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# Effect of Activated Plastic Films on Inactivation of Foodborne Pathogens

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**Abstract:** In the present study, low density polyethylene films were activated by co-extrusion with zinc oxide, zinc acetate or potassium sorbate. Films were also surface-activated with tyrosol singly or in combination with lactic acid or p-hydroxybenzoic acid. Activated films were tested on *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica* and *Pseudomonas fluorescens*. The combinations showing greatest inhibition zones and broadest inhibitory spectrum were the films activated with tyrosol plus *p*-hydroxybenzoic acid. A small delay in growth of *Listeria innocua* was observed on seabream packed in ZnO-activated films during refrigerated storage for 7 days. When films activated with 2.5% tyrosol or with 1.5% tyrosol plus 0.5 *p*-hydroxybenzoic acid were used for vacuum packaging of smoked salmon and smoked tuna challenged with cocktails of *S. enterica* and *L. monocytogenes* strains, the combination of tyrosol and *p*-hydroxybenzoic acid improved inactivation of both pathogens during chill storage compared to films singly activated with tyrosol. The best results were obtained in smoked salmon, since no viable pathogens were detected after 7 days of chill storage for the activated film. Results from the study highlight the potential of plastic films surface-activated with tyrosol and *p*-hydroxybenzoic acid in the control of foodborne pathogens in smoked seafood.

**Keywords:** active packaging; zinc oxide; tyrosol; seafood; foodborne pathogens

## 1. Introduction

Food microbial contamination most often occurs on the surface due to post-processing and handling [1]. The use of antimicrobial substances can decrease the transmission of foodborne pathogens. Antimicrobial substances can be applied on the surfaces of food products directly (e.g., by immersion or by spraying with antimicrobial solutions). Alternatively, antimicrobial substances can be immobilized in film matrices by extrusion or applied on the surfaces of films or coatings employed in food packing. This last approach has the advantage of slow release of the antimicrobial compound and a longer protection effect. At the same time, activated films or coatings also offer protection against cross contamination of the product. A variety of antimicrobial substances, including inorganic or inorganic compounds of chemical synthesis as well as natural antimicrobials have been tested for activation of food coatings [2,3]. Low density polyethylene (LDPE) films activated with benzoic or lactic acid, silver, essential oils or bacteriocins, as well as LDPE or polyvinyl chloride (PVC) films activated with ZnO, Ag, or Ag-$TiO_2$ nanoparticles are some illustrative examples [2,3].

Natural antimicrobials have always attracted the interest of researchers and food producers as hurdles against foodborne pathogens. Plant extracts have long been investigated for their antimicrobial

compounds, many of which have phenolic structures [4]. As an example, a recent study reported that carvacrol multilayer film was found to be effective in preventing mesophiles and psychrotrophs in fresh salmon fillets [5]. The olive tree and its products (table olives, olive oil, and the resulting by products of processing industries) are rich in phenolic compounds [6]. Tyrosol is a phenolic compound resulting from hydrolysis of the olive fruit's main bitter compound, oleuropein [6–8]. Nevertheless, the potential of tyrosol as a food preservative has not been exploited to a great extent. The aim of the present study was to develop activated plastic films based on chemical compounds and natural antimicrobials with strong inhibitory activity against both Gram-positive and Gram-negative bacteria and to compare the efficacy of antimicrobials incorporated by extrusion or by surface impregnation on inactivation of foodborne pathogens both in vitro and in model seafood systems.

## 2. Materials and Methods

### *2.1. Bacterial Strains*

*Listeria monocytogenes* (strains CECT 4032, CECT 911, CECT 936, and CECT 940), *Listeria innocua* (CECT 4030), *Staphylococcus aureus* CECT 976, *Escherichia coli* CECT 4783, *Salmonella enterica* (strains CECT 915, CECT 916, CECT 4000, and CECT 4300) and *Pseudomonas fluorescens* CECT 378 were supplied by the Spanish Type Culture Collection (CECT, Burjasot, Valencia, Spain). For preparation of inocula, strains were cultivated overnight at 37 °C on trypticase soy broth (TSB, Scharlab, Barcelona, Spain) and diluted one-hundred fold in sterile saline solution. The diluted cultures were spread with a sterile cotton swab on TSA agar plates, and the plates were allowed to dry at room temperature for 15 min.

