Effect of AHL-lactonase and nisin on microbiological, chemical and sensory quality of vacuum packaged sturgeon storage at 4°C

AHL-lactonase and nisin inhibit sturgeon spoilage

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ABSTRACT
Quorum sensing (QS) has been reported to be involved in bacterial food spoilage. Acetylated homoserine lactones (AHLs) are QS signals of Gram-negative bacteria. AHL lactonases AiiA196 is a QS inhibitor, and thus can theoretically be applied to control the Gram-negative bacteria caused sturgeon spoilage by inhibiting their AHL-based QS system. As Gram-negative bacteria and Gram-positive bacteria were both found in spoilage sturgeon, the combination of AiiA196 and nisin, which is an effective bactericide to control the Gram-positive bacteria, was used to control the bacteria spoilage of sturgeon. The results showed that AiiA196 at 6.6 × 10⁻², 6.6 × 10⁻¹ and 6.6 U/mL could suppress the growth of microflora and delayed the increase of TVB-N, sensory score and biogenic amine levels of sturgeon. In addition, 6.6 U/mL AiiA196 coupled with 2000 AU/mL nisin exhibited the strongest inhibition effect on spoilage and extended their shelf life by about 5 days. In conclusion, AiiA196 combined with nisin could be used as a suitable biological preservative for chilled sturgeon fillets.

Introduction
Sturgeon is farmed in China since 1990s and its production has reached to 80% of the total production of the world. However, the quality of sturgeon is a major concern to industry and consumers because the sturgeon deteriorates rapidly even under the condition of vacuum packaging and chilled storage, and that the shelf life of vacuum-packed minced sturgeon stored at 4°C was about only 4 days according to microbiological assessment. Fish deterioration is dominated by the growth of microorganisms. The micro-flora of spoilage sturgeon under vacuum packaging and chilled storage conditions was composed of Gram-negative bacteria such as Enterobacteriaceae and Aeromonas, and Gram-positive bacteria such as Lactobacillus spp. and Brochothrix thermosphacta. It is essential to apply some new preservation method to inhibit spoilage process of sturgeon and extend its shelf-life.

Quorum sensing (QS) is the mechanism used by the bacteria to respond to changes in population density and coordinate their behaviors by producing the signaling molecules and is widespread in bacteria including those commonly associated with food spoilage. It is reported that acetylated homoserine lactones (AHLs) are utilized by a diverse range of Gram-negative bacteria as QS signals, and several AHLs have been detected in bacteria from different aquatic products such as smoked salmon, fish fillets and minced fish. In our previous study, four different AHLs, C6-SHL, C8-HSL, 3-oxo-C8-HSL and 3-OH-C8-HSL, were identified from Aeromonas veronii LP-11, the specific spoilage organisms (SSO) of sturgeon stored at 4°C suggested QS was associated with sturgeon...
spoilage. Moreover, AHL 3-oxo-C6-HSL has been detected in Serratia proteamaculans B5a responsible for the proteolytic activity and spoilage of cold-smoked salmon. It was reported that adding exogenous AHLs into the shrimp increased the population percentages of SSO and the levels of TVB-N, thus accelerating the spoilage process. This accelerated spoilage is likely due to the enhanced QS regulation. Since all these reports indicate that AHL-based QS system is involved in bacterial aquatic product spoilage, it is rational to think that inhibition of the AHL-based QS system could be a potential way to prevent the bacterial spoilage. Quorum quenching enzymes such as AHL-lactonase which can degrade AHLs have been identified in some bacteria, and have shown considerable promise as quorum quenchers. AHL lactonase AiiA_{A196} which is from Bacillus, has a broad substrate spectrum specificity and was successfully applied in controlling bacterial infection in zebrafish. Thus, AHL lactonases such as AiiA_{A196} can theoretically be applied to control the spoilage Gram-negative bacteria in sturgeon by inhibiting their AHL-based QS system.

Nisin, produced by Lactococcus lactis subsp. lactis, is approved as the only bacteriocin by FDA/WHO. It has been shown that nisin kills bacteria primarily by forming pores in the cytoplasmic membrane and inhibiting peptidoglycan synthesis. Moreover, nisin can induce cell autolysis and inhibit the outgrowth of bacterial spores. Due to its unique functions against a wide range of Gram-positive bacteria, nisin plays an important role in food preservation, mainly meat and dairy products. Nisin combined with other natural preservatives possesses better quality enhancing abilities than nisin alone, and some nisin compounds exhibited good preservation effects on fish products such as Pseudoscaena crocea, snakefish and Trachinotus ovatus. Therefore, nisin can be an effective bactericide to control the Gram-positive bacteria in sturgeon.

