also reported in vegetables, fruits, and fruit puree [3]. Consumption of fruits and vegetables contaminated with *L. monocytogenes* resulted in outbreaks of listeriosis [4,5]. Therefore, it is of the utmost importance to reduce the risks for transmission of this bacterium through the food chain.

One approach to reduce transmission of foodborne pathogens is the application of biologically-derived compounds like bacteriocins and phytochemicals in antimicrobial food packagings [6–8]. Antimicrobial packaging increases the shelf life, safety, and quality of many food products by reducing microbial growth in non-sterile foods and minimizing the hazard of post-contamination of processed products [9]. Antimicrobial packaging achieves a slow migration of antimicrobial agents from an area of high concentration (packaging material) to an area of low concentration (food), providing a more prolonged exposure of bacteria to antimicrobials and decreasing

the loss of antimicrobial activity upon interaction with the food matrix [10]. When used as part of hurdle technology [11], activated films can improve microbial inactivation in foods.

Films activated with natural antimicrobials have been tested for inhibition of foodborne pathogens in several studies, as exemplified by bacteriocins [12–17] and plant essential oils, or their bioactive compounds [18–24]. Enterocins are the bacteriocins produced by enterococci. Enterocin AS-48 is a 7.2-kDa circular peptide produced by *Enterococcus faecalis* and *E. faecium* strains [25]. Enterocin AS-48 (either singly, or in combination with other antimicrobials) is active against foodborne pathogens and food spoilage bacteria [25]. The anti-*Listeria* activity of enterocin AS-48 can be potentiated by thymol [26] and by high-hydrostatic pressure (HHP) [27]. The combined application of films activated with natural antimicrobials and HHP treatments of low intensity seems a feasible approach for non-thermal preservation of foods and for decreasing the risks of proliferation of survivors during storage, while at the same time reducing the impact of treatments on the food physico-chemical properties.

The aim of the present study was to determine the anti-*Listeria* effects of films activated with a mixture of enterocin AS-48 and thymol (a cheap, commercially available antimicrobial), singly, or in combination with a mild HHP treatment. Foods having pH values close to neutral (sea bream fillets) or acidic pH (fruit puree) were chosen as model food systems for the assays.

2. Materials and Methods

2.1. Inoculum Preparation

Listeria innocua (CECT 4030) was used as a surrogate of *L. monocytogenes*. The strain was supplied by the Spanish Type Culture Collection (CECT, Burjasot, Valencia, Spain). The bacterium was cultivated overnight at 37 °C on trypticase soy broth (TSB, Scharlab, Barcelona, Spain) and diluted ten-fold in sterile saline solution.

2.2. Preparation of Activated Plastic Bags

Enterocin AS-48 was prepared by cation exchange chromatography following a previously-described procedure [28]. Thymol was from Sigma-Aldrich (Madrid, Spain). Polyethylene-polyamide bags (10 × 15 cm^2) were activated by addition of 1 mL enterocin AS-48 solution (0.5 μg/μL) plus 1 mL 0.5% thymol followed by hand rubbing to ensure impregnation of the whole inner bag surface. After incubation for one hour at ambient temperature, the remaining liquid inside the bags was removed and the bags were allowed to dry for one hour in a biosafety cabinet (Telstar, Madrid, Spain) under UV irradiation in order to avoid contamination.

2.3. Sample Preparation, Treatments, and Microbiological Analysis

The efficacy of activated bags against *Listeria* was tested in two model food systems: a fruit puree, having an acidic pH, and fish fillets having a pH close to neutrality. Fruit puree was prepared from Golden Delicious apples, pears, and bananas bought at a local supermarket. Fruits were peeled under aseptic conditions and mixed in equal amounts. The fruit mixture was processed with a domestic blender for 1 min to obtain the fruit puree. Fillets from sea bream (*Sparus aurata*) with skin were purchased at a local supermarket and cut into 5 g portions with a sterile knife under aseptic conditions. The pH of samples was measured with a pH meter (Crison, Barcelona, Spain). All food samples (5 g fruit puree, 5 g sea bream portions) were spiked with *L. innocua* to yield approximately 5.3–5.5 log CFU/g, packed in the activated bags and sealed under vacuum. In parallel, food samples spiked with *L. innocua* were packed in bags that had not been activated with any antimicrobial treatment. For each type of food and treatment, two batches (each one in triplicate) were prepared. Half of the samples (packed in films activated with antimicrobials, or not) were treated by high-hydrostatic pressure (HHP) at 300 MPa for 5 min while the rest were not treated. HHP treatment was applied with a Stansted Fluid Power LTD HHP equipment (SFP, Essex, UK) operating under standard conditions

