

## Article

# Improving the Quality and Safety of Fish Products with Edible Coatings Incorporating Piscicolin CM22 from a Psychrotolerant *Carnobacterium maltaromaticum* Strain

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**Abstract:** The consumption of raw or smoked fish entails significant microbiological risks, including contamination by pathogens such as *Listeria monocytogenes*, which can cause severe foodborne illnesses. This study explores the preservative use of piscicolin CM22, a novel bacteriocin derived from the psychrotolerant strain *Carnobacterium maltaromaticum* CM22, in two types of edible coatings (EC): chitosan-based and fish gelatin-based. An initial in vitro characterization of the technological and antimicrobial properties of these ECs with and without bacteriocin was conducted. The efficacy of the edible coatings was subsequently evaluated through shelf life and challenge tests against *L. monocytogenes* in raw and smoked fish products. The results demonstrated significant antimicrobial activity, with the chitosan-based coating containing piscicolin CM22 being the most effective in reducing microbial counts and maintaining pH and color stability. Furthermore, in the challenge test studies, both ECs effectively controlled *L. monocytogenes*, showing significant reductions in bacterial counts compared to the controls in fresh tuna, salmon, and smoked salmon. The ECs containing piscicolin CM22 reduced *Listeria* counts by up to 4 log CFU/g in raw and smoked fish samples, with effective control in smoked salmon for up to 15 days at refrigeration temperature. While further research is required to fully assess their preservation potential, these findings strongly indicate that piscicolin CM22-functionalized edible coatings hold significant potential for improving the quality and safety of fish products.



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**Keywords:** piscicolin CM22; *Listeria*; raw fish; smoked salmon; edible coating; biopreservation

## 1. Introduction

The contamination of fish and seafood by pathogenic microorganisms is a significant public health concern. Numerous studies have reported a high prevalence of *Listeria monocytogenes* in both raw and processed fish and seafood, conclusively identifying these foods as potential carriers of this pathogen [1]. Common routes for contamination of fish with *Listeria* include its spread from intestinal contents to other tissues, as well as cross-contamination through contact with various surfaces and processing equipment, such as conveyor belts, trays, and skinning and slicing machines [2]. The current strategies for controlling *L. monocytogenes* in fish and seafood include chemical treatments such as preservatives, chlorinated products, ozone, or electrolyzed oxidizing water, among others [3,4]. In addition, physical treatments such as steam application or pulsed light exposure have also been evaluated [5,6]. However, very few technologies achieve total

*Listeria* elimination, highlighting the need for continued research into new preservation methods that ensure its control.

Among the emerging methods for *Listeria* control are biopreservation strategies, which employ microorganisms and/or their metabolites, such as antimicrobial peptides or bacteriocins [7]. In this regard, the use of bacteriocins from lactic acid bacteria (LAB) could represent an effective and cost-efficient alternative to help reduce the incidence of this pathogen in fish and seafood products [8]. In fact, some authors have described the potential of several bacteriocins from LAB, such as enterocin AS-48, nisin, or piscicolin, among others [9–12], for controlling *Listeria* in fish products.

Piscicolin CM22, a new cationic bacteriocin similar to piscicolin 126, is produced by the psychrotolerant strain *Carnobacterium maltaromaticum* CM-22, which was previously isolated by our team from salmon (*Salmo salar*). This bacteriocin exhibits strong bactericidal activity against several Gram-positive bacteria, including *L. monocytogenes* [13–15]. As with other piscicolins, CM22 is anticipated to effectively control this pathogen in food products. However, given that contact surfaces are the primary source of microbiological contamination in the fish industry [16,17], it is crucial to optimize the application methods of these bacteriocins for surface treatments, such as direct spraying or its incorporation into edible coatings.

Edible coatings (ECs) are food-grade suspensions that may be delivered by spraying, spreading, or dipping, which upon drying form a thin layer on the food surface. They have been proven to be able to extend the shelf life and improve the safety of food in different ways. Furthermore, ECs are used to improve the appearance and mechanical properties of food products, reducing water loss [18,19]. While the primary function of edible coatings is to serve as a physical barrier to protect food from gases and dehydration, in recent years, ECs have also begun to be used as carriers for functional ingredients, such as preservatives [20].

Considerable research has been conducted, providing insights into the potential utilization of several food ingredients such as chitosan and plant extracts, for incorporation into edible coatings to enhance their antimicrobial effectiveness [21–23]. Bacteriocins such as piscicolins are also promising candidates for edible coatings (ECs) due to their natural preservative qualities. Additionally, they are recognized as safe (GRAS) and are typically produced by LAB, making them suitable for food applications without compromising consumer safety. Furthermore, the incorporation of bacteriocins into ECs would enhance the antimicrobial barrier properties of these coatings and reduce the reliance on chemical preservatives. This approach addresses consumer demand for cleaner labels and more natural food products. In summary, the integration of bacteriocins into ECs represents an advanced biopreservation strategy that leverages the natural antimicrobial properties of these peptides to improve food safety and quality [24].

This study aimed to evaluate the effectiveness of incorporating piscicolin CM22 into two types of edible coatings (chitosan-based and fish gelatin-based) for the preservation and control of *Listeria* species in fish products during refrigerated storage. This study innovatively integrates piscicolin CM22, a scarcely studied bacteriocin, into these edible coatings, offering an effective and natural solution to enhance the safety and extend the shelf life of raw and smoked fish products.

## 2. Materials and Methods

### 2.1. Bacterial Strains and Culture Conditions

*Carnobacterium maltaromaticum* CM22 [13], from our collection, was used as the piscicolin CM22 producer. *L. monocytogenes* CECT 4032 supplied by the Spanish Type Culture Collection (CECT) was used as the standard indicator strain for bacteriocin activity assays. *C. maltaromaticum* CM22 was grown in BHI Brain Heart Infusion broth (Sharlau, Barcelona, Spain) at 28 °C for 24 h. For fish inoculation, *L. monocytogenes* DSM 112143 isolated from smoked organic salmon were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ), *Listeria seeligeri* CECT 5342 isolates from smoked fish *L. monocyto-*

genes DMC 05 and *Listeria innocua* DMC 04 isolated from raw salmon from our collection were used to obtain more detailed information about the anti-*Listeria* activity of piscicolin CM22. *Listeria* strains were grown overnight on brain heart infusion (BHI) (Sharlau, Barcelona, Spain) at 37 °C, washed in a sterile saline solution, and then inoculated in fillet tissue samples at the selected concentration. All bacterial cultures were maintained at 4 °C on BHI agar (BHA) slants.

## 2.2. Preparation of Piscicolin CM22

Piscicolin CM22 was obtained by culturing the strain *C. maltaromaticum* CM22 in a food grade substrate including saccharose (1%), yeast extract (0.3%), NaCl (1%), lactose (1%), and 1 mM MgSO<sub>4</sub>. Cation exchange chromatography on carboxymethyl Sephadex CM-25 (Sigma Aldrich, St. Louis, MO, USA) was used to recover piscicolin from cultures. Bacteriocin activity against the indicator strain *L. innocua* was tested by eluting and using the agar well diffusion method [25]. NaCl was eliminated before the use of the eluted fractions: they were dialyzed at 4 °C against distilled water through a 2000-Da cut-off membrane, and then sterilized by filtration (0.22 µm, Milli-pore, Belford, MA, USA). The bacteriocin was quantified and adjusted to achieve a final concentration of 10,240 AU/mL according to the method described by González-Gragera et al. (2024) [13].

## 2.3. Enzymatic and Physicochemical Stability of Piscicolin CM22

To investigate the technological applications of piscicolin CM22 in food preservation and its potential incorporation into edible coatings (ECs), its enzymatic, thermal, and pH stability were studied. Enzymatic stability was assessed using the methodology described by Zhao et al. (2020) [26]. For this purpose, papain (≥10 units/mg, Sigma, St. Louis, MO, USA), trypsin (2000 units/mg, Sigma, St. Louis, MO, USA), and proteinase K (30 units/mg, Sigma, St. Louis, MO, USA) were used. The bacteriocin was mixed with each of the enzymes at a concentration of 0.5 mg/mL at 37 °C during 2 h. Afterward, the enzymes were inactivated by heat at 80 °C for 5 min. In addition, pH and thermal stability were tested using the methodology proposed by Ahn et al. (2017) [27]. To test thermal stability, the pH of purified bacteriocin was adjusted to 6.5 using HCl 4N or NaOH, and then three aliquots were heated to 60, 80, and 100 °C for 30 min each, and one of them was autoclaved (121 °C, 15 min). To test pH stability, the pH of bacteriocin was adjusted to values of 2, 3, 5, 7, 9, and 11 with HCl 4N or NaOH and incubated for 2 h at 30 °C. Finally, before assessing the antimicrobial activity of each sample against *L. monocytogenes*, the piscicolin solution was neutralized to pH 6.5.

## 2.4. Edible Coatings (ECs)

Two edible coatings were prepared for the incorporation of piscicolin CM22.

**Fish gelatine-based coating (GC).** This coating was prepared using cold-water-soluble fish gelatin (Norland Fish Products, Cranbury, NJ, USA). The film-forming solution was formulated to contain 4.5% gelatin, 1.5% glycerol as a plasticizer, and 1% sodium alginate (Sigma, St. Louis, MO, USA). First, 10 g of sodium alginate was slowly incorporated, with stirring (200 rpm), into a beaker containing 500 mL of warm deionized water. Once the sodium alginate was fully dissolved in the deionized water, 15 g of glycerol were added, continuing to stir the solution (200 rpm) for 10 min. After cooling the solution to 4 °C, fish gelatin and piscicolin CM22, previously dissolved in 430 mL of deionized water, were added to reach a concentration of 1000 AU/mL, followed by stirring (450 rpm for 10 min). In addition, a coating without bacteriocin was prepared as a control (GC). Both the control and the edible coatings containing piscicolin CM22 (GC-CM22) were stored under refrigeration until use.

**Chitosan-based coating (CC).** A chitosan coating was prepared by dissolving 18 g of chitosan with a deacetylation degree of 90% (Sigma, St. Louis, MO, USA) in 1 Kg of a sodium diacetate (2% w/w) (Brenntag, Reading, PA, USA) to obtain the acidic conditions (pH = 5.1), which was needed to dissolve the biopolymer. It was added by rapid stirring

(450 rpm) to obtain an opalescent solution. The solution was stirred using a magnetic stirrer at a rapid stirring speed (450 rpm) to achieve an opalescent solution. The sample was allowed to stand overnight to achieve the complete solution of chitosan and then filtered through a sterilized glass filter (Whatman, UK). Finally, the sample was added to piscicolin CM22 until reaching a concentration of 1000 AU/mL (CC-CM22). Similarly, a control coating without bacteriocin was formulated (CC). All coatings were stored refrigerated until needed.

### 2.5. Physicochemical Characterization of Coatings

An initial physicochemical characterization was performed on each edible coating, involving the assessment of the following parameters:

**Thickness of the films (TFs).** The thickness of the films was measured using a micro caliper at six different points of the same sample. Results were expressed as the average  $\pm$  SD.

**Films solubility (FS).** For the determination of the solubility in water of the film, first, a square film of 2.5 cm<sup>2</sup> was dried at 80 °C for 24 h and then weighed to determine the initial dry mass ( $M_0$ ). Afterward, the dried films were immersed in 50 mL of distilled water in constant agitation for 12 h at 25 °C. The mixture was centrifuged (4500 rpm, 15 min) and the precipitate was dried at 80 °C for 24 h and weighed to determine the final dry mass ( $M_f$ ). Film solubility was determined by Equation (1):

$$FS \% = (M_0 - M_f) / M_0 \times 100 \quad (1)$$

**Films water swelling (FWS).** The film swelling rate was calculated by the methodology described by Tan et al. 2022 with some modifications [28]. Briefly, the films were dried at 60 °C over 24 h and immersed in 50 mL of distilled water for 24 h. After, the film was removed from the vase, leftover water was dried with filter paper, and the films were weighed ( $M_w$ ). Then, the films were dried at 60 °C until constant weight ( $M_f$ ). The water swelling rate was determined according to Equation (2):

$$FWS\% = (M_w - M_f) / M_w \times 100 \quad (2)$$

**Films water vapor permeability (WVP).** Water vapor permeability was measured according to the methodology described by Ma et al. 2015 with some modifications [29]. Briefly, 2.5 g of anhydrous CaCl<sub>2</sub> was added into a 50 mm  $\times$  30 mm beaker and then covered with the films. The samples were kept in a chamber at 25 °C and 75% of relative humidity. The mass of the bottle was recorded daily for 3 days until constant weight. WVP (mg cm<sup>-2</sup> h<sup>-1</sup>) was determined using the following Equation (3):

$$WVP = \Delta m / (A \times \Delta t) \quad (3)$$

where  $\Delta m$  is the sample weight difference (mg),  $\Delta t$  is the time interval (h), and  $A$  is the effective area of the films (cm<sup>2</sup>).