### *2.2. Film Activation and Testing*

Low density polyethylene (LDPE) films were supplied by Andaltec (Martos, Jaen, Spain). The supplied films had been activated previously by extrusion (SJH-20 extruder, Siepla Ingenieria, S.L., Barcelona, Spain) with zinc oxide, zinc acetate or potassium sorbate (at the concentrations indicated in Table 1) or not. Raw, non-activated LDPE films were also used for surface activation with tyrosol, *p*-hydroxybenzoic acid, and lactic acid (all from Sigma-Aldrich, Madrid, Spain). For surface activation, films were placed on top of a sterile glass plate (10 × 10 $cm^2$). Then, 1 mL of the coating solution was deposited on the surface of the film and spread uniformly by using a sterile 5 mL plastic pipette. The coated films were allowed to dry for 2 h in a biosafety cabinet (Telstar, Madrid, Spain). For assay of antimicrobial activity, films were cut under aseptic conditions as circles (approx. 1 cm diameter) or squares (approx. 1 × 1 $cm^2$) and deposited on agar plates seeded with overnight cultures of the target bacterial strains as described above, with the activated side in contact with the agar surface. Plates were incubated at 37 °C for 24 h and inspected for growth inhibition zones surrounding the film. When no growth inhibition zones were detected, the films were removed with sterile tweezers and the plates were inspected for growth inhibition underneath the film. Antibacterial activity was recorded as: − (no activity), +/− (turbid inhibition zones underneath of the activated film), + (clear inhibition zones underneath of the activated film), ++ (clear inhibition zones extending 2 to 5 mm from the border mark of the activated film) or +++ (clear inhibition zones extending >5 mm from the border mark of the activated film).

### *2.3. Testing of Activated Films in Food Systems*

Polyethylene films (10 × 10 $cm^2$) activated with 5% zinc oxide by extrusion or surface-activated with 2.5% tyrosol or a combination of 1.5% tyrosol plus 0.5% *p*-hydroxybenzoic acid were used to prepare plastic bags. Foods (seabream, smoked salmon and smoked tuna) purchased at local supermarkets were used to prepare food slices (approx. 2.0 × 2.0 × 0.5 $cm^3$). Seabream slices were surface inoculated with 10 µL of an overnight culture of *L. innocua* CECT 4030 100-fold diluted in sterile saline solution. Smoked salmon and smoked tuna were surface-inoculated with 10 µL of a cocktail of *L. monocytogenes* strains (CECT 4032, CECT 911, CECT 936, and CECT 940) or *S. enterica* strains

(CECT 915, CECT 916, CECT 4000, and CECT 4300) prepared by mixing equal volumes of 1000-fold dilutions of the corresponding overnight cultures. Food slices were allowed to dry at room temperature in a biosafety cabinet for 30 min before they were vacuum packaged in the activated plastic bags. Two independent replicates (each one of them in triplicate) were prepared for each food. Foods were stored for 7 days at 10 °C in a Peltier-refrigerated incubator (Memmert GmbH, Schwabach, Germany). At desired incubation times, bags (in triplicate) were removed and the food content was pooled and homogenized with 10 mL buffered peptone water. The resulting homogenate was serially diluted in sterile saline solution and plated in triplicate on PALCAM agar with added supplement (Scharlab) for determination of *Listeria* and brilliant green agar (Scharlab) for determination of *Salmonella*. 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 $\log_{10}$ colony forming units (CFU)/g).