described elsewhere [29]. All samples (packed in films with or without antimicrobials, and treated by HHP, or not) were stored at 5 °C for 10 days. At desired incubation times, bags (in triplicate) were removed and the food content was pooled and homogenized with 10 mL sterile buffered peptone water (Scharlab, Barcelona, Spain). The resulting homogenate was serially diluted in sterile saline solution (0.85% NaCl, Scharlab) and plated in triplicate on polymyxin B-acriflavine-lithium chloride-ceftazidime-aesculin-mannitol (PALCAM) agar with added antibiotic supplement (Scharlab). The average number of colonies obtained after 24–48 h incubation of the plates at 37 °C was used to calculate the viable cell concentration (expressed as log colony forming units (CFU)/g).

2.4. Statistical Analysis

The average data ± standard deviations from replicates were determined with Excel programme (Microsoft Corp., Redmond, WA, USA). A paired *t*-test was performed at the 95% confidence interval in order to determine the statistical significance of data (Statgraphics Plus version 5.1, Statistical Graphics Corp., Princeton, NJ, USA).

3. Results

Sea bream fillets (pH 6.48) inoculated with *L. innocua* were packed in plastic bags activated or not with antimicrobials and then treated or not by HHP (Table 1). For the controls (samples packed in plastic bags without antimicrobials and without HHP treatment), viable *Listeria* counts already increased by day 5, and were significantly higher ($p < 0.05$) by 1.7 log cycles at the end of storage period. The activated film packaging significantly ($p < 0.05$) reduced viable cell counts by 1.76 log cycles, and delayed growth of the bacterium during storage. Viable *Listeria* counts for samples packed in the activated film remained significantly ($p < 0.05$) lower than controls by 1–2.67 log cycles within the first seven days of storage. However, *Listeria* counts reached the same value as controls by the end of storage period. Application of HHP treatment to samples packed in films without antimicrobials reduced *Listeria* counts significantly ($p < 0.05$) by 1.83 log cycles. However, multiplication of survivors during storage resulted in viable counts that were not significantly lower ($p > 0.05$) than controls for storage times 3, 5, and 7. Application of HHP treatment to samples packed in activated films achieved a significant ($p < 0.05$) reduction of viable counts by 1.88 log cycles. Growth of survivors was also delayed until the end of storage, with a significant ($p < 0.05$) increase in the concentration of *Listeria* of only one log cycle. Compared to the single activated film treatment, the combined treatment achieved a more prolonged delay in growth of the surviving *Listeria* population and achieved significantly ($p < 0.05$) lower viable counts at the end of storage (day 10), but not earlier. Compared to the single HHP treatment, the HHP treatment in combination with the activated film achieved significantly ($p < 0.05$) lower *Listeria* counts (1.28–3.15 log cycles) during storage times 3, 5, and 7.

Table 1. Viable cell counts for *Listeria* in sea bream fillets challenged with the bacterium and stored under refrigeration. Fillets were packed in polyethylene-polyamide bags without any treatment (controls), or in bags activated with 0.5% thymol plus 80 mg/mL enterocin AS-48. When indicated, packed fillets were treated by HHP (300 MPa, 5 min).

Treatment	Storage Time (Days)				
	0	**3**	**5**	**7**	**10**
Control	5.30 ± 0.09	4.93 ± 0.08	6.03 ± 0.22	6.36 ± 0.12	7.02 ± 0.21
Activated film (AF)	3.54 ± 0.15	3.88 ± 0.21	3.36 ± 0.22	4.06 ± 0.46	6.85 ± 0.38
HHP	3.47 ± 0.16	4.75 ± 0.17	6.06 ± 0.39	6.56 ± 0.18	5.20 ± 0.42
AF-HHP	3.42 ± 0.21	3.47 ± 0.31	3.25 ± 0.25	3.41 ± 0.29	4.49 ± 0.22

Note: Data (log CFU/g) are the average of two replicates ± standard deviation.