### 2.6. In Vitro Antimicrobial Activity of Edible Coatings (ECs)

To determine the in vitro antimicrobial activity of ECs with and without piscicolin CM22, the target strains *L. monocytogenes* DSM 112143, *L. monocytogenes* CECT 4032, *L. seeligeri* CECT 5342, *L. monocytogenes* DMC 05, and *L. innocua* DMC 04 were used. First, the agar well diffusion method was carried out following the procedure described by Tagg and MacGiven (1979) [30]. Stainless steel cylinders with a diameter of 8 mm and a height of 10 mm were placed on plates containing Mueller Hinton Agar (MHA, Scharlau, Barcelona, Spain) (10 mL). Next, 6 mL of soft BHA (BHA plus 0.8% agar) medium inoculated with approximately 10<sup>7</sup> CFU of bacteria was poured onto the plates. The cylinders were removed after the overcoating layer solidified, and the holes were individually filled with 100  $\mu$ L of each edible coating. Finally, the plates were incubated aerobically at 37 °C for 24 or 48 h.

After incubation, the clear zones were measured using a microcaliper. Each sample was tested in triplicate.

### 2.7. Food Effectiveness Trials

Raw tuna and salmon were purchased from local retailers (Alhendín, Granada, Spain). For each experiment, a series of fish 3 cm<sup>2</sup> blocks were prepared by slicing iced flesh with a sterile knife. After that, the four treatments that were used were prepared: fish gelatin-based coating (GC), fish gelatine-based coating with piscicolin CM22 (GC-CM22), chitosan-based coating (CC), and chitosan-based coating with piscicolin CM22 (CC-CM22). In addition, an untreated sample was used as a control. After preparing the treatments, the fish blocks were submerged by dipping for 30 s. Then, the different samples were placed on polystyrene dishes and sealed in a Ziploc bag in modified atmosphere packaging (MAP) (30% CO<sub>2</sub>, 70% N<sub>2</sub>) (Tecnotrip, Barcelona, Spain), and immediately stored at 4 °C and 8 °C. Two independent studies were conducted.

#### 2.7.1. Influence on the Shelf Life of Raw Tuna and Salmon

**pH.** The determination of pH was carried out during sampling each time. For pH determination, 10 mL of the homogenized sample was used. A pH-meter (Model HI 99163, Hanna, Póvoa de Varzim, Portugal) calibrated with buffer solutions at pH 4.0 and 7.0 was used to determine the pH value of the suspension in triplicates.

**Color analyses.** For instrumental color determination, a colorimeter (Model CR 410, Konica Minolta, Tokyo, Japan) was employed. The color was measured at three different points on the surface of the fish block. Measurement parameters were color space—CIE L\*a\*b\*, light source—D65, opening diameter—50 to 53 mm, and angle of observation—2°. L\* represents the clarity or luminosity of the subject measured—100 corresponds to white and 0 corresponds to black. a\* is the green/red color component—a\* < 0 is green and a\* > 0 red. Finally, b\* represents the blue/yellow color component—b\* > 0 yellow, b\* < 0 blue.

**Microbiological counts.** The evolution of the microbiology for each treatment was determined in duplicate on days 0, 1, and 3. We followed the same sampling process in the tuna and salmon, mixing 25 g of fish with 225 mL of peptone water in a 400 mL plastic sterile bag (VWR Blender Bag, Barcelona, Spain) and then they were homogenized for 1 min in a Masticator blender (IUL, Barcelona, Spain). Subsequently, each sample was collected in 100 mL plastic containers. Afterward, decimal dilutions were prepared and plated on various media, including Plate Count Agar (PCA) at 30 °C for total aerobic mesophiles, PCA at 4 °C for total psychrophiles, MacConkey agar at 30 °C for enterobacteria, Sabouraud agar with chloramphenicol at 30 °C for yeast, and Rose Bengal Agar at 25 °C for fungi (all media were obtained from Sharlab, Barcelona, Spain).

#### 2.7.2. Challenge Test in Food

Challenge test assays were performed in duplicate according to Baños et al. (2016) [31]. Fish were purchased from local retailers. Fish blocks (25 cm<sup>2</sup>) were prepared in the same manner as previously described. Raw tuna and salmon were inoculated with an adjusted concentration of 5 Log<sub>10</sub> CFU/mL to obtain a final 10<sup>3</sup> CFU/cm<sup>2</sup> concentration from a pool of *L. monocytogenes* (DSM 112143, DMC 05, CECT 5342, DMC 04, CECT 4032), using a sterile handle Digrafsky. Afterwards, different fish fillets were dipped in different treatment solutions for 30 s, including batches with ECs (GC and CC) and batches with ECs with added piscicolin CM22 (GC-CM22 and CC-CM22) at a concentration of 1000 AU/mL. Finally, a distilled water treatment was performed as a control. Additionally, challenge tests were conducted on smoked salmon using a similar procedure. The samples were individually placed on polystyrene dishes, MAP-packed for raw fish, and vacuum-packed for smoked salmon (TECNOTRIP, Barcelona, Spain). The samples were then stored at 4 °C for 3 days for raw tuna and salmon, and 28 days for smoked salmon.

For pathogen monitoring, samples from each treatment were withdrawn in triplicate at selected times to determine viable counts of *Listeria* and mixed (1/10, w/v) with a dilution

medium (0.1% peptone, 0.85% NaCl). Homogenization was performed in a Masticator blender (IUL, Barcelona, Spain) for 1 min followed by serial 10-fold dilution and plating on chromogenic selective agar COMPASS Listeria Agar (Biokar Diagnostics, Allone, France). Results were expressed as Log<sub>10</sub> CFU/cm<sup>2</sup> versus time. When it was not possible to quantify the bacteria below the detection limit (<1 Log<sub>10</sub> CFU/cm<sup>2</sup>), an investigation was carried out by pre-enrichment in Fraser broth (Biokar Diagnostics), expressing the results as presence or absence.

### 2.8. Statistical Analyses

The statistics were extracted from the results of two independent experiments. In each one, three samples (food tested) for each treatment ( $n = 6$ ) and sampling time were used. Statistical analysis and figures were performed with GraphPad Prism 8.0 software (GraphPad Software Inc., San Diego, CA, USA). Data on microbiological counts were subjected to ANOVA. Error probability values less than 0.05 were considered not significant.

## 3. Results

### 3.1. In Vitro Trials

Before assessing the efficacy of the ECs enhanced with piscicolin CM22, an in vitro characterization of the bacteriocin's technological properties was conducted. Initially, the stability of the bacteriocin was evaluated under various conditions of temperature, pH, and enzyme treatment. The results presented in Table 1 show that bacteriocin activity was maintained across a wide pH range of 2 to 10, although it was sensitive to protease enzymes such as pepsin, papain, and trypsin. The antimicrobial activity was not affected by heating at 80 °C for 30 min. However, at higher temperatures, its activity began to decrease, with a complete loss of efficacy observed at 121 °C for 15 min. Bacteriocin activity was optimal at pH 5 and pH 7 ( $23 \pm 0.5$ ), demonstrating high stability. Activity decreased significantly at pH 10 and was completely lost at pH 11, indicating instability under highly alkaline conditions. Finally, the bacteriocin was entirely degraded by trypsin, papain, and proteinase K, confirming its susceptibility to proteolytic enzymes.

**Table 1.** Antimicrobial activity stability of piscicolin CM22 under different conditions of temperature, pH, and treatment with enzymes.

Treatments	Bacteriocin Activity <sup>a</sup>
Control (only bacteriocin)	26 ± 0.5
<b>Heat</b>	
60 °C for 30 min	26 ± 1
80 °C for 30 min	25 ± 0.5
100 °C for 30 min	20 ± 1
121 °C for 15 min	-
<b>pH</b>	
2	25 ± 0.5
3	26 ± 1
5	26 ± 1
7	26 ± 0.5
9	35 ± 0.2
10	20 ± 0.5
11	-
<b>Enzymes</b>	
Trypsin, papain, and proteinase K	-

<sup>a</sup> Diameter of inhibition zone in mm against *L. monocytogenes* DSM 112142.

Following the assessment of bacteriocin stability under different conditions, we developed two types of edible coatings (ECs) to explore their potential applications in food preservation. These coatings were based on fish gelatin (GC) and chitosan (CC), incorporat-

ing piscicolin CM22, resulting in the formulations GC-CM22 and CC-CM22, respectively. After formulating the edible coatings, both with and without the bacteriocin, we conducted a comprehensive physicochemical characterization. The results, presented in Table 2, indicate that the incorporation of piscicolin did not adversely affect the technological properties of the ECs. Specifically, attributes such as film morphology, thickness, solubility, water film swelling, and water vapor permeability remained unaffected.

**Table 2.** Physicochemical characteristics of Edible Coating (EC) with and without piscicolin CM22. Thickness of the Films (TFs), Films Solubility (FS), Films Water Swelling (FWS), Films Water Vapor permeability (WVP).

Edible Coatings (ECs)	TF $\mu\text{m}$	FS, %	FWS, %	WVP, $\text{mg cm}^{-2} \text{h}^{-1}$
Gelatin coating (GC)	32.5 $\pm$ 0.6	8.7 $\pm$ 1.2	63.4 $\pm$ 2.6	8.7 $\pm$ 1.3
Gelatin-CM22 coating (GC-CM22)	31.9 $\pm$ 1.3	7.8 $\pm$ 0.9	60.9 $\pm$ 4.1	8.8 $\pm$ 1.7
Chitosan coating (CC)	21.7 $\pm$ 0.9	5.3 $\pm$ 0.7	39.7 $\pm$ 1.4	4.2 $\pm$ 1.1
Chitosan-CM22 coating (CC-CM22)	22.3 $\pm$ 1.8	4.8 $\pm$ 0.6	41.2 $\pm$ 2.3	4.3 $\pm$ 1.5

Subsequently, the antimicrobial activity of both edible coatings (ECs), with and without the incorporation of piscicolin CM22, was assessed. Given the inherent antimicrobial properties of certain EC components, initial in vitro assays were conducted to verify this activity and determine any enhancement in effectiveness resulting from the inclusion of the bacteriocin in the coatings.

The antimicrobial effectiveness of ECs against several *L. monocytogenes* strains were assessed, and the results are summarized in Table 3. The fish gelatin coating (GC) alone exhibited no inhibition, while the GC-CM22 formulation, incorporating piscicolin CM22, showed significant antimicrobial activity with inhibition zones ranging from 19  $\pm$  1 mm to 23  $\pm$  1.3 mm. Although the chitosan coating (CC) alone demonstrated modest inhibition against *Listeria*, with inhibition zones ranging from 10  $\pm$  1 mm to 12  $\pm$  1 mm, the combination of chitosan with piscicolin CM22 (CC-CM22) exhibited significantly higher antimicrobial activity, with inhibition zones extending from 26  $\pm$  1.5 mm to 29  $\pm$  2 mm. These results indicate that while chitosan itself has inherent antimicrobial properties, the addition of piscicolin CM22 markedly enhances its antibacterial effectiveness, suggesting a synergistic interaction between the two components.

**Table 3.** In vitro effectiveness of different ECs against several *Listeria* strains, determined by the size of inhibition zones using the diffusion method in solid media. Results expressed as mm.