### *2.4. Statistical Analysis*

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

## 3. Results

### *3.1. Antimicrobial Activity of Activated Films*

Films activated by co-extrusion with zinc oxide, zinc acetate or potassium sorbate showed only weak antimicrobial activity or no activity when tested on agar plates inoculated with bacterial strains (Table 1).

**Table 1.** Inhibition zones obtained by plastic films activated with different antimicrobials.

| Films | Target Bacteria | | | | |
|---|---|---|---|---|---|
| | ***Salmonella Enterica*** **CECT 915** | ***Escherichia Coli*** **CECT 4783** | ***Pseudomonas Fluorescens*** **CECT 378** | ***Listeria Innocua*** **CECT 4030** | ***Staphylococcus Aureus*** **CECT 976** |
| ZnO (5.0%) | +/− | +/− | +/− | +/− | + |
| ZnAc (0.5%) | +/− | +/− | +/− | +/− | + |
| PS (0.25%) | +/− | + | +/− | − | +/− |
| TY (0.5%) | +/− | − | − | − | + |
| TY (0.5%) + L (0.1%) | +/− | − | − | − | + |
| TY (0.5%) + L (0.5%) | +/− | +/− | + | − | + |
| TY (1.5%) + L (0.1%) | ++ | + | + | − | + |
| TY (1.5%) + L (0.5%) | ++ | ++ | ++ | +/− | + |
| TY (0.5%) + PHB (0.1%) | ++ | ++ | ++ | ++ | ++ |
| TY (0.5%) + PHB (0.5%) | ++ | ++ | ++ | ++ | ++ |
| TY (1.5%) + PHB (0.1%) | ++ | ++ | ++ | ++ | ++ |
| TY (1.5%) + PHB (0.5%) | ++ | ++ | ++ | ++ | ++ |

ZnO, zinc oxide; ZnAc, zinc acetate; PS, potassium sorbate; TY, tyrosol; L, lactic acid; PHB, *p*-hydroxybenzoic acid.

Films that were surface-activated with 0.5% tyrosol also showed weak or no activity, except for *S. aureus*. Addition of 0.1% lactic acid improved antimicrobial activity against *S. enterica*. Antimicrobial activity improved considerably against all gram-negative bacteria (*S. enterica*, *E. coli*, *P. aeruginosa*) when 1.5% tyrosol was tested in combination with 0.5% lactic acid. However, activity against *L. monocytogenes* and *S. aureus* was not enhanced. Best results were obtained when tyrosol was tested in combination with *p*-hydroxybenzoic acid. All combinations containing tyrosol (at 0.5% or

1.5%) and *p*-hydroxybenzoic acid (0.1% or 0.5%) achieved strong inhibition against all the bacterial strains tested.

### *3.2. Effect of Activated Films on Model Food Systems*

Based on preliminary results of antimicrobial activity of agar diffusion assays, two activated films and food systems were selected for further testing: (i) films co-extruded with 0.5% zinc oxide were tested for packaging of raw seabream slices challenged with *L. innocua* CECT 4030; and (ii) films activated by surface application of tyrosol or a combination of tyrosol and *p*-hydroxybenzoic acid were tested for packaging smoked fish (salmon and tuna) challenged with *L. monocytogenes* and *S. enterica*.

During refrigerated storage of seabream slices challenged with *L. innocua* CECT 4030 and vacuum packaged in LDPE films, viable counts of *Listeria* decreased by ca. one log cycles during the first day of storage, but then increased by ca. 1.5 log cycles by day 7 (Table 2). In the samples packed in LDPE films co-extruded with 5% zinc oxide, viable counts decreased progressively from time 0 to day 4 of storage by ca. 1.2 log cycles ($p < 0.05$). After 7 days of storage, viable *Listeria* counts in seabream samples packed with the activated film were non-significantly lower ($p > 0.05$) by ca. 0.5 log cycles compared to samples packed in the control film.