Listeria was unable to proliferate in the fruit puree having an acidic pH of 4.52. (Table 2). The concentrations of viable *Listeria* in the control fruit puree (films not activated) decreased slowly

during the first seven days of storage (being 0.9 log cycles lower at day 7) and more rapidly during late storage (being 2 log cycles lower at day 10). For samples packed in the activated film, viable *Listeria* counts were significantly lower ($p < 0.05$) than controls by 1.8 and 2.0 log cycles at times 0 and 3, respectively. Furthermore, counts were below the detection limit (1.0 log CFU/g) for the remaining storage period. For the samples treated by HHP singly or in combination with the activated film, no viable *Listeria* were detected at any sampling point.

Table 2. Viable cell counts for *Listeria* in fruit puree challenged with the bacterium and stored under refrigeration. Fruit puree was packed in polyethylene-polyamide bags without any treatment (controls), or in bags activated with 0.5% thymol plus 80 mg/mL enterocin AS-48. When indicated, packed puree was treated by HHP (300 MPa, 5 min).

Treatment	Storage Time (Days)				
	0	3	5	7	10
Control	5.49 ± 0.08	4.77 ± 0.12	4.38 ± 0.11	4.60 ± 0.14	3.27 ± 0.29
Activated film (AF)	3.69 ± 0.12	2.69 ± 0.14	<1.0	<1.0	<1.0
HHP	<1.0	<1.0	<1.0	<1.0	<1.0
AF-HHP	<1.0	<1.0	<1.0	<1.0	<1.0

Note: Data (log CFU/g) are the average of two replicates ± standard deviation.

4. Discussion

Results from the present study indicate that both the activated film and the HHP treatment achieved similar initial reductions in the concentration of viable *L. innocua* as a surrogate of *L. monocytogenes* in sea bream fillets. While the HHP treatment did not prevent further proliferation of survivors during food storage, the activate film delayed growth at least until day 7. Compared to other methods for bacteriocin addition such as dipping or spraying with bacteriocin solutions, activated films provide the advantage of a slow release of antimicrobials into the medium while, at the same time, can also provide some protection against bacteriocin degradation by tissue proteases or by complexation with food components [6]. Although the combined treatment of activated films with HHP did not improve inactivation of *Listeria* significantly compared to the activated films alone, it still delayed proliferation of survivors for the whole storage period, indicating a longer protection against *Listeria*. A previous study reported that spray application of enterocin AS-48 at 0.37 μg/cm^2 reduced viable counts of *L. monocytogenes* by 1.68 and 1.9 log cycles in raw hake and raw salmon fillets, respectively [30]. This effect was similar to the one obtained in the present study for films activated with a higher concentrated bacteriocin solution in combination with thymol. These results would suggest that the amount of antimicrobials adsorbed on the plastic films was low and/or that diffusion of bacteriocin and thymol into the fish tissue was slower compared to the more direct application by spray.

In the present study, *Listeria* was inoculated in the fish fillets at a concentration high enough to allow determination of log reductions of at least 4 log cycles. However, the initial concentrations of the bacterium in raw foods are expected to be much lower. For example, one recent study [31] reported that out of the 17.6–18.4% of the 301 marine fresh and smoked fish samples positive for *L. monocytogenes*, only three samples had viable *Listeria* counts above 2 log CFU/g, the highest concentration being 3.88 log CFU/g. Considering an initial concentration of 3 log CFU/g in the raw material, the combined treatment of enterocin and HHP would reduce the viable cell concentration below the threshold level of 100 viable cells per gram of food.