Our previous study showed that spoilage sturgeon contains a large number of Gram-negative bacteria (>7 log CFU/g) and a certain number of Gram-positive bacteria (>6 log CFU/g). Therefore, the combination of AiiA_{A196} and nisin has the potential to control both Gram-negative and -positive bacteria, and maintain the quality of sturgeon (Figure 1). In this study, the effect of AiiA_{A196} combined with nisin treatment on the quality of vacuum-packaged sturgeon storage at 4°C was investigated. Microbial count, sensory evaluation, TVB-N and biogenic amines were examined as preservation evaluation indices. Our results indicated that 6.6 U/mL AiiA_{A196} coupled with 2000 AU/mL nisin...

Figure 1. The theoretical hypothesis of the combined inhibition of AHL-lactonase and nisin on sturgeon spoilage.
exhibited strong inhibition effect on microbial growth and spoilage, and extended the shelf life of samples by about 5 days.

Materials and methods

Sample preparation

Sturgeon weighing approximately 1.5 kg was purchased from Hui Longguan wholesale market in Beijing, China. The duration between catch and arrival of the fish at the laboratory was less than 4 hours where they were always kept in ice. Upon arrival, the whole fish were washed under sterile water, headed, gutted, filleted (approximately each fillet of weight 50 g) and rinsed again. Then, the sturgeon fillets were kept at 0–2°C until use.

Preparation and quantification of nisin and AHL Lactonases AiiA_{A196}

Nisin (Sigma-Aldrich, St. Louis, MO) was dissolved in 20 mM sodium phosphate buffer (pH 7.0). Lactobacillus plantarum pl-2 was used as the indicator strain in the bacteriocin assay. The nisin was serially diluted and 200 μL from each dilution was spotted onto a lawn of appropriate indicator bacteria. Activity units (AU) per milliliter were calculated as previously reported.[17]

AHL lactonases AiiA_{A196} was acquired by cloning the gene that responsible for the AHL lactonase activity of Bacillus sp. strain A196 and then expressed in Escherichia coli, and the enzyme was finally purified by Ni-NTA chromatography.[10] The purified AiiA_{A196} was donated by Zhigang Zhou (Chinese Academy of Agricultural Sciences, Beijing, China). AHL lactonase activity was determined as described previously.[10] One unit of AHL lactonase activity was defined as the amount of enzyme that hydrolyzed 1 nmol 3-oxo-C8-HSL per minute.

Treatment of fish samples

Sturgeon samples were randomly divided into eight treatment groups as shown in Table 1: (A) 20 mM sodium phosphate buffer (pH 7.0), control; (B) 2000 AU/mL nisin; (C) 6.6 × 10^{-2} U/mL AiiA_{A196}; (D) 6.6 × 10^{-1} U/mL AiiA_{A196}; (E) 6.6 U/mL AiiA_{A196}; (F) 6.6 × 10^{-2} U/mL AiiA_{A196} and 2000 AU/mL nisin; (G) 6.6 × 10^{-1} U/mL AiiA_{A196} and 2000 AU/mL nisin; (H) 6.6 U/mL AiiA_{A196} and 2000 AU/mL nisin. The eight groups were immersed in different preservative solutions for 3 min and drained for 15 s. Afterward, they were vacuum-packaged in polyacrylamide-polyethylene bags individually. Samples were analyzed immediately, and the data collected were denoted as day 0. The rest of the samples were then stored at 4°C and analyzed on days 3, 6, 8, 10, 12, 14. All samples were analyzed in triplicate.

| Group | Treatment conditions                                    |
|-------|--------------------------------------------------------|
| A     | 20 mM sodium phosphate buffer (pH 7.0)                 |
| B     | 2000 AU/mL nisin                                       |
| C     | 6.6 × 10^{-2} U/mL AiiA_{A196}                         |
| D     | 6.6 × 10^{-1} U/mL AiiA_{A196}                         |
| E     | 6.6 U/mL AiiA_{A196}                                   |
| F     | 6.6 × 10^{-2} U/mL AiiA_{A196} and 2000 AU/mL nisin    |
| G     | 6.6 × 10^{-1} U/mL AiiA_{A196} and 2000 AU/mL nisin    |
| H     | 6.6 U/mL AiiA_{A196} and 2000 AU/mL nisin             |
**Microbiological analysis**

