Effect of antioxidant extracted from bamboo leaves on the quality of box-packaged sturgeon fillets stored at 4 °C

Xiao Ping Zhang[1,2,3], Xiao Yi Li[2,3], Ming Ju Yang[2,3], Xing Yang[2,3], Feng Zhao[2,3]

[1]Nanjing Agricultural University, Nanjing 210095, China; [2]Guizhou Academy of Agricultural Sciences, Guizhou fisheries research institute, Guiyang 550025, China; [3]Guizhou special aquatic products engineering technology center, Guiyang 550025, China

Corresponding Author: Xiao Ping Zhang, 306849590@qq.com

Received: 27 December 2019 / Accepted: 8 April 2020 / Published: 11 June 2020

RESEARCH ARTICLE

Abstract

The effect of antioxidant extracted from bamboo leaves (AOB) on the quality of sturgeon fillets during chilled storage was examined via three parameters: total volatile basic nitrogen (TVB-N), pH and bacterial community composition. The samples treated with 0.05% (w/v) AOB exhibited the best quality, as evidenced by retarding both pH changes and the production of TVB-N. *Pseudomonas* and *Aeromonas* were the dominant indigenous bacteria present in fresh sturgeon fillets, whereas the presence of *Janthinobacterium* and *Pedobacter* increased gradually throughout the storage process. A significant ($P < 0.05$) positive correlation was observed between the pH, TVB-N and two bacterial groups, *Pseudomonas* and *Pedobacter*. Based on the TVB-N assessment, the fillets began to decompose at the ninth day in the control samples and the twelfth day in the 0.05% AOB-treated samples. The relative abundance of *Pseudomonas*, *Janthinobacterium* and *Pedobacter* in the 0.05% AOB treatment group was lower than in the control group. Therefore, our results showed that treatment with 0.05% AOB improved the quality of fillets during the box-packaged storage.

Keywords: AOB, Sturgeon, Chilled-fillets storage, Microbiota composition, Quality changes

1. Introduction

Freshwater fish are characterised by loose tissue structure and high protein content. After being contaminated by microorganisms during processing, transportation and storage, the meat is easily oxidised, causing fish meat to spoil and deteriorate, gradually lose nutritional and commodity value, and shorten the shelf life (Thiansilakul *et al.*, 2011; Wang *et al.*, 2009). Microorganisms are the main cause of spoilage in fish meat. Fish bodies are initially contaminated by a variety of microorganisms, but only some of the bacteria are involved in the process of decay during storage. These specific spoilage organisms also produce spoilage odours or odour metabolites (Dalgaard, 1995). The composition of spoilage bacteria is complex and depends on the fish species, aquatic environment and storage conditions. Therefore, controlling the growth of spoilage microorganisms is especially important in food preservation.

Sturgeon is a high-protein, fatty fish, having high economic value. Sturgeon meat is thick and soft, and contains many amino acids essential for humans. However, because it is rich in unsaturated fatty acids and protein, sturgeon meat is prone to deterioration during transportation and storage (Benjakul and Sutthipan, 2009). To retard this process, there is an increasing amount of research being directed towards suitable preservatives: a gelatin–chitosan–rosemary extract composite film can significantly improve the quality of sturgeon meat (Chang *et al.*, 2019), and ascorbic acid and vacuum packaging can delay lipid oxidation and increase the shelf-life of sturgeon fillets (Rostamzad *et al.*, 2010). At present, the common methods of fish preservation mainly include preservation under air conditioned environments, low temperature preservation and nanotechnology application (Ceylan *et al.*, 2018; Meral *et al.*, 2019a, 2019b). Biopreservatives are derived from natural products; therefore they are safe, non-toxic and easily degradable. As a result, their popularity in fish preservation is gradually increasing.

Antioxidants of bamboo leaves (AOB) are extracted from bamboo leaves. Flavonoids, phenolic acids and lactones, the major components of AOB, have anti-oxidant, anti-microbial and anti-ulcer properties (Nirmala *et al.*, 2018). Antioxidants of bamboo leaves have different degrees of inhibition on pathogenic bacteria such as *Staphylococcus epidermidis*, *S. aureus*, *Bacillus cereus* and *Salmonella typhi* (Pang, 2017). As food additives, they can improve quality and colour. In China, AOB have been officially certified as natural antioxidants and can be used in edible oils, fish, puffed foods, meat products, baked products, fruit and vegetable juices, cereals and tea (Gong *et al.*, 2015; Lou *et al.*, 2005; Lu *et al.*, 2009; Sun and Ye, 2011; Zhang *et al.*, 2018). In recent years, the research on the effect of AOB on the preservation of aquatic products has gradually increased, and preservation effects on the large yellow croaker, sea bream, fish balls and prawns are remarkable (Guan *et al.*, 2017; Jiang *et al.*, 2013; Lin *et al.*, 2016; Wang *et al.*, 2013). Chitosan coatings incorporated with AOB and potassium sorbate could retard scallop nutrient loss by inhibiting lipid oxidation and hydrolysis (Wu *et al.*, 2019b). Pan *et al.* stated that bamboo leaf extracts had certain antioxidant and nitrosamine-inhibiting effects in cured foods (Pan *et al.*, 2019).