**Table 2.** Survival of *Listeria innocua* CECT 4032 inoculated on seabream slices vacuum-packaged in low density polyethylene films activated or not with 5% zinc oxide. Samples were stored at 4 °C for 7 days.

| **Indicator Strain** | **Storage Time (Days)** | | | |
|---|---|---|---|---|
| ***Listeria innocua*** | **0** | **1** | **4** | **7** |
| Raw seabream | | | | |
| Control | 5.49 ± 0.17 | 4.47 ± 0.23 | 4.93 ± 0.34 | 6.03 ± 17 |
| Zinc oxide (5%) | 5.47 ± 0.17 | 4.60 ± 0.15 | 4.27 ± 25 | 5.67 ± 22 |

In separate experiments, LDPE films surface-activated with 2.5% tyrosol or with 1.5% tyrosol in combination with 0.5% *p*-hydroxybenzoic acid were tested for packaging of smoked salmon and smoked tuna slices challenged with cocktails of *L. monocytogenes* and *S. enterica* strains (Table 3). Counts of *L. monocytogenes* increased non-significantly ($p > 0.05$) in smoked salmon after 7 days of refrigerated storage. For samples packed in films activated with 2.5% tyrosol, viable *Listeria* counts decreased by 0.7 log cycles at day 7 and were significantly lower ($p < 0.05$) than the untreated controls by 1.5 log cycles. Tyrosol in combination with *p*-hydroxybenzoic acid achieved greatest inactivation effect, that was noticed after 7 days of storage. At that point, viable *Listeria* counts were reduced below detectable levels in the smoked salmon. For the control smoked tuna samples, viable *Listeria* counts decreased slightly during storage (Table 3). Vacuum packaging in films activated with tyrosol alone accelerated inactivation of the listeriae, achieving viable counts that were significantly lower ($p < 0.05$) by 1.7 log cycles compared to the untreated control at day 7 of storage. A greater effect was also observed for films activated with tyrosol in combination with *p*-hydroxybenzoic acid, reducing viable *Listeria* counts significantly ($p < 0.05$) in comparison with control films both at days 3 and 7 of storage by 1.0 and 1.7 log cycles, respectively.

When smoked salmon was challenged with the cocktail of *Salmonella* strains, viable *Salmonella* counts in controls packed in films without added antimicrobials decreased slowly and significantly ($p < 0.05$) by approximately 1 to 1.2 log cycles (Table 3). For samples packed in films activated with tyrosol, reduction of viable *Salmonella* counts increased during storage, but the differences with the untreated controls were not significant ($p > 0.05$). In contrast, *Salmonella* counts decreased below detectable levels at day 7 for the films activated with tyrosol plus *p*-hydroxybenzoic acid. *Salmonella* seemed to survive better in the smoked tuna, and packaging in films activated with tyrosol alone did not reduce viable counts significantly ($p > 0.05$) during storage. Nevertheless, viable counts for samples packaged in films activated with tyrosol in combination with *p*-hydroxybenzoic acid were

significantly lower ($p < 0.05$) by 1.2 to 1.3 log cycles compared to controls packed in coatings without added antimicrobials.

**Table 3.** Survival of *Listeria monocytogenes* and *Salmonella enterica* strains inoculated on smoked tuna and smoked salmon vacuum packaged in low density polyethylene films activated or not with tyrosol singly or in combination with *p*-hydroxibenzoic acid (PHB). Samples were stored at 4 °C for 7 days.