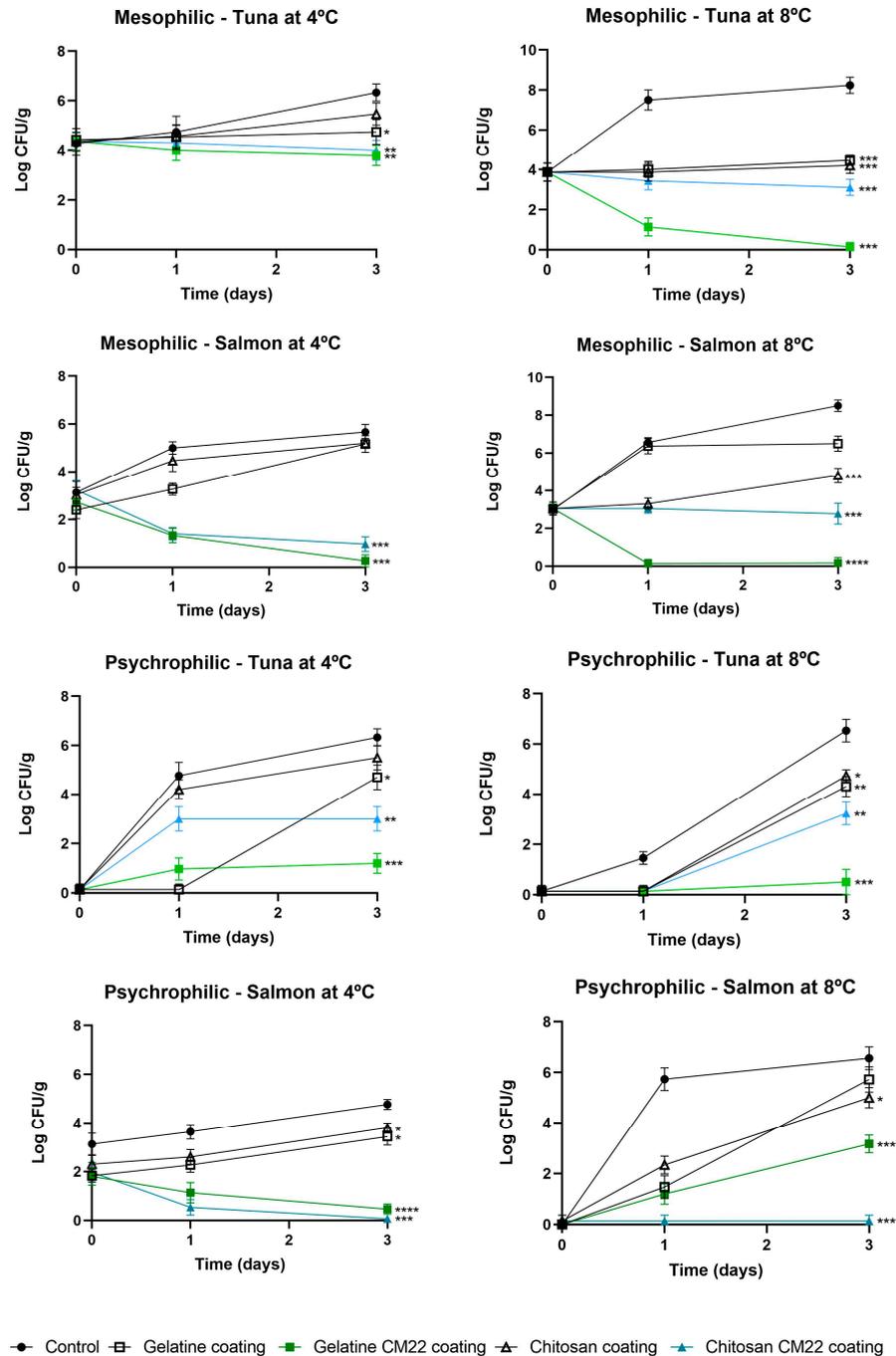
Strain	Inhibition of Halo (mm)				
	GC	GC-CM22	CC	CC-CM22	CM22
<i>L. monocytogenes</i> DZSM 112143	0	19 $\pm$ 1	10 $\pm$ 1	28 $\pm$ 1	24 $\pm$ 1
<i>L. monocytogenes</i> DMC 04	0	19 $\pm$ 1.5	11 $\pm$ 1	29 $\pm$ 1.4	25 $\pm$ 1.4
<i>L. monocytogenes</i> DMC 05	0	19 $\pm$ 2	11 $\pm$ 1.2	29 $\pm$ 2	22 $\pm$ 2
<i>L. monocytogenes</i> CECT 4032	0	22 $\pm$ 1	11 $\pm$ 1.2	27 $\pm$ 1	25 $\pm$ 1
<i>L. monocytogenes</i> CECT 5342	0	23 $\pm$ 1.3	12 $\pm$ 1	26 $\pm$ 1.5	26 $\pm$ 1.5

### 3.2. Shelf-Life Trials

#### 3.2.1. Influence on Microbial Counts

After completing the characterization and in vitro efficacy tests, the effectiveness of the new edible coatings (ECs) in extending the shelf life of raw fish stored at 4 °C and 8 °C was evaluated. Figure 1 illustrates the evolution of microbiological counts for mesophilic and psychrophilic microorganisms in raw tuna and salmon. For mesophilic bacteria, tuna stored at 4 °C showed significant reductions in bacterial counts when treated with gelatin-CM22 and chitosan-CM22 coatings. The chitosan-CM22 coating was the most effective, reducing the counts to approximately 2 log CFU/g on day 3 compared to the control, which

reached nearly 6 log CFU/g ( $p < 0.001$ ). Salmon stored at 4 °C exhibited similar trends, with chitosan-CM22 reducing mesophilic counts to about 2 log CFU/g, while the control increased to approximately 5 log CFU/g ( $p < 0.001$ ).



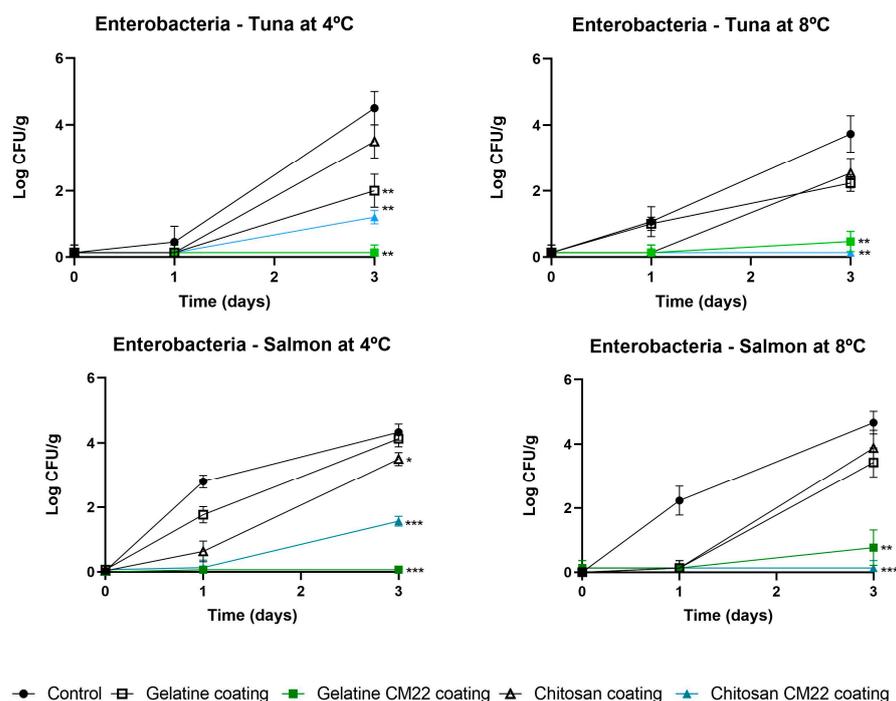
**Figure 1.** Effect of treatments with different edible coatings (ECs) containing piscicolin CM22 on mesophiles and psychrophiles in raw tuna and salmon stored at 4 °C and 8 °C. Values are the average  $\pm$  SD (error bars) from independent experiments. Significant differences compared to control: \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; \*\*\*\*  $p < 0.0001$ .

At 8 °C, the effectiveness of the coatings was even more pronounced. Tuna treated with chitosan-CM22 showed a substantial reduction in mesophilic bacteria, with counts remaining around 3 log CFU/g, whereas the control exceeded over 8 log CFU/g ( $p < 0.001$ ). Gelatin-CM22 also significantly inhibited bacterial growth, though not as effectively as

chitosan-CM22. In salmon, both coatings demonstrated strong antimicrobial activity, with gelatin-CM22 and chitosan-CM22 reducing mesophilic counts to 3 log CFU/g and 2 log CFU/g, respectively, while the control exceeded 7 log CFU/g ( $p < 0.001$ ).

For psychrophilic microorganisms, tuna stored at 4 °C and 8 °C exhibited significant inhibition when treated with chitosan-CM22, reducing bacterial counts to approximately 1 log CFU/g at both temperatures, compared to controls, which increased to 5 log CFU/g ( $p < 0.001$ ). Gelatin-CM22 also showed effectiveness, reducing counts to about 2 log CFU/g, though not as significantly as chitosan-CM22. Salmon treated with these coatings displayed similar results, with significant reductions in psychrophilic counts at both temperatures, particularly with chitosan-CM22, which maintained levels around 1 log CFU/g, while controls reached 6 log CFU/g ( $p < 0.001$ ).

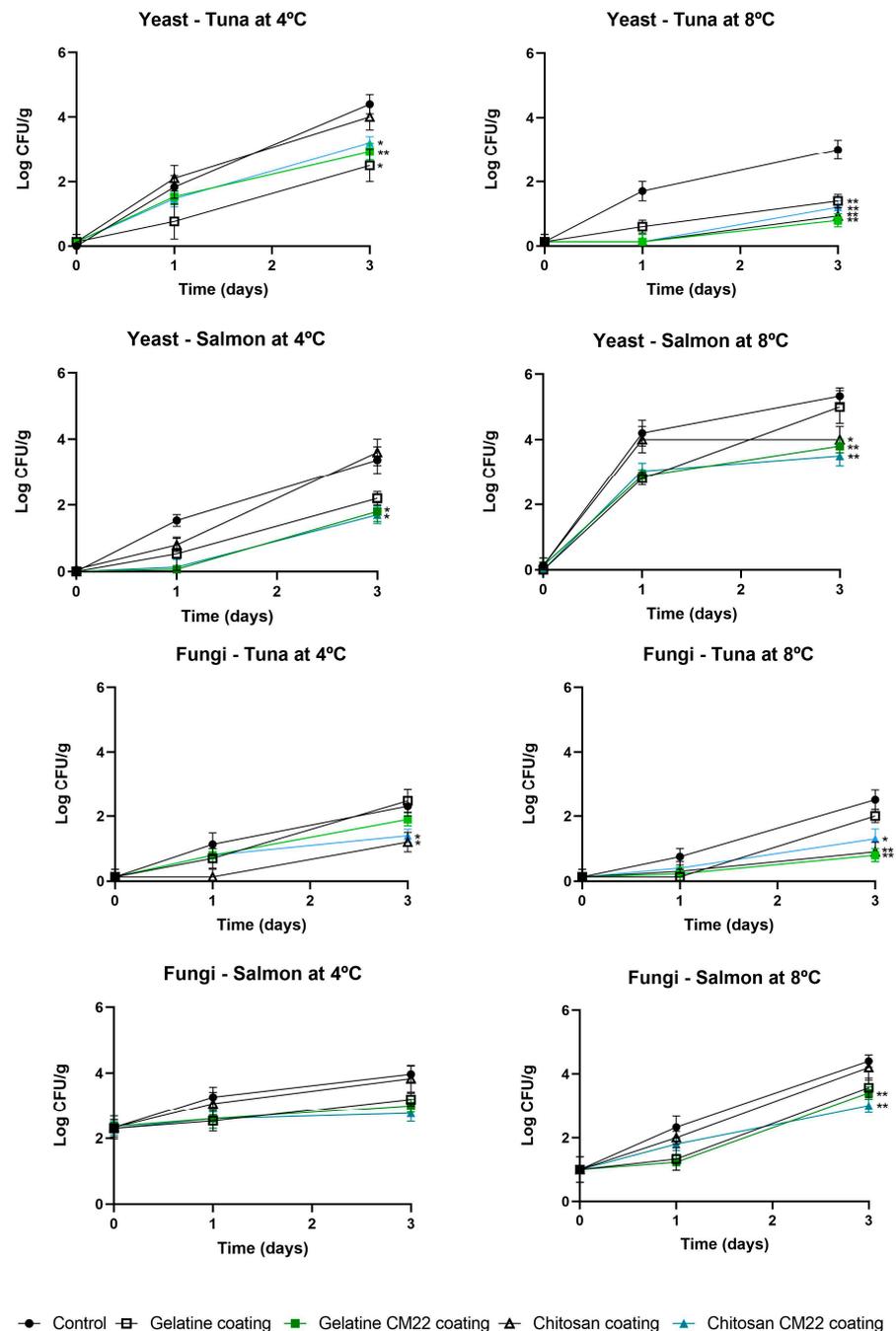
In the case of enterobacteria, the results in Figure 2 show a significant impact of edible coatings (ECs) containing piscicolin CM22 on raw tuna and salmon stored at 4 °C and 8 °C. Both gelatin-CM22 and chitosan-CM22 coatings demonstrated significant reductions in enterobacterial counts. For tuna stored at 4 °C, chitosan-CM22 reduced counts to approximately 1 log CFU/g on day 3, compared to the control at around 4 log CFU/g. Gelatin-CM22 also showed significant inhibition, reducing counts to about 1.5 log CFU/g. Similar results were observed for salmon, with chitosan-CM22 reducing counts below 1 log CFU/g, while controls reached nearly 5 log CFU/g. At 8 °C, both coatings continued to exhibit strong antimicrobial activity, maintaining low enterobacterial counts compared to significantly higher counts in control samples.