The objective of this study was to assess the impacts of AOB on the physicochemical and microbiological quality of refrigerated sturgeon fillets, thus providing a theoretical basis and technical support for the preservation of sturgeon meat.

2. Materials and methods

AOB preparation

AOB was purchased from Zhe Jiang Sheng Shi Biological Technology Co. Ltd (Model Name: AOB-1d). The flavonoid content was 20.8 g/100 g. Antioxidants of bamboo leaves were diluted in sterile water at concentrations of 0%, 0.05%, 0.10%, 0.15%, 0.20% (w/v) and then sterilised by filtration.

Preparation of sturgeon fillets, coating and storage

Fifteen live sturgeons with an average weight of 1,500±200 g and length of 60±10 cm were obtained from the Hui Shui research base of the Guizhou Fisheries Research Institute (Guizhou, China). Sturgeons were stunned, gutted, peeled, washed with sterile water and filleted. Fillets were divided into five groups containing 15 fish each. Five different treatments were used: (1) control (CK); (2) treated with a 0.05% AOB solution; (3) treated with a 0.10% AOB solution; (4) treated with a 0.15% AOB solution and (5) treated with a 0.20% AOB solution. The sturgeon fillets were immersed in their respective treatment solutions for 5 min at 4 °C, packed in sterile polyethylene boxes and stored at 4±1 °C. Triplicate samples were studied at days 0, 3, 6, 9 and 12.

pH value

The pH values were measured by using the method of Cai *et al.* with slight modifications (Cai *et al.*, 2014): 20 g of fish meat samples were mixed with 100 ml distilled water, stirred for 30 min and filtered. Analyses were performed in duplicate.

TVB-N measurement

Fish meat samples (20 g) were mixed with 100 ml distilled water, homogenised properly, left at room temperature for 30 min with regular stirring and finally filtered and saved in bottles. Total volatile basic nitrogen (TVB-N) was determined by using the method described by Ali (2009).

DNA extraction and PCR amplification of the 16S rRNA gene

Extraction of bacterial DNA followed the procedure of Huang *et al.* (2016). Polymerase chain reaction (PCR) amplicons were obtained from the V4 regions of the 16S rRNA gene. Sequencing primers for PCR amplification were 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806 R (5'-GGACTACHVGGGTWTCTAAT-3') with different barcodes. The PCR mixture (50 µl) contained 25 µl Master Mix, 4 µl Primer Cocktail, 2 µl DNA and 19 µl ddH2O. PCR was performed at 98 °C for 3 min, followed by 30 cycles of 98 °C for 45 s, 55 °C for 45 s, 72 °C for 45 s and a final extension step of 72 °C for 7 min. The PCR products from the three reactions were pooled, visualised using 1% agarose electrophoresis and purified with Qiaex II Gel Extraction Kit (Qiagen, Germany).

High-throughput sequencing on the Illumina HiSeq PE 250 platform

The library was sequenced on an IlluminaHiSeq 2500 platform (Novogene Technology Co., Ltd., Beijing, China) based on standard protocols. Operational taxonomic units (OTUs) were clustered with 97% similarity analysed by using UPARSE (version 7.0.1001). Good's coverage, Chao1, ACE, Simpson, Shannon, observed-species were computed by using QIIME (Version 1.7.0) and displayed with R software (Version 2.15.3).