In the fruit puree, *Listeria* was unable to multiply possibly because of the acidic pH of the fruit, but it did survive quite well during the first seven days of storage. Previous studies have shown that *L. monocytogenes* can grow in a number of vegetables [32–34], in non-acidic fruits [35,36] and in acidic fruits such as tomatoes [37], peeled Hamlin oranges [38], sliced raspberries, pear or kiwi [36], and in Golden Delicious apple slices [39,40]. In 2014, a listeriosis outbreak occurring in the USA was linked

to commercially-produced, prepackaged whole caramel apples [4]. A previous study showed that edible coatings activated with enterocin AS-48 at concentrations of 20 or 40 μg/mL reduced the viable cell concentrations of *L. monocytogenes* on apple cubes and improved inactivation of the bacterium in a concentration-dependent way during storage [41]. Results from the present study indicated that, under acidic pH condition, *L. innocua* was much more sensitive to treatment with activated films containing enterocin AS-48 and thymol and also to HHP treatment compared to results obtained on sea bream fillets having a pH value close to neutrality. It would be expected that the stress conditions imposed by acidic pH could decrease the capacity of *Listeria* to repair cell damage caused by enterocin AS-48 and thymol as well as by the HHP treatment. Previous studies have shown that inactivation of microorganisms by HHP treatments greatly depends on the food matrix and the pH of the food product [42]. Acid stress may enhance tolerance to HHP, but at the same time organic acids may act synergistically with HHP [42]. Another study [43] reported an additional 3 log CFU/g reduction in *L. monocytogenes* when pressurized at (353 MPa, 10 min, 45 °C) in buffer at pH 4.0 as compared with pH 6.0. A faster diffusion of antimicrobials into the puree could also contribute to the observed effects. Previous studies have shown that the anti-*Listeria* effect of enterocin AS-48 greatly depends on the medium, with bactericidal concentrations ranging from as low as 0.8 μg/mL in brain-heart infusion broth [44] to 0.8 μg/g in cooked ham [45]. According to results from the present study, and presuming a low *Listeria* load in fruit purees, both the activated film and the HHP treatment of low intensity would be effective in improving the safety of purees.

5. Conclusions

Thymol plus enterocin AS-48 incorporated in activated films delayed the proliferation of *Listeria* population in seabream fillets and induced gradual inactivation of the bacterium in fruit puree. Combination of activated films with HHP treatment provided a longer protection against *Listeria* proliferation in fillets. HHP treatment was more effective in fruit puree, achieving complete inactivation of *Listeria* population.

Acknowledgments: This work was supported by research grant UJA2014/06/25 and the University of Jaen's Plan de Apoyo a la Investigación (research structure AGR230). We also acknowledge the Campus de Excelencia Internacional Agroalimentario CeiA3.

Author Contributions: Rosario Lucas and María José Grande Burgos conceived and designed the experiments; Irene Ortega Blázquez and Rubén Pérez-Pulido performed the experiments; Rosario Lucas and Antonio Gálvez analyzed the data; Rosario Lucas and Antonio Gálvez wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. McLauchlin, J.; Mitchell, R.T.; Smerdon, W.J.; Jewell, K. *Listeria monocytogenes* and listeriosis: A review of hazard characterisation for use in microbiological risk assessment of foods. *Int. J. Food Microbiol.* **2004**, *92*, 15–33. [CrossRef]
2. Gandhi, M.; Chikindas, M.L. *Listeria*: A foodborne pathogen that knows how to survive. *Int. J. Food Microbiol.* **2007**, *113*, 1–15. [CrossRef] [PubMed]
3. European Food Safety Authority (EFSA); European Centre for Disease Prevention and Control (ECDC). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. *EFSA J.* **2016**, *14*, 4634.
4. Garner, D.; Kathariou, S. Fresh produce-associated listeriosis outbreaks, sources of concern, teachable moments, and insights. *J. Food Prot.* **2016**, *79*, 337–344. [CrossRef] [PubMed]
5. McCollum, J.T.; Cronquist, A.B.; Silk, B.J.; Jackson, K.A.; O'Connor, K.A.; Cosgrove, S.; Gossack, J.P.; Parachini, S.S.; Jain, N.S.; Ettestad, P.; et al. Multistate outbreak of listeriosis associated with cantaloupe. *N. Engl. J. Med.* **2013**, *369*, 944–953. [CrossRef] [PubMed]
6. Gálvez, A.; Abriouel, H.; López, R.L.; Ben Omar, N. Bacteriocin-based strategies for food biopreservation. *Int. J. Food Microbiol.* **2007**, *120*, 51–70. [CrossRef] [PubMed]

