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Research Article

Effects of Bioactive Packaging Films Incorporated with Bifidocin A on Microbial Reduction and Quality Parameters of Chill-Stored Spanish Mackerel (*Scomberomorus niphonius*) Fillets

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To evaluate the potential of bifidocin A for preservation of fresh Spanish mackerel fillets, the bioactive packaging films incorporated with bifidocin A (1 × minimal inhibition concentration (MIC), 2 × MIC and 4 × MIC) were developed, and their effects on the microbiological and physicochemical properties and sensory profile of mackerel fillets at refrigerated storage were investigated. Results showed that the incorporation of bifidocin A in cellulosic matrix films did not affect the thickness and elongation of the films, but reduced slightly the tension strength. The films incorporated with 2 × MIC and 4 × MIC bifidocin A presented a broad spectrum of activity against most tested bacteria, including some fish-borne specific spoilage bacteria such as *Pseudomonas fluorescens*, *Shewanella putrefaciens*, *Brochothrix thermosphacta*, and *Micrococcus luteus*, and maintained their 100% activity for 28 days during storage at 4°C. The bioactive packaging films incorporated with bifidocin A could generally suppress the growth of microflora, especially *Pseudomonas* and *Enterobacteriaceae*, as well as substantially inhibit the accumulation of total volatile basic nitrogen (TVB-N), thiobarbituric acid reactive substances (TBARS) and hypoxanthine, during chilled mackerel fillets storage. Overall, from a microbiological and physicochemical point of view, a much more effective treatment was achieved with 4 × MIC bifidocin A, extending the shelf life to 12 days and maintaining the relatively low TVB-N value (≤13.2 mg/100 g), TBARS value (≤0.45 mg MDA/kg), and *K*-value (≤32.8%), as well as a relatively high sensory score (≥7.1) during the whole storage. Hence, the bioactive packaging films incorporated with bifidocin A could be a promising hurdle technology and alternative to conventional processes used for improving the safety and quality of chilled mackerel fillets.

1. Introduction

Spanish mackerel (*Scomberomorus niphonius*) is a major marine fish and is widely distributed in the Bohai Sea and East China Sea. The fish constitutes desirable components of a healthy diet, including polyunsaturated fatty acids, essential amino acids, vitamins, and minerals and is deeply loved by consumers and producers in China. However, because of its high nutritive content, fresh mackerel fillets are highly perishable even under refrigerated storage [1].

The various techniques, including addition of chemical preservatives [2, 3], modified atmosphere packaging [4], high hydrostatic pressure [5], vacuum packing [6], and dielectric barrier discharge [7], have been introduced to preserve fish fillets. With regard to the microbial contamination of fish fillets occurring primarily at the surface, due to postprocessing handling, the use of bioactive packaging films containing antimicrobial agents could be more efficient, by slow migration to the food surface, thus helping to maintain high concentrations where they are needed [8].

Bacteriocins are safe, natural antimicrobial peptides for food preservation, and some of them present high potential to inhibit the growth of fish-borne spoilage or pathogenic bacteria [9–14]. During the last decades, several innovative bioactive packaging films enriched with bacteriocins have been developed and have received a considerable attention

for improving the safety of food products, such as goat milk, fresh beef, raw sliced pork, white semifirm cheese, frozen shrimp, sliced ham, and sausage [15–22]. However, at present there are no studies available that evaluated the antimicrobial packaging film containing bacteriocin on microbial reduction and quality parameters during the storage of a marine fish.

Bifidocin A, a novel 1198.68 kDa bacteriocin, is produced by *Bifidobacterium animalis* BB04, isolated from a centenarian's faeces. The potential of bifidocin A to control food-borne spoilage and pathogens has been reviewed and underlined in our recent work [9]. It exhibits inhibitory activity against a broad range of Gram-positive and Gram-negative bacteria in the genera *Escherichia*, *Listeria*, *Salmonella*, *Pseudomonas*, *Staphylococcus*, *Bacillus*, *Clostridium*, *Brochothrix*, *Shewanella*, *and Micrococcus*, especially *Pseudomonas fluorescens*, *Shewanella putrefaciens*, *Brochothrix thermosphacta*, and *Micrococcus luteus*, which were fish-borne specific spoilage bacteria.

The current study was designed to develop a bioactive packaging film incorporated with bifidocin A and evaluate its application feasibility in chilled Spanish mackerel (*Scomberomorus niphonius*) fillets. The physical properties and antimicrobial activity of the films incorporated with bifidocin A was evaluated, and their effects on the microbiological and physicochemical properties and sensory profile of mackerel fillets at refrigerated storage for up to 15 days were investigated.

2. Materials and Methods

2.1. Bacterial Culture and Growth Condition. The bacteriocin producer *B. animalis* BB04 was incubated anaerobically in de Man Rogosa and Sharpe (MRS) broth (LuQiao, Beijing, China) supplemented with 0.05% (w/v) L-cysteine hydrochloride (Sigma-Aldrich, Shanghai, China) (MRS-C) at 37°C. The bacteriocin-sensitive *Pseudomonas fluorescens* CGMCC 1.55 was used as the indicator strain for estimating bacteriocin activity and grown in Luria-Bertani (LB) medium (LuQiao, Beijing, China) at 30°C.