**Figure 2.** Effect of treatments with different edible coatings (ECs) containing piscicolin CM22 on enterobacteria in raw tuna and salmon stored at 4 °C and 8 °C. Values are the average  $\pm$  SD (error bars) from independent experiments. Significant differences compared to control: \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

The results in Figure 3 highlight the effectiveness of ECs in controlling yeast and fungi in raw tuna and salmon stored at 4 °C and 8 °C, although this effect was less pronounced compared to bacteria. For yeast control, chitosan-CM22 was the most effective, reducing yeast counts to approximately 1 log CFU/g in tuna at 4 °C on day 3, compared to 3.5 log CFU/g in untreated samples ( $p < 0.01$ ). Gelatin-CM22 also provided some inhibition, reducing counts to around 1.5 log CFU/g. At 8 °C, chitosan-CM22 kept yeast counts in tuna

around 1 log CFU/g, while controls increased to approximately 4 log CFU/g. Regarding fungi, tuna stored at 4 °C and treated with chitosan-CM22 coatings showed significant reductions in fungal counts. Chitosan-CM22 maintained counts to about 1 log CFU/g by day 3, compared to the control at 2.5 log CFU/g. Finally, at 8 °C, tuna treated with chitosan-CM22 maintained fungal counts around 1 log CFU/g, while control samples increased to about 2.5 log CFU/g.



**Figure 3.** Effect of treatments with different edible coatings (ECs) containing piscicolin CM22 on yeast and fungi in raw tuna and salmon stored at 4 °C and 8 °C. Values are the average  $\pm$  SD (error bars) from independent experiments. Significant differences compared to control: \*  $p < 0.05$ ; \*\*  $p < 0.01$ .

Since bacteriocins typically do not possess antifungal properties, the observed protective effect against fungi and yeasts cannot be attributed to these compounds. Instead, it is more likely attributable to the inherent antifungal properties of chitosan itself. Chitosan

is well-documented for its broad-spectrum antifungal activity [32–35]. Additionally, the barrier effect provided by the ECs plays a crucial role, as it impedes the proliferation of spoilage microorganisms, particularly aerobic ones [36]. This dual action—direct antifungal activity of chitosan combined with the protective barrier effect—likely accounts for the observed reduction in fungal and yeast growth.

### 3.2.2. Influence in Technological Properties

The pH stability for both salmon and tuna with different ECs at storage temperatures of 4 °C and 8 °C (Table 4) revealed that the variations in pH were minimal and statistically insignificant. For salmon, the treatments including gelatin coating (GC), gelatin coating with CM22 (GC-CM22), chitosan coating (CC), and chitosan coating with CM22 (CC-CM22) showed only minor deviations from the control group. At 4 °C, the pH remained largely consistent across all treatments, with fluctuations that did not substantially impact overall pH stability. Similarly, at 8 °C, while there were slight changes in pH values among the treatments, these differences were not significant. These results suggest that, under the tested conditions, the use of these ECs does not substantially alter the pH of the products.

**Table 4.** Effect of treatments of edible coatings (ECs) with piscicolin CM22 on pH of raw tuna and salmon stored at 4 °C and 8 °C.

Salmon		Time (Days)			
		0	1	3	
4 °C	Control	6.72	6.72	6.85	
	Gelatin coating (GC)	6.72	6.72	6.83	
	Gelatin coating with CM22 (GC-CM22)	6.94	6.94	6.75	
	Chitosan coating (CC)	6.79	6.79	6.77	
	Chitosan coating with CM22 (CC-CM22)	6.87	6.87	6.86	
	Control	6.85	6.85	7.15	
8 °C	Gelatin coating (GC)	6.88	6.88	6.81	
	Gelatin coating with CM22 (GC-CM22)	6.87	6.87	6.81	
	Chitosan coating (CC)	6.75	6.75	6.78	
	Chitosan coating CM22 (CC-CM22)	6.73	6.73	6.79	
	Control	6.54	6.54	6.59	
Tuna	4 °C	Gelatin coating (GC)	6.38	6.38	6.67
		Gelatin coating with CM22 (GC-CM22)	6.71	6.71	6.72
		Chitosan coating (CC)	6.38	6.38	6.35
		Chitosan coating with CM22 (CC-CM22)	6.42	6.42	6.64
		Control	6.57	6.57	6.56
		8 °C	Gelatin coating (GC)	6.53	6.53
Gelatin coating CM22 (GC-CM22)	6.68		6.68	6.76	
Chitosan coating (CC)	6.74		6.74	6.79	
Chitosan coating with CM22 (CC-CM22)	6.49		6.49	6.77	
Control	6.57		6.57	6.56	

Color is a critical attribute of fresh salmon and tuna, greatly affecting consumer perception and its marketability [37]. Maintaining the natural color during storage is essential, as any variations may indicate spoilage or decreased quality, making it a key parameter to monitor throughout the shelf life of these seafood products. The analysis of the color evolution in fresh salmon under different treatments revealed significant variations in the colorimetric parameters  $L^*$ ,  $a^*$ , and  $b^*$  (Table 5 and Figure 4). In terms of lightness ( $L^*$ ), the control exhibited a notable decrease in brightness, with values reduced to 42.41 and 38.66 on day 3 at 4 °C and 8 °C, respectively. In contrast, the chitosan coating (CC) and gelatin coating (GC) demonstrated better color preservation. The chitosan coating maintained relatively stable lightness at 4 °C (52.20 on day 3) and at 8 °C (48.65 on day 3) compared to the control. The gelatin coating also showed a positive effect, with  $L^*$  values indicating less loss of brightness at 8 °C.

**Table 5.** Color modification of treatments with edible coatings (ECs) over time in raw salmon stored at 4 and 8 °C.

Parameter		Time (Days)	Control	CC	CC-CM22	GC	GC-CM22
$L^*$	4 °C	0	50.77 ± 0.87	52.94 ± 0.87	52.93 ± 0.87	51.29 ± 0.87	51.31 ± 0.87
		1	53.68 ± 1.92	51.68 ± 2.34	52.57 ± 0.98	51.15 ± 3.01	51.4 ± 3.01
		3	42.41 <sup>a</sup> ± 2.03	52.20 <sup>b,c</sup> ± 3.73	55.66 ± 4.03	50.35 <sup>c</sup> ± 2.27	50.38 <sup>b</sup> ± 2.27
	8 °C	0	52.63 ± 1.08	52.94 ± 1.37	52.94 ± 0.13	51.25 ± 0.88	51.11 ± 0.88
		1	42.49 <sup>a</sup> ± 0.97	43.83 <sup>a</sup> ± 1.02	49.33 <sup>b</sup> ± 0.90	50.35 <sup>b</sup> ± 0.53	50.96 <sup>b</sup> ± 0.53
		3	38.66 <sup>a</sup> ± 0.85	48.65 <sup>b</sup> ± 0.48	48.55 <sup>b</sup> ± 1.47	50.55 <sup>c</sup> ± 0.48	49.81 <sup>b,c</sup> ± 0.48
$a^*$	4 °C	0	24.35 <sup>b</sup> ± 0.98	21.81 <sup>a</sup> ± 0.98	21.81 <sup>a</sup> ± 0.98	24.76 <sup>b</sup> ± 0.98	24.93 <sup>b</sup> ± 0.98
		1	11.15 <sup>a</sup> ± 0.75	15.65 <sup>b</sup> ± 0.10	15.85 <sup>b</sup> ± 1.31	19.85 <sup>c</sup> ± 0.7	19.82 <sup>c</sup> ± 0.7
		3	12.71 <sup>a</sup> ± 0.65	14.11 <sup>a,b</sup> ± 0.85	13.50 <sup>a</sup> ± 1.44	17.69 <sup>b</sup> ± 1.13	17.57 <sup>b</sup> ± 1.13
	8 °C	0	23.44 ± 1.75	21.81 ± 1.01	21.81 ± 0.66	24.27 ± 0.43	24.96 ± 0.43
		1	13.42 <sup>a</sup> ± 0.41	18.55 <sup>b</sup> ± 0.74	17.58 <sup>b</sup> ± 0.34	18.21 <sup>b</sup> ± 0.65	13.20 <sup>a</sup> ± 0.65
		3	11.49 <sup>a</sup> ± 0.13	15 <sup>b</sup> ± 0.34	15.52 <sup>b</sup> ± 0.42	11.51 <sup>a</sup> ± 0.70	11.52 <sup>a</sup> ± 0.70
$b^*$	4 °C	0	13.35 ± 0.40	14.46 ± 0.40	14.46 ± 0.40	14.9 ± 0.40	14.58 ± 0.40
		1	12.58 ± 0.68	14.71 ± 0.67	13.75 ± 1.34	14.88 ± 1.75	13.71 ± 1.75
		3	6.31 <sup>a</sup> ± 0.88	15.27 <sup>b</sup> ± 1.88	13.3 <sup>b</sup> ± 1.11	13.63 <sup>b</sup> ± 0.58	13.64 <sup>b</sup> ± 0.58
	8 °C	0	13.52 ± 0.33	13.46 ± 0.69	13.46 ± 0.47	14.81 ± 0.23	13.57 ± 0.23
		1	8.79 <sup>a</sup> ± 0.24	11.97 <sup>b</sup> ± 0.27	13.28 <sup>b</sup> ± 0.17	12.68 <sup>b</sup> ± 0.47	13.68 <sup>b</sup> ± 0.47
		3	1.49 <sup>a</sup> ± 0.1	9.51 <sup>b</sup> ± 0.3	9.48 <sup>b</sup> ± 0.26	10.48 <sup>b</sup> ± 0.46	10.48 <sup>b</sup> ± 0.46