7. Irkin, R.; Esmer, O.K. Novel food packaging systems with natural antimicrobial agents. *J. Food Sci. Technol.* **2015**, *52*, 6095–6111. [CrossRef] [PubMed]
8. Malhotra, B.; Keshwani, A.; Kharkwal, H. Antimicrobial food packaging: Potential and pitfalls. *Front. Microbiol.* **2015**, *6*, 611. [CrossRef] [PubMed]
9. Hotchkiss, J. Food packaging interactions influencing quality and safety. *Food Addit. Contam.* **1997**, *14*, 601–607. [CrossRef] [PubMed]
10. Han, J.H. Antimicrobial food packaging. *Food Technol.* **2000**, *54*, 56–65.
11. Leistner, L.; Gorris, L.G.M. Food preservation by hurdle technology. *Trends Food Sci. Technol.* **1995**, *6*, 2–46. [CrossRef]
12. Mauriello, G.; de Luca, E.; la Storia, A.; Villani, F.; Ercoloni, D. Antimicrobial activity of a nisin-activated plastic film for food packaging. *Lett. Appl. Microbiol.* **2005**, *41*, 464–469. [CrossRef] [PubMed]
13. Neetoo, H.; Ye, M.; Chen, H.Q. Effectiveness and stability of plastic films coated with nisin for inhibition of *Listeria monocytogenes*. *J. Food Prot.* **2007**, *70*, 1267–1271. [CrossRef] [PubMed]
14. Santiago-Silva, P.; Soares, N.F.F.; Nobrega, J.E.; Junior, M.A.W.; Barbosa, K.B.F.; Volp, A.C.P.; Zerdas, E.R.M.A.; Würlitzer, N.J. Antimicrobial efficiency of film incorporated with pediocin (ALTA 2351) on preservation of sliced ham. *Food Control* **2009**, *20*, 85–89. [CrossRef]
15. Natrajan, N.; Sheldon, B.W. Efficacy of nisin coated polymer films to inactivate *Salmonella typhimurium* on fresh broiler skin. *J. Food. Prot.* **2000**, *63*, 1189–1196. [CrossRef] [PubMed]
16. Perez Espitia, P.J.; Soares, N.F.F.; Coimbra, J.S.R.; Andrade, N.J.; Cruz, R.S.; Medeiros, E.A.A. Bioactive peptides: Synthesis, properties and applications in the packaging and preservation of food. *Compr. Rev. Food Sci. Food Saf.* **2012**, *11*, 187–204. [CrossRef]
17. Viedma, P.M.; Ercolini, D.; Ferrocino, I.; Abriouel, H.; Omar, N.B.; Lopez, R.L.; Galvez, A. Effect of polyethylene film activated with enterocin EJ97 in combination with EDTA against *Bacillus coagulans*. *LWT Food Sci. Technol.* **2010**, *43*, 514–518. [CrossRef]
18. Guarda, A.; Rubilar, J.F.; Miltz, J.; Galotto, M.J. The antimicrobial activity of microencapsulated thymol and carvacrol. *Int. J. Food Microbiol.* **2011**, *146*, 144–150. [CrossRef] [PubMed]