2.2. Preparation of Partially Purified Bacteriocin and Quantification of Bacteriocin Activity

2.2.1. Bacteriocin Production in 5 L Fermentor. A 5-liter Biostat B fermentor (B. Braun Biotech International, Melsungen, Germany) containing 3.5 liters of MRS-C medium with the constant temperature (37°C), and pH 6.5 was used to study the kinetics of *B. animalis* BB04 growth and bacteriocin production. Aerating nitrogen and slow agitation (100 rpm) was maintained to keep the anaerobic environment and the fermentation broth homogenous, respectively. At appropriate time intervals, samples were taken for determining cell growth by the viable cell count and bacteriocin production by the antimicrobial activity. The antimicrobial activity was defined as the reciprocal of the highest serial two-fold dilution producing a distinct inhibition of *P. fluorescens* CGMCC 1.55 and expressed in terms of arbitrary units per milliliter (AU/mL).

2.2.2. Partial Purification of Bacteriocin. Partially purified bifidocin A was obtained from the above 24 h old culture of *B. animalis* BB04 by pH-mediated adsorption and desorption onto/from producer cells, as described previously [9]. Firstly, the cultures were adjusted to pH 8.0 and stirred overnight at 4°C. After being harvested (10,000 × g, 10 min) and washed twice with sterile 20 mM phosphate buffer (pH 8.0), the cells were resuspended in 200 mL of 100 mM·NaCl (pH 3.0) and agitated for 24 h at 4°C. Then, the supernatant including bacteriocin was obtained by centrifugation (10,000 × g, 15 min). Finally, the bacteriocin samples were determined for antimicrobial activity as described above, and then they were lyophilized and stored at −20°C before further experiment.

2.2.3. Minimal Inhibition Concentration (MIC) Determination. The MIC of partially purified bifidocin A against *P. fluorescens* CGMCC 1.55 was determined as described by Liu et al. [9]. The MIC was defined as the lowest concentration of partially purified bifidocin A at which the growth of the indicator strain was inhibited completely.

2.3. Preparation of Bioactive Films Incorporated with Bifidocin A. Cellulose acetate, acetone, and partially purified bifidocin A were employed on film production. The films were prepared by the casting process, according to Santiago-Silva et al. [21], with some modifications. Briefly, a cellulose acetate solution was firstly prepared by adding the flakes of cellulose acetate to acetone in a proportion of 1 : 10 (10% w/v) and left to stand for about 12–18 h. And then, the bifidocin A was incorporated in the cellulosic emulsion with the final concentrations ranging from 1 × MIC to 4 × MIC. After that, the mixture was homogenized and spread on glass plates, previously cleaned with acetone. Finally, the films were removed from the plates, after evaporation of the solvent under ambient conditions, and were stored in packages of polyethylene (PE) at 4°C for further analysis. A film without bifidocin A incorporation was used as the negative control.

2.4. Physical Properties of Bioactive Films Incorporated with Bifidocin A. Films were characterized in terms of their thickness and mechanical properties. The thickness of the film samples was determined using a thickness gauge (CH-1-ST, China) with a precision of 0.01 mm. Five thickness-measured values were randomly taken from each film, and the average of all measurements was used in the calculations of mechanical properties. The tensile strength and elongation at break were determined with a slight modification using a Universal Testing Machine (UTM2502, China) as described by Woraprayote et al. [17]. The initial grip length and cross-head speed of ten film samples ($10 \times 150\ mm^2$) were set at 100 mm and 5 mm/min, respectively. Tensile strength value was calculated by dividing the maximum stress by cross-sectional area of the specimen, and elongation values were expressed as percent units, with the ratio of extended length at break point of initial length.

2.5. Evaluation of the Antimicrobial Activity of the Films Incorporated with Bifidocin A

2.5.1. Spectrum of Antimicrobial Activity. The spectrum of activity of the antimicrobial films incorporated with/without bifidocin A (1 × MIC, 2 × MIC and 4 × MIC) was determined, respectively, against the selected food-borne spoilage and pathogenic strains (Table 1) by using an adaptation of the agar diffusion method [23]. Fragments of 1 cm^2 of each film were cut, sterilized under UV light, and placed over solidified LB/TSYEB/TSB agar. The agar was previously inoculated with each bacteria (Table 1) in order to obtain a lawn (10^7–10^8 cfu/mL) of the strain. After 24 h incubation at 30°C, the presence of clear zone of inhibition was evaluated.

2.5.2. Stability of Antimicrobial Activity. To evaluate the stability of antimicrobial activity, the films, incorporated with bifidocin A (2 × MIC and 4 × MIC), were sterilized under UV light for 15 min each side and incubated at 4°C and 25°C for up to 28 days. The residual activity was then assayed against the indicator strain *P. fluorescens* CGMCC 1.55 at two days interval and reported as a percentage of antimicrobial activity of the samples after storage compared with that of time zero.

2.6. Chilled Mackerel Fillets Preparation and Sampling. Fresh mackerel was purchased from an aquatic product wholesale market in Beijing, China, in June 2017, and was immediately transported to the laboratory alive. Fish were killed by a blow to the head, scaled, gutted, filleted, and washed within 2 h. After washing, fish fillets were left to drain on sterile stainless steel wire mesh for 10 min. The average weight for each fillet was 100 g. And then, these fish fillets were divided into three groups. The treatment groups were packed by the bioactive films incorporated with 2 × MIC and 4 × MIC bifidocin A, respectively. The fish fillets samples packed using the films without bifidocin A incorporation were used as the negative control. Packed fish fillets were stored at 4°C in refrigerators for up to 15 days. Three fillets were selected randomly from each group for analysis every three days.