| Treatment Conditions | Storage Time (Days) | | |
|---|---|---|---|
| | 0 | 3 | 7 |
| *Salmonella enterica* | | | |
| Smoked salmon | | | |
| Control | 3.84 ± 0.24 | 2.47 ± 0.14 | 2.69 ± 0.34 |
| Tyrosol (2.5%) | 3.84 ± 0.38 | 1.84 ± 0.19 | 2.27 ± 0.31 |
| Tyrosol (1.5%) + PHB (0.5%) | 3.84 ± 0.24 | 1.69 ± 0.27 | <1.00 |
| Smoked tuna | | | |
| Control | 3.66 ± 0.14 | 3.17 ± 0.24 | 3.00 ± 0.18 |
| Tyrosol (2.5%) | 3.66 ± 0.14 | 3.00 ± 0.21 | 2.95 ± 0.40 |
| Tyrosol (1.5%) + PHB (0.5%) | 3.66 ± 0.14 | 1.84 ± 0.28 * | 1.77 ± 0.42 * |
| *Listeria monocytogenes* | | | |
| Smoked salmon | | | |
| Control | 3.14 ± 0.19 | 3.20 ± 0.18 | 4.0 ± 0.21 |
| Tyrosol (2.5%) | 3.14 ± 0.19 | 2.54 ± 0.28 | 2.42 ± 0.31 * |
| Tyrosol (1.5%) + PHB (0.5%) | 3.14 ± 0.19 | 2.44 ± 0.42 | <1.00 |
| Smoked tuna | | | |
| Control | 3.17 ± 0.13 | 2.47 ± 0.24 | 2.69 ± 0.37 |
| Tyrosol (2.5%) | 3.17 ± 0.13 | 1.60 ± 0.14 | 1.47 ± 0.16 * |
| Tyrosol (1.5%) + PHB (0.5%) | 3.17 ± 0.13 | 1.47 ± 0.17 * | 1.00 ± 0.12 * |

* Significant reduction of viable counts ($p < 0.05$).

## 4. Discussion

Activated coatings have attracted greatest attention for preservation of ready-to-eat (RTE) refrigerated food products, as hurdles against human pathogenic and food spoiling bacteria. Results from the present study indicated that the type of antimicrobial and the way it was applied on the coating had a strong influence on its antimicrobial activity. Zinc oxide, zinc acetate and potassium sorbate are amenable for incorporation into plastic films in the extrusion process, since they will withstand the temperature (of around 185 °C) required for melting of plastic materials. Furthermore, ZnO particles have been incorporated into a number of different polymers used in food packaging, including low-density polyethylene (LDPE) [9,10]. Nevertheless, in our experiments, incorporation of these antimicrobials into LDPE offered poor results and low antimicrobial activity. This could be possibly due to very slow diffusion of the antimicrobials into the substrate where the bacteria grow. A recent study indicated that the main problem to be solved in adding ZnO nano/microparticles to a polymeric matrix seems therefore related to the formation of agglomerated domains that occur because of the strong intermolecular interactions among the ZnO particles in combination with their high surface area [11]. As a matter of fact, in our experiments using a model food system, LDPE activated with zinc oxide only induced a small delay on growth of *L. innocua* (as a surrogate of *L. monocytogenes*) in the vacuum-packaged seabream fillets. Control of *Listeria* in fresh fish is important in order to avoid food cross contamination and also to prevent transmission of the pathogen through consumption of raw seafood. In contrast, the different combinations of tyrosol and *p*-hydroxybenzoic acid applied on the surface of LDPE all elicited strong antimicrobial activity against both Gram-positive and Gram-negative bacteria including foodborne pathogens, toxicogenic bacteria and spoilage bacteria. These results are of interest for practical application since tyrosol is abundant in natural products

such as by-products from the olive oil industry and is also a cheap compound compared to other phenolic antimicrobials.