19. Cerisuelo, J.P.; Bermudez, J.M.; Aucejo, S.; Catala, R.; Gavara, R.; Hernandez-Munoz, P. Describing and modeling the release of an antimicrobial agent from an active PP/EVOH/PP package for salmon. *J. Food Eng.* **2013**, *116*, 352–361. [CrossRef]
20. Otero, V.; Becerril, R.; Santos, J.A.; Rodríguez-Calleja, J.M.; Nerin, C.; García-López, M.L. Evaluation of two antimicrobial packaging films against *Escherichia coli* O157:H7 strains in vitro and during storage of a Spanish ripened sheep cheese (Zamorano). *Food Control* **2014**, *42*, 296–302. [CrossRef]
21. Muriel-Galet, V.; Cerisuelo, J.P.; López-Carballo, G.; Lara, M.; Gavara, R.; Hernández-Muñoz, P. Development of antimicrobial films for microbiological control of packaged salad. *Int. J. Food Microbiol.* **2012**, *157*, 195–201. [CrossRef] [PubMed]
22. Muriel-Galet, V.; López-Carballo, G.; Gavara, R.; Hernández-Muñoz, P. Antimicrobial food packaging film based on the release of f LAE from EVOH. *Int. J. Food Microbiol.* **2012**, *157*, 239–244. [CrossRef] [PubMed]
23. Ramos, M.; Jiménez, A.; Peltzer, M.; Garrigós, M.C. Characterization and antimicrobial activity studies of polypropylene films with carvacrol and thymol for active packaging. *J. Food Eng.* **2012**, *109*, 513–519. [CrossRef]
24. Theinsathid, P.; Visessanguan, W.; Kruenate, J.; Kingcha, Y.; Keeratipibul, S. Antimicrobial activity of lauric arginate-coated polylactic acid films against *Listeria monocytogenes* and *Salmonella typhimurium* on cooked sliced ham. *J. Food Sci.* **2012**, *77*, M142–M149. [CrossRef] [PubMed]
25. Grande-Burgos, M.J.; Pérez-Pulido, R.; López-Aguayo, M.C.; Gálvez, A.; Lucas, R. The cyclic antibacterial peptide enterocin AS-48: Isolation, mode of action, and possible food applications. *Int. J. Mol. Sci.* **2014**, *15*, 22706–22727. [CrossRef] [PubMed]
26. Molinos, A.C.; Abriouel, H.; López, R.L.; Omar, N.B.; Valdivia, A.; Gálvez, A. Enhanced bactericidal activity of enterocin AS-48 in combination with essential oils, natural bioactive compounds and chemical preservatives against *Listeria monocytogenes* in ready-to-eat salad. *Food Chem. Toxicol.* **2009**, *47*, 2216–2223. [CrossRef] [PubMed]
27. Ananou, S.; Garriga, M.; Jofré, A.; Aymerich, T.; Gálvez, A.; Maqueda, M.; Martínez-Bueno, M.; Valdivia, E. Combined effect of enterocin AS-48 and high hydrostatic pressure to control food-borne pathogens inoculated in low acid fermented sausages. *Meat Sci.* **2010**, *84*, 594–600. [CrossRef] [PubMed]