2.7. Microbiological Analyses. At each selected time, 10 g of each sample was taken aseptically and homogenized in 90 mL 0.1% (w/v) sterile peptone water containing 1% (v/v) Tween 80 and 0.85% (w/v) NaCl. After disintegration for 2 min in a Stomacher (LAB-BLENDER, 400, London, UK), the resulting suspension was serially diluted in 10-fold and then plated in triplicate on total count and selective agar plates. Total viable counts (TVCs) were determined on plate count agar (PCA, Difco), incubated at 30°C for 48 h. *Enterobacteriaceae* were enumerated on violet red bile glucose agar (VRBGA, Difco), incubated at 37°C for 48 h. *Pseudomonads* were enumerated on cephaloridine-fucidin-cetrimide agar (CFC, Difco), incubated at 30°C for 48 h. H_2S-producing bacteria survival was monitored by enumeration on iron agar (IA, Difco), incubated at 25°C for 3 days. *Photobacteria* were enumerated on conductance medium (BCM, Difco), incubated at 15°C for 5 days.

Table 1: Thickness, tensile strength, and elongation at break of the films incorporated without/with 1 × MIC, 2 × MIC, and 4 × MIC bifidocin A.

Treatments	Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)
Control	0.66 ± 0.02^a	7.17 ± 0.26^a	3.64 ± 0.15^a
1 × MIC	0.66 ± 0.01^a	5.68 ± 0.42^b	3.59 ± 0.23^a
2 × MIC	0.69 ± 0.02^a	5.46 ± 0.31^b	3.38 ± 0.13^a
4 × MIC	0.71 ± 0.03^a	4.89 ± 0.25^c	3.20 ± 0.19^a

Values are expressed as means ± standard deviation. Different superscripts (a, b, and c) in the same column indicate significant difference ($P < 0.05$).

2.8. Physicochemical Analyses. At each selected time, 10 g of each sample was blended with 20 mL of distilled water in a blender for 30 s, and the pH value of homogenate filtrate was determined using a HI 8420 Microcomputer pH meter (Hanna instruments, Germany). Total volatile basic nitrogen (TVB-N) was measured using a microtitration methodology including distillation in a Foss 2300 Kjeldahl Analyzer Unit (Foss Tecator AB, Sweden) and titration with hydrochloric acid. TVB-N value was determined according to the consumption of hydrochloric acid and was expressed as mg/100 g flesh [24]. Thiobarbituric acid reactive substances (TBARS) value was determined according to the method of Shi et al. [25] and was expressed as mg malondialdehyde (MDA)/kg flesh. *K*-values were determined according to the method of Huang et al. [26].

2.9. Sensory Evaluation. Sensory analysis was carried out by nine trained panelists from the laboratory staff according to the method of Duman and Özpolat [27] with minor modification. Appearance, odor, texture, and overall quality were scored on a 9-point descriptive scale as follows: 8.0–9.0 = good, 6.0–7.9 = acceptable, 4.0–5.9 = unacceptable, and 1.0–3.9 = completely spoiled.

2.10. Statistical Analysis. All experiments were conducted at least in triplicate (except microbiological analyses, which were performed in duplicate). Data were expressed as means ± standard deviation, analysis of variance was used to test for differences on SPSS 20.0, least significant difference (LSD) test for multiple comparison at the 0.05 level of significance.

3. Results and Discussion

3.1. Partially Purified Bacteriocin Preparation and Bacteriocin Activity Assay. Cell growth and bacteriocin production properties of *B. animalis* BB04 were measured at 37°C in 5 L fermentor with controlled pH. As shown in Figure 1, strain BB04 started to produce bacteriocin (70 AU/mL) at 12 h during the early exponential growth phase. The maximal bacteriocin production (640 AU/mL) was observed after

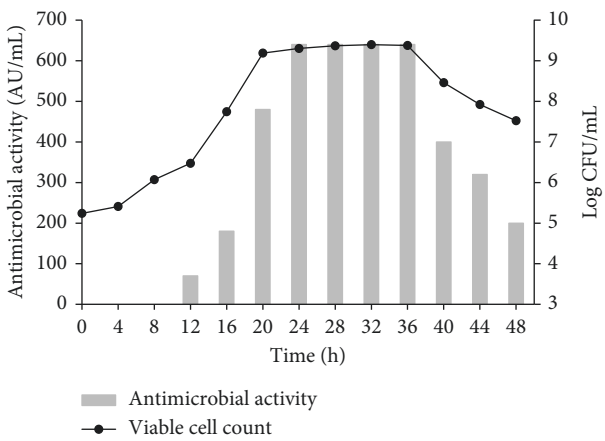

Figure 1: Production of bacteriocin by *B. animalis* BB04 in 5 L fermentor with controlled pH at 37°C.

24–36 h of incubation during the stationary phase, and then the activity decreased significantly in the following 12 h. Each 64.13 mg of partially purified bifidocin A powder was obtained from every above 24 h old culture of *B. animalis* BB04 by adsorption and desorption onto/from producer cells, and the specific activity was 798.38 AU/mg. The MIC of bifidocin A against *P. fluorescens* CGMCC 1.55 was 0.13 mg/mL. Like other bacteriocins, the MIC value of bifidocin A against *P. fluorescens* was maintained at a relatively low concentration level [28], which indicated this bacteriocin has great potential as effective and natural preservative in food industry.