Based on results from preliminary tests with bacterial strains on TSA plates, LDPE films surface-activated with tyrosol and tyrosol plus *p*-hydroxybenzoic acid were selected for further study, choosing two smoked RTE seafood as models. Smoked salmon was chosen because it is a RTE fish product commonly sold in vacuum packages. Furthermore, if smoked salmon is not processed and handled properly, it can be contaminated with *Listeria monocytogenes* [12,13]. A recent EU survey on the prevalence of *L. monocytogenes* in certain RTE foods found a prevalence in smoked fish of 10.4% [14]. Smoked seafood products can also carry other foodborne pathogens. As an example, *Salmonella* outbreaks have been linked to consumption of smoked salmon [15,16]. Therefore, a cocktail of *S. enterica* strains was also included in the challenge tests. Results from the present study suggested that the LDPE activated with tyrosol plus *p*-hydroxybenzoic acid could significantly reduce viable counts of *L. monocytogenes* and *S. enterica* during refrigerated storage both in smoked salmon and in smoked tuna packed under vacuum. Furthermore, the concentrations of inocula used in the present study (3.1 to 3.8 $\log_{10}$ CFU/g) exceed the expected levels for a cross-contamination during processing (which may range between 1 and 2 $\log_{10}$ CFU/g) [14]. It is also important to note that the antimicrobial effects were obtained on cocktails of strains. This is relevant when testing antimicrobial substances in foods, since strain differences in sensitivity to antimicrobial substances may exist.

It is also worth noting the strong antibacterial effects shown by LDPE activated with all combinations of tyrosol plus *p*-hydroxybenzoic acid against pseudomonads. This is important since the shelf life of seafood products can be shortened considerably by spoilage bacteria. Pseudomonads have been reported from vacuum and modified atmosphere packaged salmon products [17–19]. Under chilled, aerobic storage conditions, *Pseudomonas* and related genera such as *Shewanella* are the most common spoilage organisms [19]. The strong antimicrobial activity against *Pseudomonas* shown by plastic films activated with combinations of tyrosol and *p*-hydroxybenzoic acid encourage further studies on their possible application as hurdles against this spoiling bacterium in refrigerated food products.

In conclusion, results from the study highlight the potential of surface-activated plastic films containing tyrosol in the control of foodborne pathogens and spoilage bacteria in seafood.

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**Author Contributions:** Belén Soriano Cuadrado prepared plastic films co-extruded with different antimicrobials and evaluated the technical feasibility of incorporating different antimicrobials in various types of plastic films. Pilar Martínez Viedma, Mari Carmen López Aguayo and Irene Ortega Blazquez tested antimicrobial activity of activated films and carried challenge tests with activated films in food systems. Maria José Grande Burgos and Rubén Pérez Pulido contributed with data analysis and preparation and correction of manuscript. Antonio Gálvez and Rosario Lucas López conceived and designed the experiments.

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

## References

1. Perez-Perez, C.; Regalado-Gonzalez, C.; Rodriguez-Rodriguez, C.A.; Barbosa-Rodriguez, J.R.; Villasenor-Ortega, F. Incorporation of antimicrobial agents in food packaging films and coatings. In *Advances in Agricultural and Food Biotechnology*; Guevara-González, R.G., Torres-Pacheco, L., Eds.; Research Signpost: Trivandrum, India, 2006; pp. 193–216.
2. Appendini, P.; Hotchkiss, J.H. Review of antimicrobial food packaging. *Innov. Food Sci. Emerg. Technol.* **2002**, *3*, 113–126. [CrossRef]
3. De Azeredo, H.M.C. Antimicrobial nanostructures in food packaging. *Trends Food Sci. Technol.* **2013**, *30*, 56–69. [CrossRef]
4. Burt, S. Essential oils: Their antibacterial properties and potential applications in foods—A review. *Int. J. Food Microbiol.* **2004**, *94*, 223–253. [CrossRef] [PubMed]