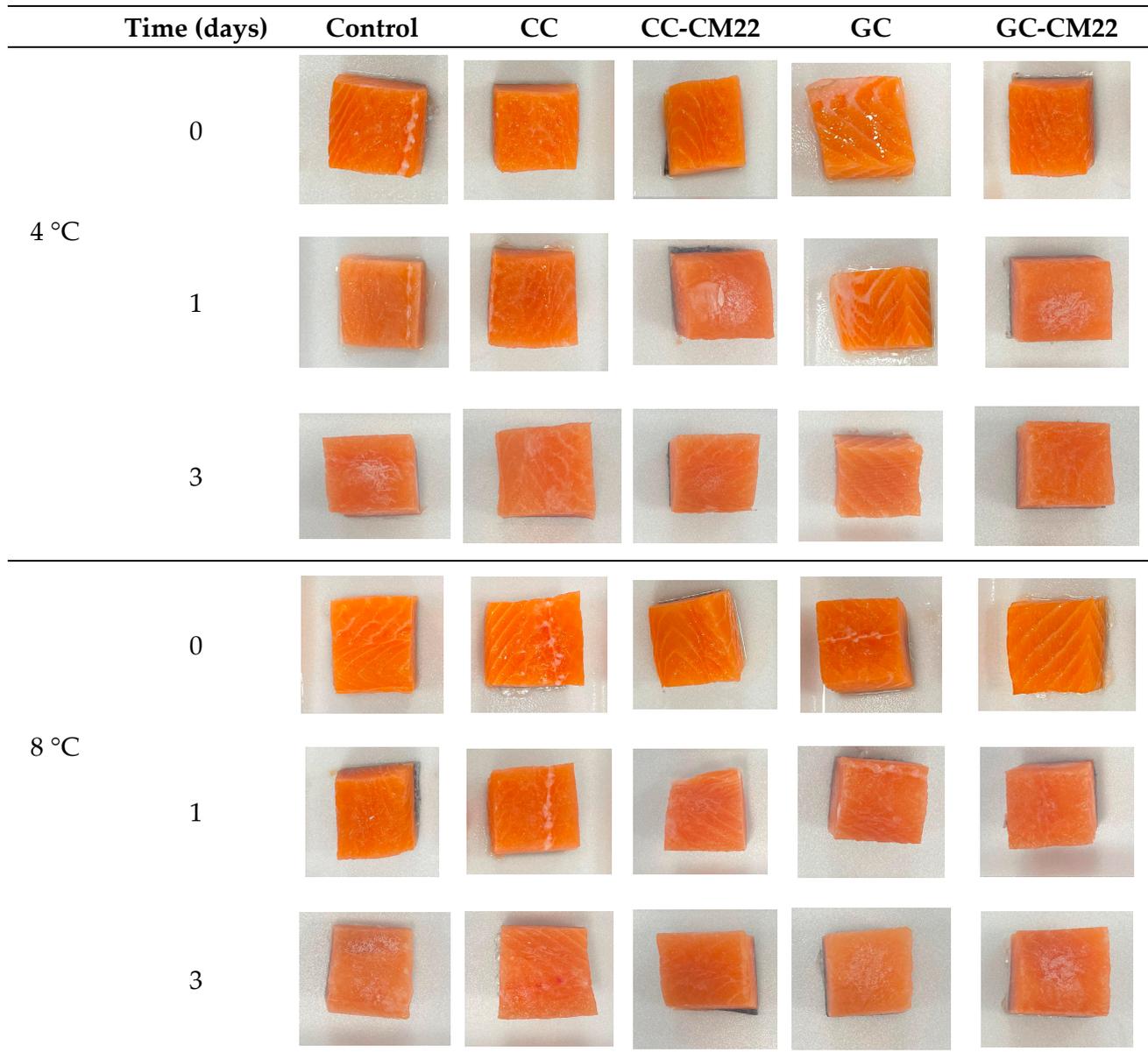
Different letters indicate statistically significant differences ( $p < 0.05$ ) between treatments.

Regarding  $a^*$  and  $b^*$  parameters (red and yellow intensity), the control exhibited a significant decrease in redness over time, with  $a^*$  values falling from 24.35 to 11.49 at 8 °C on day 3. In contrast, the chitosan and gelatin coatings demonstrated better preservation of red color, with the gelatin coating maintaining higher  $a^*$  values at 4 °C and the chitosan coating showing relative stability at 8 °C. For the  $b^*$  parameter, which measures yellow intensity, both the control and the treatments experienced a loss of yellow intensity; however, the chitosan and gelatin coatings were more effective in preserving this parameter.

Ultimately, it is important to highlight that the incorporation of piscicolin CM22 into the coatings did not substantially affect color preservation. The values for  $L^*$ ,  $a^*$ , and  $b^*$  were similar between coatings with and without CM22, demonstrating that this additive had a negligible impact on maintaining color stability.

The color values for tuna during shelf life at 4 °C and 8 °C, as detailed in Table 6 (Figure 5), reveal that chitosan coatings (CC and CC-CM22) were highly effective in preserving lightness ( $L^*$ ), particularly at 4 °C, where values remained between 38.16 and 40.04. In contrast, the control and gelatin coatings showed significant reductions in lightness, especially at 8 °C, where the control's  $L^*$  value fell to 18.48 by day 3, potentially impact-

ing perceived freshness. Chitosan coatings also maintained a superior red color intensity (a\*) compared to gelatin coatings. Although the preservation of yellow color intensity (b\*) showed less variation among treatments, chitosan coatings slightly outperformed the control and gelatin coatings, indicating a modest benefit in maintaining yellow color.

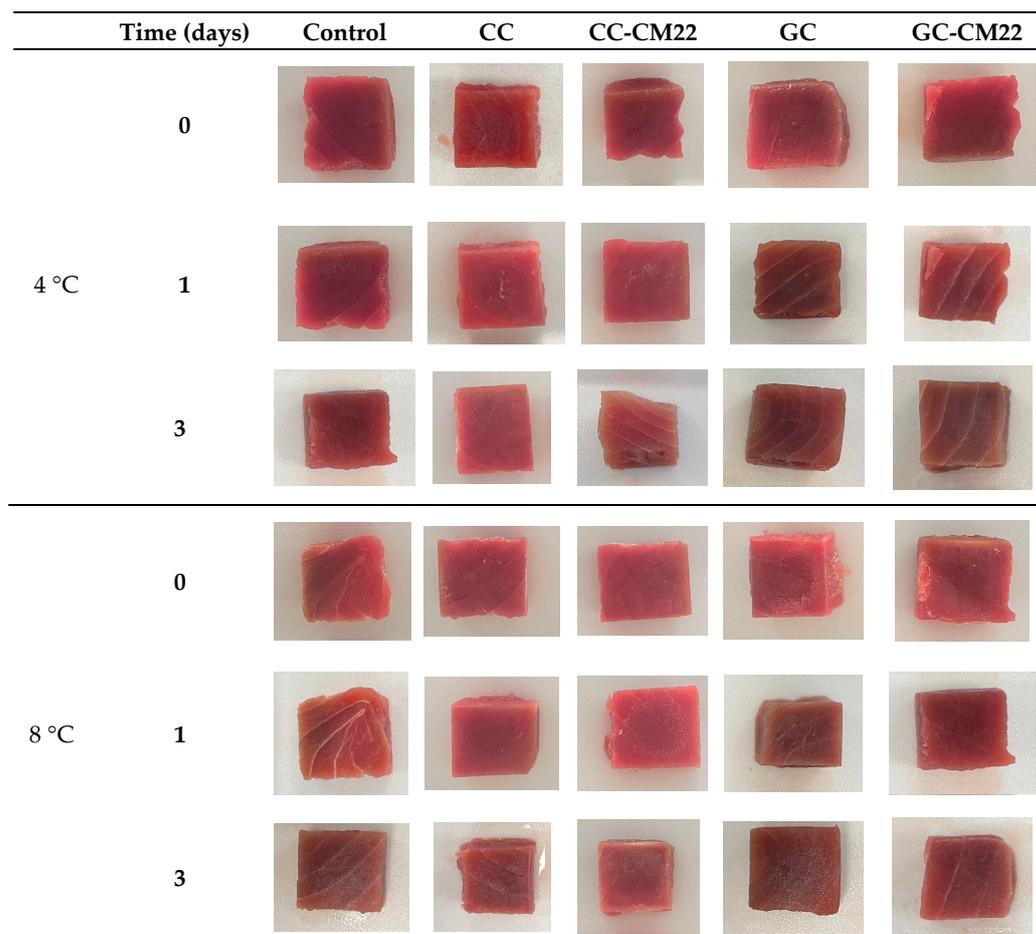


**Figure 4.** Photographs of salmon illustrating the progression of color under various treatments, stored at 4 °C and 8 °C.

**Table 6.** Color modification of treatments with edible coatings (ECs) over time in raw tuna stored at 4 and 8 °C.

Parameter	Time (Days)	Control	CC	CC-CM22	GC	GC-CM22	
L*	4 °C	0	27.05 <sup>a</sup> ± 0.87	40.55 <sup>b</sup> ± 0.87	40.55 <sup>b</sup> ± 0.87	27.76 <sup>a</sup> ± 0.87	27.45 <sup>a</sup> ± 0.87
		1	26.62 <sup>a</sup> ± 1.92	38.16 <sup>b</sup> ± 2.34	38.57 <sup>b</sup> ± 0.98	28.79 <sup>a</sup> ± 3.01	28.44 <sup>a</sup> ± 3.01
		3	25.17 <sup>a</sup> ± 2.03	38.60 <sup>b</sup> ± 3.73	40.04 <sup>b</sup> ± 4.03	28.6 <sup>a</sup> ± 2.27	28.31 <sup>a</sup> ± 2.27
	8 °C	0	27.5 <sup>a</sup> ± 1.08	42.55 <sup>b</sup> ± 1.37	42.55 <sup>b</sup> ± 0.13	27.5 <sup>b</sup> ± 0.88	27.48 <sup>a</sup> ± 0.88
		1	11.50 <sup>a</sup> ± 0.97	37.90 <sup>c</sup> ± 1.02	33.66 <sup>c</sup> ± 0.90	22.2 <sup>b</sup> ± 0.53	23.66 <sup>b</sup> ± 0.53
		3	18.48 <sup>a</sup> ± 0.85	38.45 <sup>b</sup> ± 0.48	38.47 <sup>b</sup> ± 1.47	18.46 <sup>a</sup> ± 0.48	18.49 <sup>a</sup> ± 0.48
a*	4 °C	0	16.24 <sup>b</sup> ± 0.98	15.47 <sup>b</sup> ± 0.98	15.47 <sup>b</sup> ± 0.98	5.71 <sup>a</sup> ± 0.98	5.13 <sup>a</sup> ± 0.98
		1	16.89 <sup>b</sup> ± 0.75	16.07 <sup>b</sup> ± 0.10	16.35 <sup>b</sup> ± 1.31	9.61 <sup>a</sup> ± 0.7	9.97 <sup>a</sup> ± 0.7
		3	15.14 <sup>b</sup> ± 0.65	16.51 <sup>b</sup> ± 0.85	16.24 <sup>b</sup> ± 1.44	11.52 <sup>a</sup> ± 1.13	12.4 <sup>a</sup> ± 1.13
	8 °C	0	16.64 <sup>b</sup> ± 1.75	16.79 <sup>b</sup> ± 1.01	16.79 <sup>b</sup> ± 0.66	5.99 <sup>a</sup> ± 0.43	5.08 <sup>a</sup> ± 0.43
		1	9.24 <sup>a</sup> ± 0.41	14.49 <sup>b</sup> ± 0.74	14.63 <sup>b</sup> ± 0.34	8.11 <sup>a</sup> ± 0.65	8.49 <sup>a</sup> ± 0.65
		3	0.52 <sup>a</sup> ± 0.13	12.52 <sup>b</sup> ± 0.34	12.52 <sup>b</sup> ± 0.42	0.53 <sup>a</sup> ± 0.70	0.53 <sup>a</sup> ± 0.70
b*	4 °C	0	5.29 <sup>a</sup> ± 0.40	5.47 <sup>b</sup> ± 0.40	5.55 <sup>b</sup> ± 0.40	2.16 <sup>a</sup> ± 0.40	2.81 <sup>a</sup> ± 0.40
		1	6.66 <sup>a</sup> ± 0.68	5.06 <sup>a</sup> ± 0.67	6.04 <sup>a</sup> ± 1.34	6.60 <sup>a</sup> ± 1.75	10.27 <sup>b</sup> ± 1.75
		3	2.07 <sup>a</sup> ± 0.88	4.51 <sup>b</sup> ± 1.88	4.44 <sup>b</sup> ± 1.11	1.51 <sup>a</sup> ± 0.58	15.68 <sup>c</sup> ± 0.58
	8 °C	0	5.78 <sup>a</sup> ± 0.33	5.14 <sup>b</sup> ± 0.69	5.14 <sup>b</sup> ± 0.47	2.05 <sup>a</sup> ± 0.23	2.77 <sup>a</sup> ± 0.23
		1	6.94 <sup>a</sup> ± 0.24	5.14 <sup>b</sup> ± 0.27	5.11 <sup>b</sup> ± 0.17	1.17 <sup>a</sup> ± 0.47	1.43 <sup>a</sup> ± 0.47
		3	1.48 <sup>a</sup> ± 0.1	4.51 <sup>b</sup> ± 0.3	4.48 <sup>b</sup> ± 0.26	1.52 <sup>a</sup> ± 0.46	1.49 <sup>a</sup> ± 0.46

Different letters indicate statistically significant differences ( $p < 0.05$ ) between treatments.