3.2. Physical Properties of Bioactive Films Incorporated with Bifidocin A. Bioactive films must possess adequate properties to maintain durability and withstand the external stresses that prevail during food processing, handling, and storage. As shown in Table 1, the incorporation of bifidocin A in cellulosic matrix films did not affect the thickness and elongation values of the films ($P \geq 0.05$). However, there was a slight difference ($P < 0.05$) in tensile resistance between the films with bifidocin A and the film control. The films incorporated with 1 × MIC, 2 × MIC, and 4 × MIC bifidocin A showed a reduction in the tension strength value in relation to the control film. This may be ascribed to low interaction between bifidocin A and the polymer, allowing the formation of aggregates. Similar results were obtained by Gouvêa et al. [29] on basis of acetate cellulose film with added bacteriophages, which showed reductions in the tensile resistance as compared to the control.

3.3. The Antimicrobial Activity of the Bioactive Films Incorporated with Bifidocin A. The films incorporated without bifidocin A did not exhibit the inhibitory activity against tested bacteria (data not shown). The antimicrobial spectrum of the films incorporated with bifidocin A is presented in Table 2. The films incorporated with 2 × MIC and 4 × MIC bifidocin A showed a broad spectrum of activity against most tested bacterial strains, which is in consistence with the antimicrobial activity of bacteriocin only as described previously [9]. However, for the treatments at 1 × MIC, the films were not effective against *Shewanella putrefaciens* CGMCC 1.6515, *S. piezotolerans* CGMCC 1.6160, *Aeromonas hydrophila* CICC 10868, *Salmonella enteritidis* ATCC 13076, *S. enteritidis* CGMCC 1.106031, and *S. aureus* CGMCC 1.169. A similar observation has been made by de Lima Marques et al. [30]. This probably related to the relatively low migration level of bacteriocin transferred from the packaging material to the solidified medium.

The films incorporated with 2 × MIC and 4 × MIC bifidocin A maintained their 100% activity against *P. fluorescens* CGMCC 1.55 for 28 days during storage at 4°C. However, the bacteriocin activity could not be detected after incubation for 18 days at 25°C. The residual activity of the treatments with 2 × MIC and 4 × MIC bifidocin A was 36% and 41%, respectively, after 15 days of incubation at 25°C. These results indicated the bioactive films incorporated with bifidocin A are more suitable for preservation of chilled food.

Based on these result, additional studies assessing the effect of bioactive films incorporated with bifidocin A on chilled mackerel fillets were performed at 2 × MIC and 4 × MIC concentration.

3.4. Microbiological Analyses. As shown in Figure 2(a), the initial TVC in control and treatment groups were 3.36, 2.54, and 2.43 log CFU/g, respectively. The TVC increased with storage time and reached up to 7.93 log CFU/g on the 9th day for the control samples, which exceeded the maximum acceptable level of 7.0 log CFU/g for freshwater and marine fish [6, 26, 27]. Compared with the control, all the treatments with bifidocin A significantly reduced the initial bacteria counts ($P < 0.05$) and caused inhibition of the growth of bacteria in mackerel fillets and extension of the shelf life under storage at 4°C. The treatment with 4 × MIC bifidocin A was much more effective and prolonged the chilled shelf life of mackerel fillets to 12 days. Lv et al. [14] reported that the addition of bacteriocin DY4-2 produced by *Lb. plantarum* DY4-2 in turbot (*Scophthalmus maximus*) fillets could achieve a microbiological shelf life extension of 4 days. And Gui et al. [11] reported that the addition of paraplantaricin L-ZB1 produced by *Lb. paraplantarum* L-ZB1 could extend the shelf life of chilled rainbow trout fillets to 10 days. These results demonstrated that bacteriocin from lactic acid bacteria may be considered as a suitable candidate for its application as fresh fish biopreservative.

Compared with the control, all treatments with bifidocin A significantly inhibited the growth of *Pseudomonas* bacteria during the whole storage ($P < 0.05$) (Figure 2(b)). Moreover, *Pseudomonas* bacteria were first detected on the 3rd day in bifidocin A treatment groups. These results suggested that the films incorporated with bifidocin A had a better inhibitory effect on *Pseudomonas* bacteria than others, which correlated well with the high inhibition activity of bifidocin A for *P. fluorescens* as described previously. However, there were no significant differences in *Pseudomonas* bacteria

Table 2: Antimicrobial spectrum of the films incorporated with bifidocin A.

Indictor strains	Source[a]	Media	Sensitivity[b]		
			1 × MIC	2 × MIC	4 × MIC
Gram-negative bacteria					
Pseudomonas fluorescens 1.55	CGMCC	LB	+	+++	+++
P. fluorescens 1.1802	CGMCC	LB	+	+++	+++
Shewanella putrefaciens 1.6515	CGMCC	LB	−	+	++
S. piezotolerans 1.6160	CGMCC	LB	−	−	+
Aeromonas hydrophila 10868	CICC	LB	−	+	++
Salmonella enteritidis 13076	ATCC	LB	−	+	++
S. enteritidis 1.106031	CGMCC	LB	−	+	++
Escherichia coli 1.90	CGMCC	LB	+	+++	+++
E. coli 80739	ATCC	LB	+	+++	+++
Gram-positive bacteria					
Brochothrix thermosphacta 11509	ATCC	LB	+	++	++
Clostridium butyricum 1.5205	CGMCC	LB	+	++	+++
Micrococcus luteus 1.193	CGMCC	LB	+	+++	+++
Listeria monocytogenes 35152	ATCC	TSYEB	+	+++	+++
L. monocytogenes 54002	CMCC	TSYEB	+	+++	+++
Staphylococcus aureus 1.128	CGMCC	TSB	+	++	+++
S. aureus 1.169	CGMCC	TSB	−	+	++
Bacillus subtilis 1.7740	CGMCC	LB	+	++	++
B. cereus 1.229	CGMCC	LB	+	++	+++