Twenty-five grams of each sample were transferred aseptically to 225 ml of sterile physiological saline (0.85%, w/v) and homogenized for 60 s. For microbial enumeration, 0.1 ml samples of serial dilutions (1:10, diluent, 0.85% physiological saline) of fish homogenates were spread on the appropriate plates. Total viable bacterial counts and Psychrotrophs were determined using plate count agar (PCA, code HB0101, Qingdao Hope Biol-Technology Co., Ltd. China). For total viable counts (TVC), plates were incubated at 30°C for 2 days, while for Psychrotrophs, plates were kept at 7°C for 7 days, and then the colonies were counted. *Aeromonas* were investigated in ampicillin macconey agar base (AMA, code HB8576) at 28°C for 2 days. Enterobacteriaceae were investigated in violet red bile glucose agar (VRBGA, code HB0176) after incubation at 37°C for 24 h. Microbiological data were transformed to logarithms of the number of colony-forming units (log CFU/g). All plates were examined visually for typical colony types and morphology characteristics associated with each growth medium.

**Sensory analyses**

Sensory analyses were conducted by a taste panel consisting of six experienced judges, according to the method as previously described. Four levels (0–3) and four parameters (texture, odor, color and gapping) were used to evaluate the sensory of sturgeon fillets.

**TVB-N**

TVB-N value was measured by semi-micro steam distillation. The method was performed by distillation after adding MgO to the homogenized samples (10 g). TVB-N values were determined with Kjeldahl apparatus (KDY-9820, Beijing, China). The results are expressed as mg TVB-N per 100 g rainbow trout.

**Biogenic amines**

Putrescine, cadaverine, tryptamine, tyramine, histamine, phenethylamine, spermine and spermidine were extracted as previously described and stored at −20°C prior to analysis. Biogenic amines were analyzed by using a high performance liquid chromatographic (HPLC) method as previously described. Results are expressed as mg per kg sturgeon. Total level of biogenic amines (BA) was calculated as the sum of putrescine, cadaverine, tryptamine, tyramine, histamine, phenethylamine, spermine and spermidine.

**Statistical analyses**

Analyses were performed with the SPSS 13.0 statistics software (SPSS, Chicago, IL, USA). Descriptive statistics (mean and standard deviation), one-way ANOVA and Pearson correlation analysis were applied. The significance level was set at 0.05.

**Results**

**Microbiological analysis**

The changes in total viable counts of sturgeon fillets are shown in Figure 2a. All sturgeon samples with different treatments were divided into different groups denoted as A–H, as detail described in material and methods (2.3) (Table 1). All samples started with microbial counts less than 4 log CFU/g, suggesting that these samples were in good quality. If 7 log CFU g−1 are considered as the maximum level for acceptability of fish during the storage period (ICMSF, 1986), the shelf life was 3–6 days for untreated samples (group A), 6–8 days for group B, C, D and E, 8–10 days for group F and
G, and 10–12 days for group H. It could be concluded that the shelf life of group C, D and E was longer than that of A, indicating that AiiA<sub>AI96</sub> had an inhibitory effect on microbial growth in sturgeon fillets. Compared to group A, group B and E resulted in a shelf-life extension of about 2 days, and group H had the longest shelf-life extension of about 4 days. These results indicated that the antibacterial effective of 6.6 U/mL AiiA<sub>AI96</sub> was close to that of 2000 AU/mL nisin, and the combination of AiiA<sub>AI96</sub> and nisin had better antibacterial effect than AiiA<sub>AI96</sub> or nisin alone.