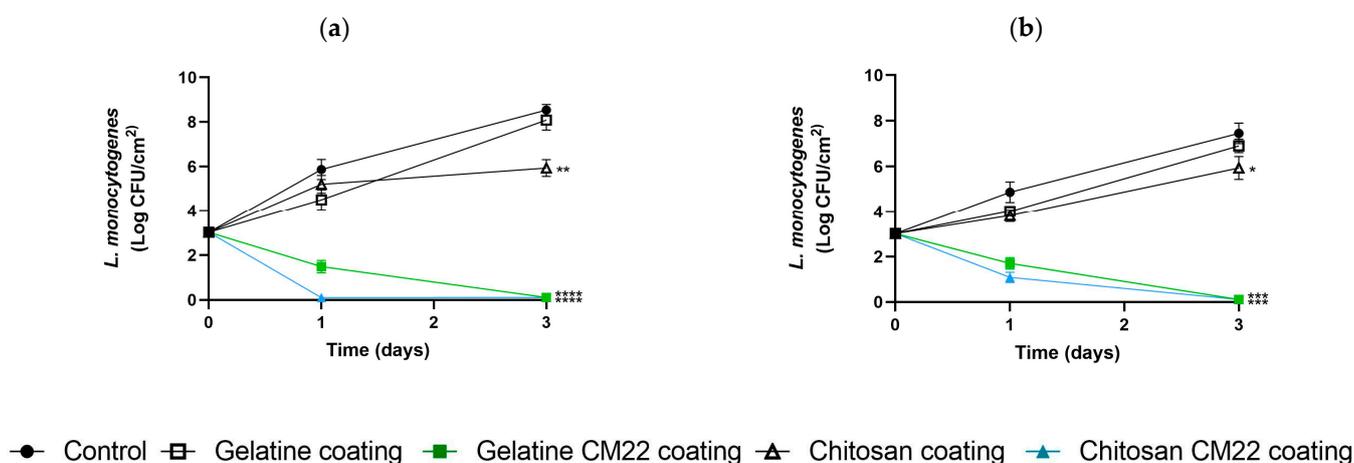
**Figure 5.** Photographs of tuna illustrating the progression of color under various treatments, stored at 4 °C and 8 °C.

The results obtained suggest that gelatin coatings (GC and GC-CM22) could be counterproductive in this case, accelerating color degradation faster than the control and potentially negatively affecting the perceived freshness and quality of the final product. The data imply that while both chitosan and gelatin coatings might be suitable for fresh salmon, the application of gelatin-based coatings for fresh tuna would be entirely inadvisable.

These findings underscore the critical importance of conducting tailored sensory studies for each specific food matrix, offering valuable insights into the seafood industry. Furthermore, they emphasize the necessity of developing preservation strategies that are not only effective but also align with the high-quality standards expected by consumers.

### 3.3. Challenge Test

Challenge tests were conducted by superficially inoculating *L. monocytogenes* to assess the level of protection offered by each coating, both with and without piscicolin CM22, against the pathogen. Figure 6 shows the results expressed in Log CFU/cm<sup>2</sup>.



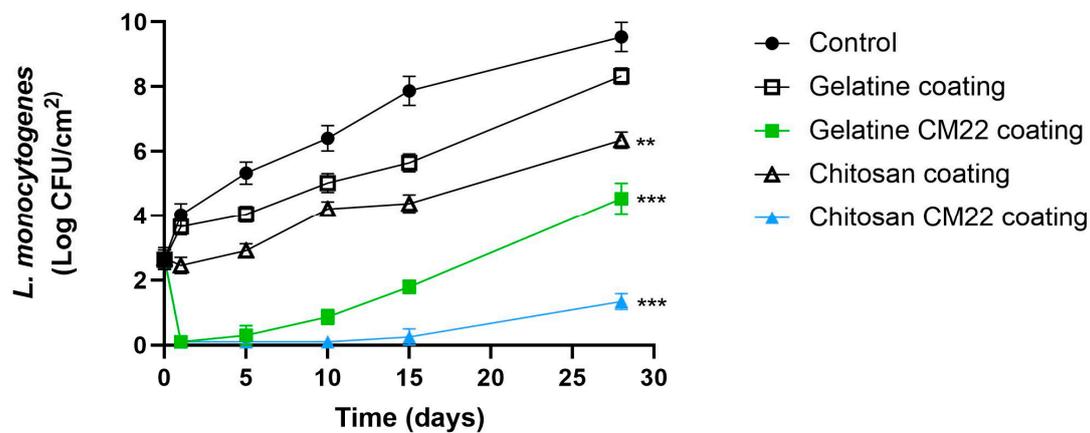
**Figure 6.** Effect of treatments with edible coatings (ECs) with and without piscicolin CM22 on the viability of *Listeria monocytogenes* in raw tuna (a) and raw salmon (b) stored at 4 °C and packaged in MAP. Values are the mean  $\pm$  SD (error bars) from independent experiments. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; \*\*\*\*  $p < 0.0001$  compared to control.

For tuna, the results indicated that ECs containing piscicolin CM22 were significantly more effective in reducing *L. monocytogenes* compared to ECs without bacteriocin and the control. The chitosan coating with piscicolin CM22 (CC-CM22) was the most effective, achieving a drastic and significant reduction in bacterial counts in three days. The gelatin coating with piscicolin CM22 (GC-CM22) also showed a notable and significant reduction, with bacterial counts decreasing below the limit of detection. However, the elimination was not complete in both cases, as the pre-enrichment test returned positive for *Listeria*. In contrast, the coatings without piscicolin CM22 (CC and GC) and the control exhibited a significant increase in bacterial counts, reaching nearly 9 log CFU/cm<sup>2</sup> in the control.

For salmon, a similar pattern was observed. The coatings containing piscicolin CM22 (CC-CM22 and GC-CM22) demonstrated the highest effectiveness, significantly reducing *L. monocytogenes* counts below the limit of detection after three days of storage, compared to the coatings without piscicolin CM22 (CC and GC) and the control, which showed significant increases in bacterial counts (approx. 8 log CFU/cm<sup>2</sup>). Despite the significant reduction observed, the enrichment test again tested positive for the presence of *L. monocytogenes*.

Finally, challenge tests were conducted on smoked salmon, which is characterized by a longer shelf life and different packaging and preservation conditions. The primary aim of these assays was to evaluate whether the efficacy of the coatings (ECs) remained effective

over time. To this end, vacuum-packed smoked salmon was superficially inoculated with *L. monocytogenes*. Figure 7 presents the results expressed in Log CFU/cm<sup>2</sup>.



**Figure 7.** Effect of treatments with piscicolin CM22 on the viability of *Listeria monocytogenes* in vacuum-packed smoked salmon stored at 4 °C. Values are the average  $\pm$  SD (error bars) of independent experiments. \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; respect to control.

The control samples exhibited a significant increase in bacterial counts over the 30 days, reaching nearly 9 log CFU/cm<sup>2</sup>. Similarly, the samples treated with chitosan (CC) and gelatin (GC) coatings without piscicolin CM22 also showed important increases in *Listeria* counts, reaching approximately 6 and 8 log CFU/cm<sup>2</sup>, respectively. In contrast, the samples treated with chitosan and piscicolin CM22 (CC-CM22) and gelatin with piscicolin CM22 (GC-CM22) demonstrated a significant reduction in bacterial counts compared to the controls. However, it is noteworthy that *Listeria* began to recover after day 15 of storage at 4 °C (Figure 7), with concentrations reaching approximately between 1 and 4 log CFU/cm<sup>2</sup>. This recovery of the pathogen could be due to the repair of *Listeria* cells with sublethal injury [38] or to a loss of stability of piscicolin CM22 over an extended storage time. Therefore, further studies are needed to investigate this phenomenon.

#### 4. Discussion

The use of ECs has rapidly expanded across nearly all sectors of the food industry, primarily due to their significant ability to enhance food safety and preservation. ECs function as physical barriers that protect the microbiological quality of food, thereby extending its shelf life [18]. This protective barrier is achieved through the film-forming substances used in their production, which not only safeguard the food but may also impart inherent antimicrobial properties, such as those found in chitosan [32,39]. Furthermore, ECs offer the distinct advantage of being able to incorporate antimicrobials, such as bacteriocins, which have been extensively studied as an effective biopreservation strategy for controlling pathogenic and spoilage bacteria [33]. In this study, we functionalized chitosan-based ECs with bacteriocins, which are widely used to extend shelf life due to their antimicrobial properties, with successful applications in meat and seafood products [18,40–42]. Similarly, fish gelatin ECs have demonstrated the ability to inhibit microbial growth, providing comparable benefits in different seafood [4].

Numerous researchers have incorporated bacteriocins into ECs. For example, nisin has been shown to be effective in whey protein-based coatings for controlling pathogens such as *Listeria* and spoilage microorganisms like *Bacillus thermosphacta* [24]. Furthermore, other studies have reported the anti-*Listeria* activity of an EC based on corn protein supplemented with nisin in turkey frankfurter [43]. Similar to our work, trials have been conducted with nisin in fish gelatin-based ECs applied to ham and bologna [44], as well as turkey-type sausages [45].

As previously mentioned, one of the significant advantages of ECs is their potential to serve as carriers of antimicrobials [24]. In this context, the combined effects of chitosan with other antimicrobial agents, such as organic acids, essential oils, or bacteriocins, have also been investigated. Beverly et al. (2008) reported the anti-*Listeria* properties of chitosan-based coatings combined with acetic and lactic acids [46]. Similarly, Ojagh et al. (2010) found that applying a chitosan coating enriched with cinnamon oil reduced *L. monocytogenes* counts on refrigerated rainbow trout [47].

In our study, we have demonstrated the potential of incorporating the bacteriocin piscicolin CM22 into two types of coatings as a promising technology for food preservation. However, many aspects of their efficacy remain unknown. Although bacteriocins are effective at low concentrations, they often exhibit low *in vivo* stability and are susceptible to degradation by proteolytic enzymes [48]. Jack et al. (1996) observed that the bactericidal activity of piscicolin 126 was not destroyed by exposure to elevated temperatures at low pH values; however, this activity was lost at high pH values, especially when high pH was combined with elevated temperatures [49]. These findings align with our *in vitro* results, where piscicolin CM22 was completely degraded by proteolytic enzymes and exhibited instability under highly alkaline conditions and high temperatures.

In food trials, edible coatings enriched with piscicolin CM22 have demonstrated promising results in maintaining technological properties. Specifically, these coatings effectively preserve optimal pH and color even under suboptimal storage conditions. Quality degradation in fresh tuna and salmon is often associated with increased pH levels due to the formation of alkaline compounds such as trimethylamine, ammonia, and other basic volatile substances produced during spoilage [50,51]. Additionally, as noted earlier, color is a crucial attribute for both fresh salmon and tuna, significantly impacting consumer perception and marketability [37]. The ability of chitosan-based coatings to maintain the natural color of salmon and tuna during storage is a notable achievement. These results underscore the importance of assessing technological properties for each specific food product, as alterations to critical attributes such as color in raw tuna can significantly impact consumer acceptance.