[a]CGMCC, China General Microbiological Culture Collection Center; CICC, China Center of Industrial Culture Collection; ATCC, American Type Culture Collection; CMCC, National Center for Medical Culture Collections. [b]Inhibition zone (mm): +++, >21 mm; ++, 16–20 mm; +, 1–15 mm; −, no inhibition.

counts between the treatments with 2 × MIC and 4 × MIC bifidocin A during the first 6 days ($P \geq 0.05$).

Another more evident effect of bifidocin A treatment group was shown on *Enterobacteriaceae* bacteria (Figure 2(c)). Compared to the control, a significant decline in *Enterobacteriaceae* counts was observed in bifidocin A-treated samples ($P < 0.05$). This may be attributed to the high inhibition activity of bifidocin A for *Salmonella* and *Escherichia*. Samples treated with 4 × MIC bifidocin A had a lower *Enterobacteriaceae* counts of 3.02 log CFU/g on the 15th day compared to 3.58 (2 × MIC bifidocin A), which indicated that the treatment with 4 × MIC bifidocin A more efficiently inhibited the growth of *Enterobacteriaceae* bacteria than did the 2 × MIC bifidocin A treatment ($P < 0.05$).

In addition, the growth rate of other tested bacteria, including *Photobacterium* and H_2S-producing bacteria, was slightly lower in bifidocin A-treated groups when compared with the control group (Figures 2(d) and 2(e)). This may be related to the wide range of antimicrobial activity of bifidocin A used in this study.

The potential of the addition of bacteriocin alone to control undesirable microbes in fish product has been evaluated by a number of research groups in recent years [10–14]. However, in order to comply with actual tendency of consumers of searching for healthier foods, minimally processed and free of additives, the addition of preservatives directly to foods should be avoided as far as possible [8, 21]. In this study, we developed the antimicrobial potential of packaging films incorporated with bifidocin A to improve preservation of chilled mackerel fillets. This bioactive packaging could be a more promising preservation technology than bacteriocin alone, due to its action not only as a barrier against external agents but also by maintaining high concentrations bacteriocin on the food surface with a low migration [21].

3.5. Physicochemical Analyses. As shown in Figure 3(a), the pH value showed a rapid decrease in control and reached a minimum value of 6.04 in the first 6 days, due to the generation of an acidic substance by glycogen degradation. However, the pH increased in the further storage, which should result from alkaline substances such as ammonia and trimethylamine generated from protein degradation [10]. The change rate of pH value in the treatment groups was significantly lower than that in the control group ($P < 0.05$), which indicated the treatments with bifidocin A can effectively inhibit the degradation of glycogen and protein in chilled mackerel fillets.

TVB-N is the degradation product produced by proteins under the action of microorganisms and enzymes [24, 27]. As shown in Figure 3(b), the initial TVB-N level of all the fish fillets samples was 9.4–9.7 mg/100 g and no significant difference was observed after 3 days ($P \geq 0.05$). The TVB-N values increased gradually with the storage time, and the value of control had increased from 9.5 to 33.2 mg/100 g of fish fillets sample by day 15, had exceeded the maximum acceptable limit of 30 mg/100 g [6, 26, 27]. Compared with control, TVB-N values in all the treatments with bifidocin A could be controlled in a relatively low level (≤25.7 mg/100 g) during the whole storage. Besides, a higher decrease was observed in the treatment with 4 × MIC bifidocin A. This finding was similar to that of Ananou et al. [13] who observed sardine fillets treated with enterocin AS-48 produced by *Enterococcus faecalis* A-48-32 reduced the TVB-N content under refrigerated storage.