Aeromonas was reported to be the dominant spoilage bacteria in vacuum-packed chilled sturgeon.<sup>1</sup> The counts of Aeromonas of all sturgeon samples were below the detection limit of method (1 log CFU/g) at day 0 and significantly increased from day 0 to day 6 ($P < .05$) (Figure 2b). At day 6, Aeromonas counts reached to 5.39 log CFU/g for group A, while reached to 4.76, 4.61 and 4.50 log CFU/g for group B, C, and D, respectively, showing a reduction of less than 1 log CFU/g compared to group A. For group F, G and E, Aeromonas counts reached to 4.35, 4.16 and 3.69 log CFU/g, respectively, showing a reduction of more than 1 but less than 2 log CFU/g compared to group A at day 6. For group H, Aeromonas counts only reached to 2.97 log CFU/g, showing a reduction of more than 2 log CFU/g compared to group A at day 6. As shown in these results, the inhibition effect
of different groups against *Aeromonas* was $H > E > G > F > D > C > B$. Obviously, 6.6 U/mL AiiA$_{AI96}$ coupled with 2000 AU/mL nisin (group E) was most effective, followed by 6.6 U/mL AiiA$_{AI96}$ (group E). *Aeromonas* counts of group E were significantly lower ($P < .05$) than B during day 3 to 14, indicating *Aeromonas* in chilled sturgeon were more sensitive to 6.6 U/mL AiiA$_{AI96}$ than 2000 AU/mL nisin. These results indicated that AiiA$_{AI96}$ could significantly inhibit the growth of *Aeromonas*, and the incorporation of 2000 AU/mL nisin into AiiA$_{AI96}$ significantly enhanced the antimicrobial function of AiiA$_{AI96}$. During the whole storage, the counts of *Aeromonas* in group C and D were keeping nearly the same value ($P < .05$), indicating that the inhibition effect of *Aeromonas* was not enhanced with increasing the concentration of AiiA$_{AI96}$ from $6.6 \times 10^{-2}$ U/mL to $6.6 \times 10^{-1}$ U/mL.

Psychrotrophs were also reported to be significant part of the spoilage microflora in vacuum-packed chilled sturgeon.[1] The counts of Psychrotrophs for all sturgeon samples were significantly increased ($P < .05$) from day 0 to day 6 (Figure 2c), which was similar to the results of *Aeromonas*. At day 6, Psychrotrophs counts of group C, D and E were reduced 0.35, 0.67 and 1.41 log CFU/g, respectively, compared with that of the control group A. The results suggested that AiiA$_{AI96}$ could inhibit the growth of Psychrotrophs, and the inhibition effect was enhanced with increasing the concentration from $6.6 \times 10^{-2}$ to $6.6 \ U/mL$. The Psychrotrophs counts of group H were less than 5 log CFU/g during the whole storage and it was also the lowest value among all groups, indicating that 6.6 U/mL AiiA$_{AI96}$ combined with 2000 AU/mL nisin was the best effective treatment to inhibit the growth of Psychrotrophs.

Enterobacteriaceae were found to be responsible for spoilage of cold-stored fish such as sturgeon and large yellow croakers.[1][14] The Enterobacteriaceae counts of all groups were significantly increased ($P < .05$) during the storage from day 0 to day 8 (Figure 2d). At day 8, group C, D, and E showed significantly lower ($P < .05$) Enterobacteriaceae counts than that of A, and group E exhibited the lowest value, indicating that 6.6 U/mL AiiA$_{AI96}$ was more effective than that of the lower concentrations. During the storage from day 2 to 14 (except day 2 and day 14), Enterobacteriaceae counts of group H were always keeping the lowest value among those of all groups. These results indicated that AiiA$_{AI96}$ had inhibitory effect on Enterobacteriaceae growth, and the effect was enhanced when combining with 2000 AU/mL nisin.

**Sensory analysis**

The sensory scores of all samples undergoing different treatments started with 0 (absolutely fresh) and presented different increase patterns during storage (Figure 3). From day 2, all treatment groups gave lower sensory scores and showed better characteristics of texture, odor, color and gapping than control group A. The observed shelf life of sturgeon fillets was about 3–6 day for A, 6–8 day for B, C, D and E, 8–10 day for F and G, and 10–12 day for H, which was in accordance with the results of TVC. Based on sensory analyses, group H extended shelf life by approximately 5 days compared to A, which was the longest extension among all the groups. Interestingly, B and E showed no obvious difference ($P < .05$) in sensory during the whole storage, indicating that AiiA$_{AI96}$ treatment at 6.6 U/mL was similar to nisin treatment at 2000 AU/mL in maintaining good quality characteristics of sturgeon.