Beyond the direct findings, our study also revealed that both edible coatings (ECs) substantially improved the microbiological quality of raw tuna and salmon during storage at 4 °C and 8 °C. Notably, significant reductions were observed in the counts of enterobacteria, fungi, and yeasts. This is particularly remarkable considering that most bacteriocins produced by LAB are predominantly effective against Gram-positive bacteria. The broader antimicrobial effect observed in our study may be attributed to the intrinsic antimicrobial properties of the coating materials themselves, as well as the barrier effect that restricts microbial growth. Chitosan, in particular, is well-documented for its broad-spectrum antimicrobial activity and its effectiveness as a preservative in film-forming edible coatings [52]. While the direct antimicrobial action of piscicolin CM22 was not evident, it is plausible that a synergistic interaction between chitosan and piscicolin CM22 contributed to the observed effects. Synergism has been previously reported in studies combining chitosan with enzymes like lysozyme [53] and bacteriocins such as nisin [54], where the combined application enhanced antimicrobial efficacy against both Gram-positive and Gram-negative bacteria.

The most notable results were obtained in the challenge tests against *L. monocytogenes*, which demonstrated a significant reduction of the pathogen in raw tuna, raw salmon, and smoked salmon. Similar findings were reported by Wan et al. (1997) who described the ability of piscicolin 126 in milk and Camembert cheese to inhibit *L. monocytogenes* [55]. Additionally, studies focused on the treatment of listeriosis, such as the work by Igham (2003), have shown *in vivo* protection provided by piscicolin against *Listeria* in infected mice [11]. It is also important to emphasize that piscicolins are not the only bioactive compounds of interest produced by *C. maltaromaticum* [56]; other peptides and bacteriocins from this species have also demonstrated significant activity against *Listeria* and other food spoilage microorganisms [57–59].

In summary, given the well-documented vulnerability of raw or minimally processed fish products such as raw tuna, salmon, and smoked salmon to pathogens as *L. monocytogenes* (particularly due to its ability to grow at refrigeration temperatures) [60–62], this study presents innovative developments using bacteriocins produced by psychrotolerant LAB strains as a preservation strategy for refrigerated fish products. Additionally, the efficacy of these ECs containing piscicolin CM22 at both 4 °C and 8 °C (where 8 °C indicates potential abuse during cold chain disruptions) demonstrates their robustness under various conditions. Furthermore, these ECs have been evaluated within the framework of multiple barrier technology, which integrates different preservation methods such as refrigeration, modified atmosphere packaging (MAP), and vacuum packaging [63]. Thus, the new ECs align well with this approach, providing an additional barrier that is compatible with current preservation technologies.

Overall, the integration of piscicolin CM22 into ECs represents a significant advancement in biopreservation strategies, leveraging the natural antimicrobial properties of bacteriocins to enhance the quality and safety of seafood products. Thus, future research should focus on optimizing these coatings for different food matrices and validating their efficacy under real-world conditions to fully realize their potential in food preservation.

## 5. Conclusions

This study underscores the substantial potential of incorporating piscicolin CM22, a novel bacteriocin derived from a psychrotolerant *C. maltaromaticum* strain, into chitosan-based and fish gelatin-based edible coatings (ECs) to enhance the quality and safety of fish products. The findings demonstrate that these ECs significantly improved microbiological quality and extended the shelf life of raw fish stored at both 4 °C and 8 °C. Moreover, chitosan-based ECs maintained pH stability and enhanced color retention, thereby preserving the sensory and technological attributes of the fish products. Conversely, fish gelatin-based coatings were found to alter the color of raw tuna. Additionally, both ECs exhibited potent anti-Listerial activity, significantly reducing the microbial counts of the pathogen in raw tuna, raw salmon, and smoked salmon. These results suggest that the integration of natural antimicrobial agents such as piscicolin CM22 into biopolymer-based coatings represents a promising biopreservation strategy, meeting the increasing consumer demand for safer and more natural food products.

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## References

1. Jami, E.; White, B.A.; Mizrahi, I. Potential Role of the Bovine Rumen Microbiome in Modulating Milk Composition and Feed Efficiency. *PLoS ONE* **2014**, *9*, e85423. [[CrossRef](#)] [[PubMed](#)]
2. Gudmundsdóttir, S.; Gudbjörnsdóttir, B.; Einarsson, H.; Kristinsson, K.G.; Kristjánsson, M. Contamination of Cooked Peeled Shrimp (*Pandalus borealis*) by *Listeria monocytogenes* during Processing at Two Processing Plants. *J. Food Prot.* **2006**, *69*, 1304–1311. [[CrossRef](#)] [[PubMed](#)]

3. Poole, S.; Norhana, M.N.W.; Poole, S.E.; Deeth, H.C.; Dykes, G.A. Prevalence, Persistence and Control of Salmonella and *Listeria* in Shrimp and Shrimp Products: A Review. *Food Control* **2010**, *21*, 343–361. [[CrossRef](#)]
4. Jiang, M.; Liu, S.; Wang, Y. Effects of Antimicrobial Coating from Catfish Skin Gelatin on Quality and Shelf Life of Fresh White Shrimp (*Penaeus vannamei*). *J. Food Sci.* **2011**, *76*, 204–209. [[CrossRef](#)] [[PubMed](#)]
5. Bremer, P.J.; Monk, I.; Butler, R. Inactivation of *Listeria monocytogenes*/*Flavobacterium* spp. Biofilms Using Chlorine: Impact of Substrate, pH, Time and Concentration. *Lett. Appl. Microbiol.* **2002**, *35*, 321–325. [[CrossRef](#)]
6. Hierro, E.; Ganan, M.; Barroso, E.; Fernández, M. Pulsed Light Treatment for the Inactivation of Selected Pathogens and the Shelf-Life Extension of Beef and Tuna Carpaccio. *Int. J. Food Microbiol.* **2012**, *158*, 42–48. [[CrossRef](#)]
7. Danielski, G.M.; Henrique, P.; Maria, C.; Cavalari, D.A.; Daube, G.; Clinquart, A.; Ernlund, R.; Macedo, F. De *Carnobacterium maltaromaticum* as Bioprotective Culture in Vitro and in Cooked Ham. *Meat Sci.* **2020**, *162*, 108035. [[CrossRef](#)]
8. Reis, F.B.; De Souza, V.M.; Thomaz, M.R.S.; Fernandes, L.P.; De Oliveira, W.P.; Martinis, E.C.P. De International Journal of Food Microbiology Use of *Carnobacterium maltaromaticum* Cultures and Hydroalcoholic Extract of *Lippia sidoides* Cham. against *Listeria monocytogenes* in Fish Model Systems. *Int. J. Food Microbiol.* **2011**, *146*, 228–234. [[CrossRef](#)]
9. Baños, A.; Ariza, J.J.; Nuñez, C.; Gil-Martínez, L.; García-López, J.D.; Martínez-Bueno, M.; Valdivia, E. Effects of *Enterococcus faecalis* UGRA10 and the Enterocin AS-48 against the Fish Pathogen *Lactococcus garvieae*. Studies in Vitro and in Vivo. *Food Microbiol.* **2019**, *77*, 69–77. [[CrossRef](#)]
10. Mills, S.; Stanton, C.; Hill, C.; Ross, R.P. New Developments and Applications of Bacteriocins and Peptides in Foods. *Annu. Rev. Food Sci. Technol.* **2011**, *2*, 299–329. [[CrossRef](#)]
11. Ingham, A.; Ford, M.; Moore, R.J.; Tizard, M. The Bacteriocin Piscicolin 126 Retains Antilisterial Activity in Vivo. *J. Antimicrob. Chemother.* **2003**, *51*, 1365–1371. [[CrossRef](#)] [[PubMed](#)]
12. Zhang, J.; Zhao, L.; Tang, W.; Li, J.; Tang, T.; Sun, X. Heliyon Characterization of a Novel Circular Bacteriocin from *Bacillus velezensis* 1-3, and Its Mode of Action against *Listeria monocytogenes*. *Heliyon* **2024**, *10*, e29701. [[CrossRef](#)] [[PubMed](#)]
13. González, E.; David, G.J.; López, G.; Teso, C.; Irene, P.; Hernández, J.; Manuel, J.; Sánchez, P.; Valdivia, E.; Lopez, M.M.; et al. Genomic Characterization of Piscicolin CM22 Produced by *Carnobacterium maltaromaticum* CM22 Strain Isolated from Salmon (*Salmo Salar*). *Probiotics Antimicrob. Proteins* **2024**. [[CrossRef](#)] [[PubMed](#)]
14. Gursky, L.J.; Martin, N.I.; Derksen, D.J.; Van Belkum, M.J.; Kaur, K.; Vederas, J.C.; Stiles, M.E.; McMullen, L.M. Production of Piscicolin 126 by *Carnobacterium maltaromaticum* UAL26 Is Controlled by Temperature and Induction Peptide Concentration. *Arch. Microbiol.* **2006**, *186*, 317–325. [[CrossRef](#)]
15. Gibbs, G.M.; Davidson, B.E.; Hillier, A.J. Novel Expression System for Large-Scale Production and Purification of Recombinant Class IIa Bacteriocins and Its Application to Piscicolin 126. *Appl. Environ. Microbiol.* **2004**, *70*, 3292–3297. [[CrossRef](#)]
16. Surya, T.; Jeyasekaran, G.; Jeya, R.; Alsahli, M.S.; Devanesan, S.; Sivaraman, B.; Arisekar, U.; Huong, T. Effect of Antibiotics and Sanitizers on Salmonella Biofilms Associated with Seafood Contact Surfaces. *Microbiol. Res.* **2023**, *266*, 127213. [[CrossRef](#)]
17. Dhowlaghar, N.; Abeyundara, P.D.E.A.; Nannapaneni, R. Growth and Biofilm Formation by *Listeria monocytogenes* in Catfish Mucus Extract on Four Food Contact Surfaces at 22 and 10 °C and Their Reduction by Commercial Disinfectants. *J. Food Prot.* **2018**, *81*, 59–67. [[CrossRef](#)]
18. Wu, S. International Journal of Biological Macromolecules Effect of Chitosan-Based Edible Coating on Preservation of White Shrimp during Partially Frozen Storage. *Int. J. Biol. Macromol.* **2014**, *65*, 325–328. [[CrossRef](#)]
19. Falguera, V.; Quintero, J.P.; Jiménez, A.; Aldemar-Muñoz, J.; Ibarz, A. Edible Films and Coatings: Structures, Active Functions and Trends in Their Use. *Trends Food Sci. Technol.* **2011**, *22*, 292–303. [[CrossRef](#)]
20. Amankwaah, C.; Li, J.; Lee, J.; Pascall, M.A. Development of Antiviral and Bacteriostatic Chitosan-Based Food Packaging Material with Grape Seed Extract for Murine Norovirus, *Escherichia coli* and *Listeria innocua* Control. *Food Sci. Nutr.* **2020**, *8*, 6174–6181. [[CrossRef](#)]
21. Jasour, M.S.; Ehsani, A.; Samaneh, S. Chitosan Coating Incorporated with the Lactoperoxidase System: An Active Edible Coating for Fish Preservation. *J. Sci. Food Agric.* **2014**, *95*, 1373–1378. [[CrossRef](#)] [[PubMed](#)]
22. Olaimat, A.N.; Fang, Y.; Holley, R.A. International Journal of Food Microbiology Inhibition of *Campylobacter jejuni* on Fresh Chicken Breasts by  $\kappa$ -Carrageenan/Chitosan-Based Coatings Containing Allyl Isothiocyanate or Deodorized Oriental Mustard Extract. *Int. J. Food Microbiol.* **2014**, *187*, 77–82. [[CrossRef](#)] [[PubMed](#)]
23. Gniewosz, M.; Kra, K.; Woreta, M.; Kosakowska, O. Antimicrobial Activity of a Pullulan—Caraway Essential Oil Coating on Reduction of Food Microorganisms and Quality in Fresh Baby Carrot. *J. Food Sci.* **2013**, *78*, M1242–M1248. [[CrossRef](#)] [[PubMed](#)]
24. Murillo-Martínez, M.M.; Tello-Solís, S.R.; García-Sánchez, M.A.; Ponce-Alquicira, E. Antimicrobial Activity and Hydrophobicity of Edible Whey Protein Isolate Films Formulated with Nisin and/or Glucose Oxidase. *J. Food Sci.* **2013**, *78*, M560–M566. [[CrossRef](#)]
25. Galvez, A.; Maqueda, M.; Valdivia, E.; Quesada, A.; Montoya, E. Characterization and Partial Purification of a Broad Spectrum Antibiotic AS-48 Produced by *Streptococcus faecalis*. *Can. J. Microbiol.* **1986**, *32*, 765–771. [[CrossRef](#)]
26. Zhao, R.; Lu, Y.; Ran, J.; Li, G.; Lei, S.; Zhu, Y.; Xu, B. Purification and Characterization of Bacteriocin Produced by *Lactobacillus Rhamnosus* Zrx01. *Food Biosci.* **2020**, *38*, 100754. [[CrossRef](#)]
27. Ahn, Y.N. Effect of Alkyl Branches on the Thermal Stability of Quaternary Ammonium Cations in Organic Electrolytes for Electrochemical Double. *Phys. Chem. Chem. Phys.* **2017**, *19*, 19959–19966. [[CrossRef](#)]