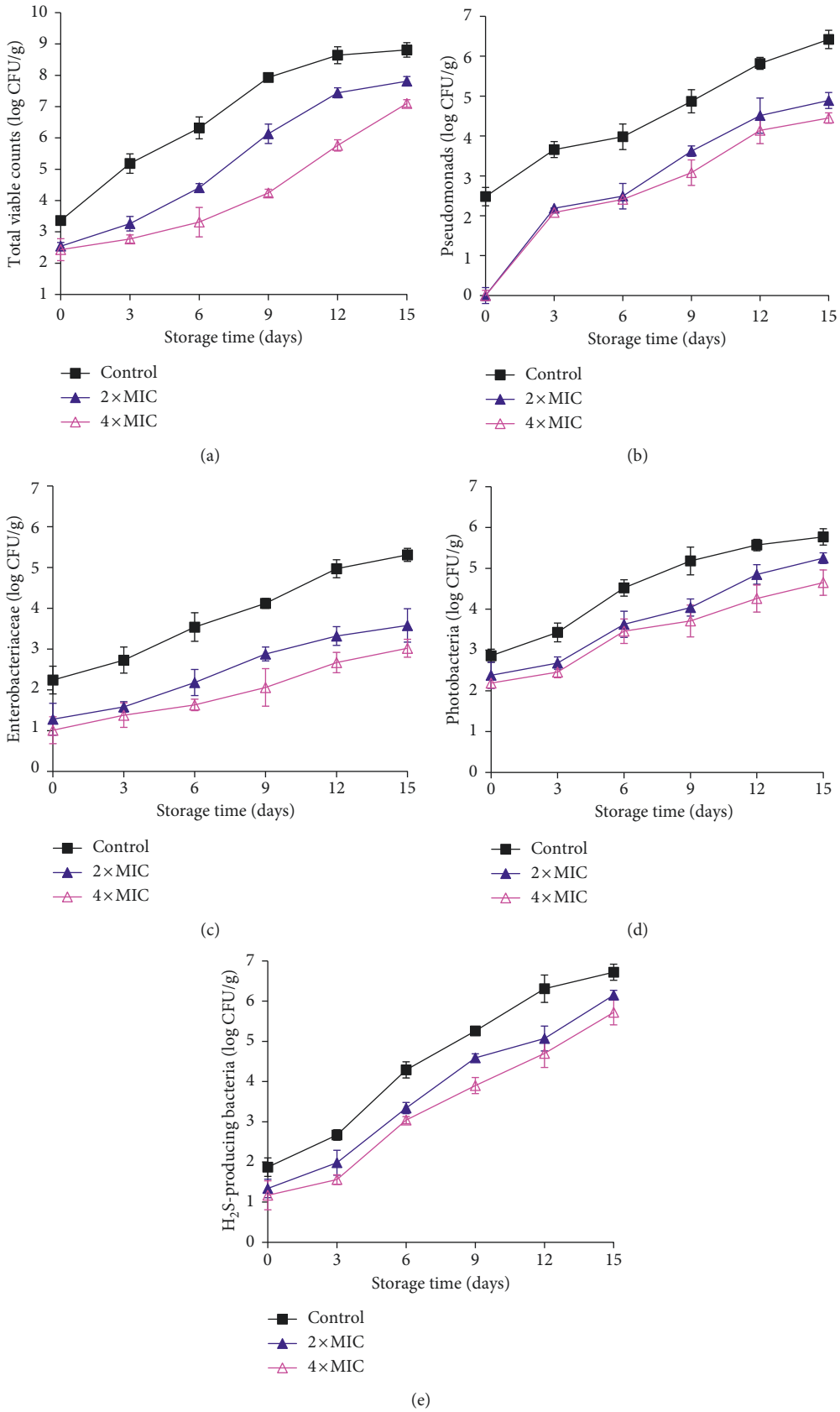

Figure 2: Changes in total viable counts (a), *Pseudomonas* (b), *Enterobacteriaceae* (c), *Photobacterium* (d), and H_2S-producing bacteria (e) of chilled mackerel fillets packed with the films incorporated without/with 2 × MIC and 4 × MIC bifidocin A.