5. Rollini, M.; Nielsen, T.; Musatti, A.; Limbo, S.; Piergiovanni, L.; Hernandez Munoz, P.; Gavara, R. Antimicrobial Performance of Two Different Packaging Materials on the Microbiological Quality of Fresh Salmon. *Coatings* **2016**, *6*, 6. [CrossRef]
6. Charoenprasert, S.; Mitchell, A. Factors influencing phenolic compounds in table olives (*Olea europaea*). *J. Agric. Food Chem.* **2012**, *60*, 7081–7095. [CrossRef] [PubMed]
7. Amiot, M.J.; Fleuriet, A.; Macheix, J.J. Importance and evolution of phenolic compounds in olive during growth and maturation. *J. Agric. Food Chem.* **1996**, *34*, 823–826. [CrossRef]
8. García, A.; Brenes, M.; García, P.; Romero, C.; Garrido, A. Phenolic content of commercial olive oils. *Eur. Food Res. Technol.* **2003**, *216*, 520–525.
9. Emamifar, A.; Kadivar, M.; Shahedi, M.; Solimanian-Zad, S. Effect of nanocomposite packaging containing Ag and ZnO on reducing pasteurization temperature of orange juice. *J. Food Process. Preserv.* **2012**, *36*, 104–112. [CrossRef]
10. Droval, G.; Aranberri, I.; Bilbao, A.; German, L.; Verelst, M.; Dexpert-Ghys, J. Antimicrobial activity of nanocomposites: Poly(amide) 6 and low density poly(ethylene) filled with zinc oxide. *E-Polymers* **2008**, *128*, 1–13. [CrossRef]
11. Silvestre, C.; Duraccio, D.; Marra, A.; Strongone, V.; Cimmino, S. Development of Antibacterial Composite Films Based on Isotactic Polypropylene and Coated ZnO Particles for Active Food Packaging. *Coatings* **2016**, *6*, 4. [CrossRef]
12. Rørvik, L.M. *Listeria monocytogenes* in the smoked salmon industry. *Int. J. Food Microbiol.* **2000**, *62*, 183–190. [CrossRef]
13. Hwang, C.A. Effect of salt, smoke compound, and storage temperature on the growth of *Listeria monocytogenes* in simulated smoked salmon. *J. Food Prot.* **2007**, *70*, 2321–2328. [PubMed]
14. European Food Safety Authority. Analysis of the baseline survey on the prevalence of *Listeria monocytogenes* in certain ready-to-eat foods in the EU, 2010–2011 Part A: *Listeria monocytogenes* prevalence estimates. *EFSA J.* **2013**, *11*, 3241.
15. Friesema, I.; de Jong, A.; Hofhuis, A.; Heck, M.; van den Kerkhof, H.; de Jonge, R.; Hameryck, D.; Nagel, K.; van Vilsteren, G.; van Beek, P.; et al. Large outbreak of *Salmonella* Thompson elated to smoked salmon in The Netherlands, August to December 2012. *Euro Surveill.* **2014**, *19*, 1–8. [CrossRef]
16. Friesema, I.H.; de Jong, A.E.; Fitz James, I.A.; Heck, M.E.; van den Kerkhof, J.H.; Notermans, D.W.; van Pelt, W.; Hofhuis, A. Outbreak of *Salmonella* Thompson in the Netherlands since July 2012. *Euro Surveill.* **2012**, *17*, 1–14.
17. Churchill, O.J.; Fernandez-Piquer, J.; Powell, S.M.; Tamplin, M.L. Microbial and sensorial models for head-on and gutted (HOG) Atlantic Salmon (*Salmo salar*) stored from 0 to 15 °C. *Food Microbiol.* **2016**, *57*, 144–150. [CrossRef] [PubMed]
18. Macé, S.; Cornet, J.; Chevalier, F.; Cardinal, M.; Pilet, M.F.; Dousset, X.; Joffraud, J.J. Characterisation of the spoilage microbiota in raw salmon (*Salmo salar*) steaks stored under vacuum or modified atmosphere packaging combining conventional methods and PCR-TTGE. *Food Microbiol.* **2012**, *30*, 164–172. [CrossRef] [PubMed]
19. Milne, D.; Powell, S.M. Limited microbial growth in Atlantic salmon packed in a modified atmosphere. *Food Cont.* **2014**, *42*, 29–33. [CrossRef]