28. Tan, C.; Faqir, Y.; Zeng, Y.; Huang, Y.; Ocloo, E.A.; Kaleri, A.R.; Kalhor, M.T.; Ma, J.; Aslam, M. Investigation of Biomechanical Characteristics of Novel Chitosan from Dung Beetle and Its Application Potential on Stored Tomato Fruit. *J. Food Meas. Charact.* **2022**, *16*, 4551–4563. [[CrossRef](#)]
29. Ma, J.; Xin, C.; Tan, C. Preparation, Physicochemical and Pharmaceutical Characterization of Chitosan from *Catharsius Molossus* Residue. *Int. J. Biol. Macromol.* **2015**, *80*, 547–556. [[CrossRef](#)]
30. Tagg, J.R.; McGiven, A.R. Assay System for Bacteriocins. *Appl. Microbiol.* **1971**, *21*, 943. [[CrossRef](#)]
31. Baños-Arjona, 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 Science and Technology* **2016**, *66*, 672–677. [[CrossRef](#)]
32. Thambiliyagodage, C.; Jayanetti, M.; Mendis, A.; Ekanayake, G. Recent Advances in Chitosan-Based Applications—A Review. *Materials* **2023**, *16*, 2073. [[CrossRef](#)] [[PubMed](#)]
33. Mohamed, A.; El Galiou, O.; Zantar, S.; Arakrak, A.; Laglaoui, A.; Zerrouk, M.H. Edible Films and Coatings: Major Challenges and Potential Applications in Food Packaging. A Review. In *Food Packaging: The Smarter Way*; Springer: Singapore, 2022; pp. 187–224. [[CrossRef](#)]
34. Nitikornwarakul, C.; Wangpradid, R.; Rakkapao, N. Impact of Molar Composition on the Functional Properties of Glutinous Rice Starch–Chitosan Blend: Natural-Based Active Coating for Extending Mango Shelf Life. *Polymers* **2024**, *16*, 1375. [[CrossRef](#)] [[PubMed](#)]
35. Qin, Y.; Li, P.; Guo, Z. Cationic Chitosan Derivatives as Potential Antifungals: A Review of Structural Optimization and Applications. *Carbohydr. Polym.* **2020**, *236*, 116002. [[CrossRef](#)]
36. Palou, L.; Valencia-Chamorro, S.A.; Pérez-Gago, M.B. Antifungal Edible Coatings for Fresh Citrus Fruit: A Review. *Coatings* **2015**, *5*, 962–986. [[CrossRef](#)]
37. Anderson, S. Salmon Color and the Consumer. In Proceedings of the IIFET 2000 Proceedings, Corvallis, OR, USA, 10–14 July 2000; p. 3.
38. Fang, T.; Wu, Y.; Xie, Y.; Sun, L.; Qin, X.; Liu, Y.; Li, H.; Dong, Q.; Wang, X. Inactivation and Subsequent Growth Kinetics of *Listeria monocytogenes* after Various Mild Bactericidal Treatments. *Front. Microbiol.* **2021**, *12*, 646735. [[CrossRef](#)]
39. Russo, G.L.; Langellotti, A.L.; Torrieri, E.; Masi, P. Emerging Technologies in Seafood Processing: An Overview of Innovations Reshaping the Aquatic Food Industry. *Compr. Rev. Food Sci. Food Saf.* **2024**, *23*, e13281. [[CrossRef](#)]
40. Moreira, R.; Pereda, M.; Marcovich, N.E.; Roura, S.I. Antimicrobial Effectiveness of Bioactive Packaging Materials from Edible Chitosan and Casein Polymers: Assessment on Carrot, Cheese, and Salami. *J. Food Sci.* **2011**, *76*, 54–63. [[CrossRef](#)]
41. Petrou, S.; Tsiraki, M.; Giatrakou, V.; Savvaidis, I.N. International Journal of Food Microbiology Chitosan Dipping or Oregano Oil Treatments, Singly or Combined on Modi Fi Ed Atmosphere Packaged Chicken Breast Meat. *Int. J. Food Microbiol.* **2012**, *156*, 264–271. [[CrossRef](#)]
42. Günlü, A.; Koyun, E. Effects of Vacuum Packaging and Wrapping with Chitosan-Based Edible Film on the Extension of the Shelf Life of Sea Bass (*Dicentrarchus labrax*) Fillets in Cold Storage (4 °C). *Food Bioprocess Technol.* **2013**, *6*, 1713–1719. [[CrossRef](#)]
43. Lungu, B.; Johnson, M.G. Potassium Sorbate Does Not Increase Control of *Listeria monocytogenes* When Added to Zein Coatings with Nisin on the Surface of Full Fat Turkey Frankfurter Pieces in a Model System at 4 °C. *J. Food Sci.* **2002**, *70*, M95–M99. [[CrossRef](#)]
44. Gill, A.O.; Holley, R.A. Surface Application of Lysozyme, Nisin, and EDTA to Inhibit Spoilage and Pathogenic Bacteria on Ham and Bologna. *J. Food Prot.* **2000**, *63*, 1338–1346. [[CrossRef](#)] [[PubMed](#)]
45. Min, B.J.; Han, I.Y.; Dawson, P.L. Antimicrobial Gelatin Films Reduce *Listeria monocytogenes* on Turkey Bologna 1. *Poult. Sci.* **2010**, *89*, 1307–1314. [[CrossRef](#)] [[PubMed](#)]
46. Beverly, R.L.; Janes, M.E.; Prinyawiwatmula, W.; No, H.K. Edible Chitosan Films on Ready-to-Eat Roast Beef for the Control of *Listeria monocytogenes*. *Food Microbiol.* **2008**, *25*, 534–537. [[CrossRef](#)]
47. Ojagh, S.M.; Rezaei, M.; Hadi Razavi, S.; Hashem Hosseini, S.M. Effect of Chitosan Coatings Enriched with Cinnamon Oil on the Quality of Refrigerated Rainbow Trout. *Food Chem.* **2010**, *120*, 193–198. [[CrossRef](#)]
48. Meade, E.; Slattery, M.A.; Garvey, M. Bacteriocins, Potent Antimicrobial Peptides and the Fight against Multi Drug Resistant Species: Resistance Is Futile? *Antibiotics* **2020**, *9*, 32. [[CrossRef](#)]
49. Jack, R.W.; Wan, J.; Gordon, J.; Harmark, K.; Davidson, B.E.; Hillier, A.J.; Wettenhall, R.E.H.; Hickey, M.W.; Coventry, M.J. Characterization of the Chemical and Antimicrobial Properties of Piscicolin 126, a Bacteriocin Produced by *Carnobacterium piscicola* JG126. *Appl. Environ. Microbiol.* **1996**, *62*, 2897–2903. [[CrossRef](#)]
50. Karamah, E.F.; Ismaningtyas, N.; Putri Ilmiyah, A. The Effect of Exposure Time and Water Replacement in the Application of Ozonated Water to Maintain the Quality of Tuna. *IOP Conf. Ser. Mater. Sci. Eng.* **2019**, *509*, 012092. [[CrossRef](#)]
51. Chan, S.S.; Jakobsen, A.N.; Roth, B.; Jessen, F. Water Holding Properties of Atlantic Salmon. *Compr. Rev. Food Sci. Food Saf.* **2021**, *21*, 477–498. [[CrossRef](#)]
52. Kong, M.; Chen, X.G.; Xing, K.; Park, H.J. Antimicrobial Properties of Chitosan and Mode of Action: A State of the Art Review. *Int. J. Food Microbiol.* **2010**, *144*, 51–63. [[CrossRef](#)]
53. Duan, J.; Park, S.I.; Daeschel, M.A.; Zhao, Y. Antimicrobial Chitosan-Lysozyme (CL) Films and Coatings for Enhancing Microbial Safety of Mozzarella Cheese. *J. Food Sci.* **2007**, *72*, M355–M362. [[CrossRef](#)] [[PubMed](#)]

54. Masterson, K.; Major, I.; Lynch, M.; Rowan, N. Synergy Assessment of Four Antimicrobial Bioactive Compounds for the Combinational Treatment of Bacterial Pathogens. *Biomedicines* **2023**, *11*, 2216. [[CrossRef](#)] [[PubMed](#)]
55. Wan, J.; Harkmark, K.; Davidson, B.E.; Hillier, A.J.; Gordon, J.B.; Wilcock, A.; Hickey, M.W.; Coventry, M.J. Inhibition of *Listeria monocytogenes* by Piscicolin 126 in Milk and Camembert Cheese Manufactured with a Thermophilic Starter. *J. Appl. Microbiol.* **1997**, *82*, 273–280. [[CrossRef](#)] [[PubMed](#)]
56. Acedo, J.Z.; Towle, K.M.; Lohans, C.T.; Miskolzie, M.; McKay, R.T.; Doerksen, T.A.; Vederas, J.C.; Martin-Visscher, L.A. Identification and Three-Dimensional Structure of Carnobacteriocin XY, a Class IIb Bacteriocin Produced by Carnobacteria. *FEBS Lett.* **2017**, *591*, 1349–1359. [[CrossRef](#)]
57. De Niederhäusern, S.; Camellini, S.; Sabia, C.; Iseppi, R. Antilisterial Activity of Bacteriocins Produced by Lactic Bacteria Isolated from Dairy Products. *Foods* **2020**, *9*, 1757. [[CrossRef](#)]
58. Regiane, L.F.; Ka, R.; Furlaneto, C. Antimicrobial Activity of Enterocins against *Listeria* sp. and Other Food Spoilage Bacteria. *Biotechnol. Lett.* **2020**, *7*, 797–806. [[CrossRef](#)]
59. Martin-visscher, L.A.; Yoganathan, S.; Sit, C.S.; Lohans, C.T.; Vederas, J.C. The Activity of Bacteriocins from *Carnobacterium maltaromaticum* UAL307 against Gram-Negative Bacteria in Combination with EDTA treatment. *FEMS Microbiol. Lett.* **2011**, *317*, 152–159. [[CrossRef](#)]
60. Gonzales-Barron, U.; Cadavez, V.; De Oliveira Mota, J.; Guillier, L.; Sanaa, M. A Critical Review of Risk Assessment Models for *Listeria monocytogenes* in Produce. *Foods* **2024**, *13*, 1111. [[CrossRef](#)]
61. Heir, E.; Jensen, M.R.; Aasli, A.W.; Berget, I.; Holck, A.L. Reduction and Growth Inhibition of *Listeria monocytogenes* by Use of Anti-Listerial Nisin, P100 Phages and Buffered Dry Vinegar Fermentates in Standard and Sodium-Reduced Cold-Smoked Salmon. *Foods* **2023**, *12*, 4391. [[CrossRef](#)]
62. Chen, R.; Skeens, J.W.; Wiedmann, M.; Guariglia-Oropeza, V. The Efficacy of Nisin against *Listeria monocytogenes* on Cold-Smoked Salmon at Natural Contamination Levels Is Concentration-Dependent and Varies by Serotype. *Front. Microbiol.* **2022**, *13*, 930400. [[CrossRef](#)]
63. Leistner, L. Basic Aspects of Food Preservation by Hurdle Technology. *Int. J. Food Microbiol.* **2000**, *55*, 181–186. [[CrossRef](#)]

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