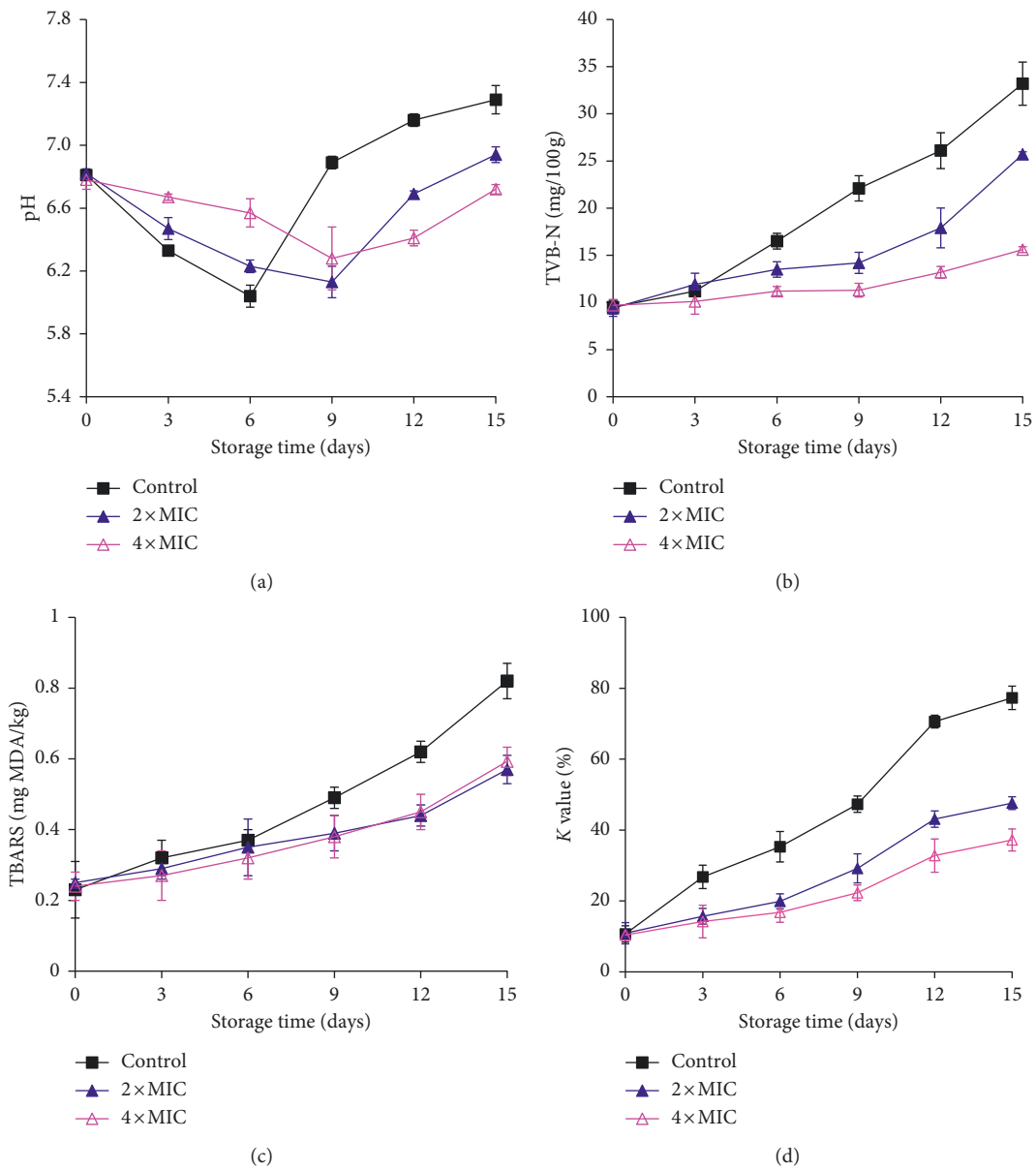

Figure 3: Changes in pH (a), TVB-N (b), TBARS (c), and K-value (d) of chilled mackerel fillets packed with the films incorporated without/with $2\times$MIC and $4\times$MIC bifidocin A.

TBARS is used widely to assess the degree of secondary lipid oxidation by measuring the content of MDA, which is the product formed by the reaction of polyunsaturated fatty acids with oxygen [25]. TBARS values of 1 mg MDA/kg are usually regarded as the limit for normal odor [10, 26]. The effect of the films incorporated with bifidocin A on TBARS values of chilled mackerel fillets is shown in Figure 3(c). Lower TBARS values were obtained for all the samples (<1 mg MDA/kg), which indicated low oxidative rancidity. The treatments with bifidocin A did not produce a significant effect on TBARS during the first 6 days. Compared to the control, the treatments with bifidocin A delayed the increase of TBARS ($P < 0.05$) after the fillets were stored for 9 days. However, no significant differences in TBARS values were observed between the treatments with $2\times$MIC and $4\times$MIC bifidocin A ($P \geq 0.05$).

K-value is widely used to evaluate the fish freshness [24, 26]. The initial K-values of the control and treatments with $2\times$MIC and $4\times$MIC bifidocin A were 10.7%, 10.9%, and 10.4%, respectively (Figure 3(d)). The K-values increased among all samples over time in this experiment. During almost the entire storage, the K-values of the treatments with bifidocin A continued below 70%, although control samples exceeded this limit on the 12th day (70.6%). These results indicated that the treatments with bifidocin A can effectively delay the degradation of inosine monophosphate (IMP) and the production of hypoxanthine (Hx) in chilled mackerel fillets.

From a technological point of view, it is important to determine the influence of bioactive packing films incorporated with bacteriocin on physicochemical parameters in the actual food system. Until now, nothing has been

Table 3: Changes in sensory scores of chilled mackerel fillets packed with the films incorporated without/with 2 × MIC and 4 × MIC bifidocin A.

Parameters	Treatments	0 days	3 days	6 days	9 days	12 days	15 days
Appearance	Control	$8.9 \pm 0.0^{A,e}$	$8.2 \pm 0.2^{A,d,e}$	$7.6 \pm 0.3^{A,d}$	$5.2 \pm 0.4^{A,c}$	$3.8 \pm 0.1^{A,b}$	$2.6 \pm 0.4^{A,a}$
	2 × MIC	$8.9 \pm 0.0^{A,e}$	$8.4 \pm 0.1^{B,d,e}$	$8.1 \pm 0.1^{B,d}$	$7.4 \pm 0.1^{B,c}$	$5.8 \pm 0.1^{B,b}$	$4.1 \pm 0.2^{B,a}$
	4 × MIC	$8.9 \pm 0.0^{A,d}$	$8.4 \pm 0.1^{B,c,d}$	$8.2 \pm 0.1^{B,c}$	$8.0 \pm 0.1^{C,c}$	$6.9 \pm 0.1^{C,b}$	$6.0 \pm 0.3^{C,a}$
Odor	Control	$8.8 \pm 0.1^{A,d}$	$8.3 \pm 0.1^{A,d}$	$7.3 \pm 0.2^{A,c}$	$5.9 \pm 0.2^{A,b}$	$3.6 \pm 0.4^{A,a}$	$3.3 \pm 0.1^{A,a}$
	2 × MIC	$8.9 \pm 0.0^{A,d}$	$8.5 \pm 0.2^{B,d}$	$8.0 \pm 0.1^{B,c,d}$	$7.7 \pm 0.1^{B,c}$	$5.9 \pm 0.1^{B,b}$	$4.5 \pm 0.2^{B,a}$
	4 × MIC	$9.0 \pm 0.0^{A,d}$	$8.5 \pm 0.3^{B,c,d}$	$8.1 \pm 0.1^{B,c}$	$8.0 \pm 0.1^{C,c}$	$7.2 \pm 0.1^{C,b}$	$6.2 \pm 0.3^{C,a}$
Texture	Control	$8.9 \pm 0.0^{A,e}$	$8.4 \pm 0.1^{A,e}$	$7.8 \pm 0.1^{A,d}$	$5.8 \pm 0.2^{A,c}$	$3.7 \pm 0.3^{A,b}$	$2.4 \pm 0.4^{A,a}$
	2 × MIC	$8.8 \pm 0.1^{A,e}$	$8.4 \pm 0.1^{A,d,e}$	$8.1 \pm 0.1^{B,d}$	$7.6 \pm 0.1^{B,c}$	$6.0 \pm 0.1^{B,b}$	$4.0 \pm 0.1^{B,a}$
	4 × MIC	$8.9 \pm 0.0^{A,d}$	$8.7 \pm 0.3^{B,c,d}$	$8.3 \pm 0.1^{B,c}$	$8.1 \pm 0.1^{C,c}$	$7.2 \pm 0.1^{C,b}$	$6.3 \pm 0.2^{C,a}$
Overall quality	Control	$9.0 \pm 0.0^{A,e}$	$8.3 \pm 0.1^{A,d}$	$7.6 \pm 0.3^{A,c}$	$5.5 \pm 0.2^{A,b}$	$3.4 \pm 0.2^{A,a}$	$3.2 \pm 0.2^{A,a}$
	2 × MIC	$8.9 \pm 0.0^{A,e}$	$8.5 \pm 0.1^{B,d,e}$	$8.1 \pm 0.1^{B,d}$	$7.5 \pm 0.3^{B,c}$	$5.9 \pm 0.2^{B,b}$	$4.4 \pm 0.2^{B,a}$
	4 × MIC	$8.9 \pm 0.0^{A,d}$	$8.6 \pm 0.2^{B,c,d}$	$8.2 \pm 0.1^{B,c}$	$8.0 \pm 0.1^{C,c}$	$7.1 \pm 0.1^{C,b}$	$6.1 \pm 0.3^{C,a}$