**TVB-N analysis**

The chemical spoilage of fish samples during storage was usually evaluated by measuring the changes in the content of TVB-N.[21] The initial TVB-N value of all samples was around 1.60 mg/100 g and then they kept significantly increasing ($P < .05$) during storage from day 0 to 12 (Figure 4). Group A showed much higher value than those of other treated groups ($P < .05$) after day 3, indicating TVB-N accumulation in sturgeon could be restrained by the AiiA$_{AI96}$ and/or nisin treatments. When group E reached sensory rejection at day 8, its TVB-N value (26.12 mg/100 g) was significantly lower than that of group C (42.50 mg/100 g) and D (40.77 mg/100 g), indicating that the inhibition effect of AiiA$_{AI96}$ on TVB-N was greatly enhanced when increasing the concentration of AiiA$_{AI96}$ from $6.6 \times 10^{-2}$ U/mL to
Interestingly, the TVB-N values of group F, G and H were significantly lower (P < .05) than C, D and E, respectively, from day 8 to day 14. These results indicated that when AiiA\textsubscript{AI96} was combined with nisin, the inhibition effect of AiiA\textsubscript{AI96} on TVB-N accumulation was enhanced.

**BA analysis**

BAs are significant compounds in fish safety and quality determination, since they can be used as indicators of freshness or microbial spoilage in fish.\textsuperscript{[22]} The BA values of all groups increased during the whole storage (Figure 4). At day 8 and day 10, among three AiiA\textsubscript{AI96} treated groups (C, D and E), only the BA value of group E was significantly lower than A (P < .05), while the other two groups showed no obvious difference (P > .05) compared with A, indicating that AiiA\textsubscript{AI96} with low concentrations (6.6 × 10\textsuperscript{-2} U/mL$\sim$6.6 × 10\textsuperscript{-1} U/mL) were ineffective but increasing concentration to 6.6 U/mL was effective to suppress BA increase. Group F, G and H had much higher (P < .05) BA values than C, D and E, respectively, from day 8 to the end of the storage, suggesting that incorporation of 2000 AU/mL nisin into AiiA\textsubscript{AI96} significantly enhanced the inhibition effect of AiiA\textsubscript{AI96} on BA, which was similar to the results of TVB-N. The BA value of group H was significantly lower than other groups during day 8 to 14, which confirmed that group H was the optimal treatment to delay the spoilage process of sturgeon.

**Discussion**

In recent years, more and more studies found that bacterial food spoilage may be regulated by QS systems, and QS inhibitors or antagonists were suggested as new food preservatives to extend food
shelf life. In our study, AiiA_{AI96}, an AHL-mediated QS inhibitor, could significantly \((P < .05)\) reduced the numbers of TVC (Figure 2a), and AiiA_{AI96} of 6.6 U/mL extended the shelf life of vacuum-packaged sturgeon fillets about 2 days (Figure 2a). These results suggested that QS inhibitor such as AiiA_{AI96} could be developed as a new food preservative to extend shelf life.

In addition to TVC, other microorganisms of *Aeromonas*, Psychrotrophs and Enterobacteriaceae in sturgeon were all inhibited by AiiA_{AI96} (Figure 2b-d). The inhibition effect of AiiA_{AI96} on these microorganisms naturally leads us to question that how this effect happened since AiiA_{AI96} was not an antibacterial. Previous studies reported that some *Aeromonas* and Enterobacteriaceae isolated from meat and vegetables could produce AHLs signals.\(^3\) In fact, four different AHLs have been extracted and identified in *Aeromonas veronii* isolated from sturgeon in our previous study.\(^6\) In addition, some researchers found that QS signals influence the growth kinetics of spoilage bacteria in food.\(^8,23,24\) Zhu et al. found that exogenous \(N\)-hexanoyl-homoserine lactone increased the growth rates and population percentages of the SSO in shrimp samples under refrigerated storage.\(^8\) Moreover, it was reported that exogenous cyclo-(L-Pro-L-Leu) shortened lag phase durations and enhanced growth rates of the dominant bacteria, \(H_2S\) producing bacteria, in *Pseudosciaena crocea* stored at 4°C.\(^23\) Dunstall et al. reported that N-benzyloxy carbonyl-\(L\)-homoserine lactone and \(N\)-3-oxyhexanoyl-\(DL\)-homoserine lactone reduced the lag phase duration and increased the exponential growth rate of *P. fluorescens* isolated from raw milk.\(^24\) Consequently, it can be deduced that the growth of the bacteria could be inhibited by blocking the QS signals. AHL lactonases AiiA_{AI96} was effective in catalyzing QS signals AHLs degradation, and it has the potential to suppress the growth of the AHL producing bacteria. In our study, the number of *Aeromonas*, Psychrotrophs and Enterobacteriaceae were reduced by AiiA_{AI96} treatment, and this was probably due to the blocking effect of AiiA_{AI96} on AHLs signals.
Levels of TVB-N, BA and sensory score are important quality parameters for the assessment of spoilage. As shown in Figure 3–5, compared with the control group A, 6.6 U/mL AiiA_{AI96} treatment could significantly reduce the production of TVB-N and BA and gave lower sensory scores. TVB-N was mainly composed of ammonia and amines, which results from the degradation of proteins, nonprotein nitrogenous compounds and endogenous enzymes, and was produced mainly by bacterial decomposition. Biogenic amines are nonvolatile compounds formed by microbial decarboxylation of amino acids. Since both TVB-N and BA are related to bacterial growth, the inhibition of TVB-N and BA accumulation in sturgeon was likely attributed to the slower growth of bacterial populations caused by 6.6 U/mL AiiA_{AI96} treatment. This was supported by the results that TVC, *Aeromonas*, Psychrotrophs and Enterobacteriaceae were all significantly suppressed by 6.6 U/mL AiiA_{AI96} (Figure 2a-d). In addition, it is reported that a number of the microbial extracellular enzymes causing food spoilage were regulated by QS, which suggested that QS could affect the spoilage capability of bacteria. Gu et al. found that the spoilage capability of *Pseudosciaena crocea* was significantly enhanced by supplement QS signals. Consequently, AiiA_{AI96} could weaken the spoilage capability of bacteria in sturgeon by hydrolyzing QS signals AHLs, thereby lower the contents of TVB-N and BA in sturgeon. Meanwhile, the better characteristics of texture, odor, color and gapping of 6.6 U/mL AiiA_{AI96} treatment than the control could also be attributed to the inhibition of bacterial growth and spoilage.