28. Abriouel, H.; Valdivia, E.; Martínez-Bueno, M.; Maqueda, M.; Gálvez, A. A simple method for semi-preparative-scale production and recovery of enterocin AS-48 derived from *Enterococcus faecalis* subsp. liquefaciens A-48-32. *J. Microbiol. Methods* **2003**, *55*, 599–605. [CrossRef]
29. Del Árbol, J.T.; Pulido, R.P.; Grande, M.J.; Gálvez, A.; Lucas, R. Survival and high-hydrostatic pressure inactivation of foodborne pathogens in salmorejo, a traditional ready-to-eat food. *J. Food Sci.* **2015**, *80*, M2517–M2521. [CrossRef] [PubMed]
30. Baños, A.; García-López, J.D.; Núñez, C.; Martínez-Bueno, M.; Maqueda, M.; Valdivia, E. Biocontrol of *Listeria monocytogenes* in fish by enterocin AS-48 and *Listeria* lytic bacteriophage P100. *LWT Food Sci. Technol.* **2016**, *66*, 672–677. [CrossRef]
31. Wieczorek, K.; Osek, J. Prevalence, genetic diversity and antimicrobial resistance of *Listeria monocytogenes* isolated from fresh and smoked fish in Poland. *Food Microbiol.* **2017**, *64*, 164–171. [CrossRef] [PubMed]
32. Harris, L.J.; Farber, J.N.; Beuchat, L.R.; Parish, M.E.; Suslow, T.V.; Garret, E.H.; Busta, F.F. Outbreaks associated with fresh produce: Incidence, growth, and survival of pathogens in fresh and fresh-cut produce. *Compr. Rev. Food Sci. Food Saf.* **2003**, *2*, 78–141. [CrossRef]
33. Penteado, A.L.; Leitao, M.F.F. Growth of *Listeria monocytogenes* in melon, watermelon and papaya pulps. *Int. J. Food Microbiol.* **2004**, *92*, 89–94. [CrossRef] [PubMed]
34. Molinos, A.C.; Abriouel, H.; Omar, N.B.; Valdivia, E.; Lucas, R.; Maqueda, M.; Cañamero, M.M.; Gálvez, A. Effect of immersion solutions containing enterocin AS-48 on *Listeria monocytogenes* in vegetable foods. *Appl. Environ. Microbiol.* **2005**, *71*, 7781–7787. [CrossRef] [PubMed]
35. Ukuku, D.O.; Fett, W. Behavior of *Listeria monocytogenes* inoculated on cantaloupe surfaces and efficacy of washing treatments to reduce transfer from rind to fresh-cut pieces. *J. Food Prot.* **2002**, *65*, 924–930. [CrossRef] [PubMed]
36. Molinos, A.C.; Abriouel, H.; Omar, N.B.; Lucas, R.; Valdivia, E.; Gálvez, A. Inactivation of *Listeria monocytogenes* in raw fruits by enterocin AS-48. *J. Food Prot.* **2008**, *71*, 2460–2467. [CrossRef] [PubMed]
37. Beuchat, L.R.; Brackett, R.E. Behavior of *Listeria monocytogenes* inoculated into raw tomatoes and processed tomato products. *Appl. Environ. Microbiol.* **1991**, *57*, 1367–1371. [PubMed]
38. Pao, S.; Brown, G.E.; Schneider, K.R. Challenge studies with selected pathogenic bacteria on freshly peeled Hamlin orange. *J. Food Sci.* **1998**, *63*, 359–362. [CrossRef]
39. Conway, W.S.; Leverentz, B.; Saftner, R.A.; Janisiewicz, W.J.; Sams, C.E.; Leblanc, E. Survival and growth of *Listeria monocytogenes* on fresh-cut apple slices and its interaction with *Glomerella cingulata* and *Penicillium expansum*. *Plant Dis.* **2000**, *84*, 177–181. [CrossRef]
40. Alegre, I.; Abadias, M.; Anguera, M.; Oliveira, M.; Viñas, I. Factors affecting growth of foodborne pathogens on minimally processed apples. *Food Microbiol.* **2010**, *27*, 70–76. [CrossRef] [PubMed]
41. Del Carmen López Aguayo, M.; Burgos, M.J.G.; Pulido, R.P.; Gálvez, A.; López, R.L. Effect of different activated coatings containing enterocin AS-48 against *Listeria monocytogenes* on apple cubes. *Innov. Food Sci. Emerg. Technol.* **2016**, *35*, 177–183. [CrossRef]
42. Alpas, H.; Kalchayanand, N.; Bozoglu, F.; Ray, B. Interactions of high hydrostatic pressure, pressurization temperature and pH on death and injury of pressure-resistant and pressure-sensitive strains of foodborne pathogens. *Int. J. Food Microbiol.* **2000**, *60*, 33–42. [CrossRef]
43. Stewart, C.M.; Jewett, F.F.; Dunne, C.P.; Hoover, G.H. Effect of concurrent high hydrostatic pressure, acidity and heat on the injury and destruction of *Listeria monocytogenes*. *J. Food Saf.* **1997**, *17*, 23–36. [CrossRef]
44. Mendoza, F.; Maqueda, M.; Gálvez, A.; Martínez-Bueno, M.; Valdivia, E. Antilisterial activity of peptide AS-48 and study of changes induced in the cell envelope properties of an AS-48-adapted strain of *Listeria monocytogenes*. *Appl. Environ. Microbiol.* **1999**, *65*, 618–625. [PubMed]
45. Ananou, S.; Baños, A.; Maqueda, M.; Martínez-Bueno, M.; Gálvez, A.; Valdivia, E. Effect of combined physico-chemical treatments based on enterocin AS-48 on the control of *Listeria monocytogenes* and *Staphylococcus aureus* in a model cooked ham. *Food Control* **2010**, *21*, 478–486. [CrossRef]