Values are expressed as means ± standard deviation. Different superscripts (A, B, and C) in the same column indicate significant difference ($P < 0.05$). Different superscripts (a, b, c, d, and e) in the same row indicate significant difference ($P < 0.05$).

reported on the effect of the films incorporated with bacteriocin on quality parameters of fish product. In this study, we described the influence of packaging films incorporated with bifidocin A on the pH, TVB-N, TBARS, and *K*-value of chilled mackerel fillets in detail. The results indicated that the bioactive packaging with bifidocin A effectively inhibits the accumulation of TVB-N, TBARS, and Hx during chilled mackerel fillets storage, which is basically consistent with the microbiological change, and achieve the similar effect as the addition of bacteriocin alone.

3.6. Sensory Evaluation. As shown in Table 3, sensory parameters (appearance, odor, texture, and overall quality) decreased during storage. The control group was able to maintain the acceptable quality at 4°C for approximately 6 days and spoiled completely in 12 days. However, the samples in treatments with 2 × MIC bifidocin A denoted as unacceptable quality by the sensory panel on the 12th day. The best organoleptic quality of mackerel fillets was observed in the treatment group with 4 × MIC bifidocin A, which could maintain a better sensory profile throughout the whole storage. Overall, the sensory score showed that the films incorporated with bifidocin A significantly improved the preservation of chilled mackerel fillets and prolonged their shelf life. A similar observation has been made by Fu et al. [10], who reported the large yellow croaker (*Pseudosciaena crocea*) fillets treated with coagulin L1208 could achieve a high sensory score and prolong the period of grade-A freshness up to 8 days at 4°C.

4. Conclusion

In this study, a novel antimicrobial packaging film by the incorporation of bifidocin A in the cellulosic matrix was successfully developed. The films incorporated with 2 × MIC and 4 × MIC bifidocin A presented a broad spectrum of activity *in vitro* against most of tested food-borne spoilage and pathogenic bacteria, and maintained their 100% activity for 28 days during storage at 4°C. The bioactive packaging films incorporated with bifidocin A could generally suppress the growth of microflora, especially *Pseudomonas* and *Enterobacteriaceae*, as well as substantially inhibit the accumulation of TVB-N, TBARS, and Hx, during chilled mackerel fillets storage. Overall, from a microbiological and physicochemical point of view, a much more effective treatment was achieved with 4 × MIC bifidocin A, extending the shelf life to 12 days and maintaining a better sensory profile during the whole storage. In summary, the present work demonstrates for the first time the potential of bifidocin A as biopreservatives for fresh marine fish during refrigerated storage. The bioactive packaging films incorporated with bifidocin A could be a promising hurdle technology and alternative to conventional processes used for extending the safety and quality of refrigerated mackerel fillets. In our laboratory, a detailed study on the mechanism of bifidocin A on the inactivation of fish-borne specific spoilage bacteria is currently underway.

Data Availability

The data in our manuscript have been deposited and shared in the Figshare database (https://doi.org/10.6084/m9.figshare.7306415; https://figshare.com/s/ebd074e1dcb08cb73e6b).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Mrs. Xue Li carried out preparation of partially purified bacteriocin and bacteriocin activity quantification guided by Dr. Guorong Liu. Ms. Xu Hao carried out the preparation of bioactive films incorporated with bifidocin A and evaluation of the antimicrobial activity of the films guided by Dr. Chengtao Wang. Mrs. Xue Li carried out the experimental design and the investigation of the effects of bioactive films on the microbiological and physicochemical properties and sensory profile of mackerel fillets at refrigerated storage guided by Dr. Baoguo Sun. The manuscript was written by Dr. Guorong Liu.

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