Nisin, produced by *Lactococcus lactis* subsp. *Lactis*, is active against Gram-positive organisms and is not generally active against Gram-negative bacteria. However, when it is associated with chelators, the activity of nisin against Gram-negative bacteria can increase. In our study, lower values of Gram-negative bacteria count of *Aeromonas* and Enterobacteriaceae were detected in 2000 AU/mL nisin treated group between day 6 and day 12 of storage. In the previous study, nisin prepared by solubilized in sodium phosphate buffer could also inhibit the growth of Gram-negative bacteria,

![Figure 5. Changes in biogenic amines of vacuum-packed sturgeon fillets with different treatments stored at 4°C. Group A (■) was a control group treated with 0.1 M pH 7.0 PBS; group B (○), were treated with 2000 IU/mL nisin; group C (●), D (▲) and E (□) were treated with $6.6 \times 10^{-2}$, $6.6 \times 10^{-1}$ and 6.6 U/mL AiiA_{AI96}, respectively; group F (○), G (△) and H (□) were treated with 2000 IU/mL nisin combined with $6.6 \times 10^{-2}$, $6.6 \times 10^{-1}$ and 6.6 U/mL AiiA_{AI96}, respectively.](image-url)
Enterobacteriaceae and Pseudomonas in rainbow trout stored at 4°C. This inhibition effect of nisin on Gram-negative bacteria may due to the synergistic action of nisin with phosphate, since it has been reported that trisodium phosphate increases the sensitivity of Escherichia coli, Pseudomonas fluorescens to nisin. Interestingly, group F, G and H presented lower TVC, Aermonas, Psychrotrophs and Enterobacteriaceae than C, D, and E, respectively, during the storage (Figure 2), indicating that nisin combined with AiiA_{AI96} showed enhanced antibacterial effect than AiiA_{AI96} alone. This may be due to the synergy antibacterial effect of AiiA_{AI96} and nisin. In addition, as Gram-positive bacteria such as Lactobacillus spp. and Brochothrix thermosphacta increased significantly in sturgeon during the spoilage process[1] and the high inhibition efficiency of nisin on the growth of Gram-positive bacteria, the presence of nisin could improve the inhibition effect on Gram-positive bacteria of AiiA_{AI96} and thus decreased the TVC of sturgeon.

Conclusion

The combination treatment of AiiA_{AI96} and nisin could maintain the quality of sturgeon better than that of AiiA_{AI96} or nisin treatment alone. Among all treatments, 6.6 U/mL AiiA_{AI96} coupled with 2000 AU/mL nisin was the most effective and could extend the shelf life of sturgeon by about 5 days. Consequently, it could be concluded that AiiA_{AI96} combined with nisin has the potential to be developed as a suitable biological preservative strategy for chilled vacuum-packaged sturgeon fillets.

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