3. Results and discussion

The effect of antioxidants on pH

The change of pH during the storage of fish is closely related to microbial metabolism, enzyme activity and accumulation of acidic substances, which is considered as an important indicator for evaluating the spoilage

and deterioration of fish. The pH value of all treatments gradually increased during the storage (Figure 1). Volatile bases produced by endogenous or microbial enzymes can probably increase the pH value (Riebroy *et al.*, 2007). Similar patterns in pH change have also been reported for sardine, sea bream and blue fish (Erkan *et al.*, 2011; Goulas and Kontominas, 2007; Özyurt *et al.*, 2012). However, another study has reported that pH at first decreased, and then increased during chilled storage of sturgeon treated with radish seed protein extract (Li *et al.*, 2018b). This was assumed to be affected by the seasons, diet, species, level of activity and post-mortem evolution of the flesh (Nie *et al.*, 2018). In this study, the initial pH of fish samples was about 6.40. This observation is in agreement with that of Nie *et al.*, who reported a pH value of 6.48 for fresh Japanese sea bass before treatment (Nie *et al.*, 2018). No significant differences were observed between the control and 0.05%, 0.10%, 0.15%, 0.20% AOB groups during the first 6 days. The pH values of 0.05%, 0.10% and 0.15% groups were significantly lower at 9 and 12 days than in the control samples. During the entire time of storage, the 0.05% and 0.10% samples exhibited the lowest pH levels.

Effect of antioxidant on TVB-N production

TVB-N is closely related to the freshness and the degree of spoilage of fish muscle; therefore, it serves as an important indicator for the assessment of freshness of fish (Cheng *et al.*, 2017). Overall, the TVB-N values increased during the storage (Figure 2). Significantly lower TVB-N values were recorded in 0.05% AOB samples as compared to the control till the end of the storage, suggesting that AOB has significant antimicrobial effect. TVB-N values in 0.05% AOB samples changed from 6.01 to 9.80 mg/100 g within the first 6 days, and gradually increased to 14.76 mg/100 g at day 9. TVB-N values in 0.10% AOB samples changed from 7.33 to 8.12 mg/100 g within the first 6 days, and gradually increased to 15.59 mg/100 g at day 9. The values of control, 0.15% and 0.20% AOB samples increased to 25.83, 28.92, 31.98 mg/100 g at day 9, thus exceeding the limits of acceptability for human consumption of freshwater fish in China (20 mg N/100 g) (Cheng *et al.*, 2017). The results showed that 0.05% and 0.10% AOB treatments effectively slowed down the increase of TVB-N during storage. This observation is in agreement with that of Lin *et al.*, who reported that AOB could inhibit the increase of TVB-N in *Litopenaeus vannamei* stored at 4±1 °C (Lin *et al.*, 2016). Wen *et al.* also reported similar findings, with the initial TVB-N values (7.30 mg/100 g) significantly increased without crossing the limit of acceptability in silver carp samples treated with chitosan coating + AOB during the chilled storage (Wen *et al.*, 2013). The present study shows that a high concentration of AOB could reduce the effect of inhibition and antioxidation to bacteria. This can probably be explained based on the oxidative rancidity caused by a large amount of AOB, which accelerated the deterioration of the product (Jiang *et al.*, 2011; Lin *et al.*, 2016).

Bacterial OTU clustering

We compared the composition of microbiota between the control and the 0.05% AOB treatment. Through reads splicing, an average of 62,365 tags were measured per sample. After quality filtering, the valid data amounted to 53,316 tags, with the effective rate of quality control being 85.42%. The sequences were clustered into 4,553 OTUs with the cut-off of 97% identity. Among this, 4,516 OTUs (99.19%) could be annotated through BLAST analysis against the Silva 132 rRNA gene database. The Good's coverage of all samples was greater than 0.98, suggesting that there was sufficient sampling of the bacterial communities. A total of 91.19% of all taxa could be annotated to the phylum level, and 37.71% to the genus level. The mean

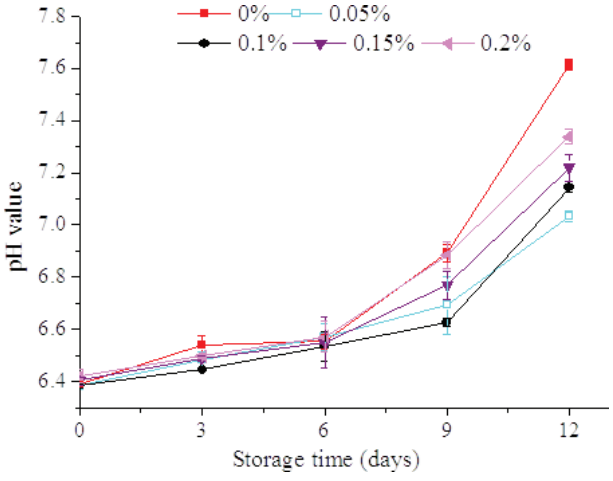

Figure 1. Changes in pH of sturgeon fillets during storage at 4 °C.

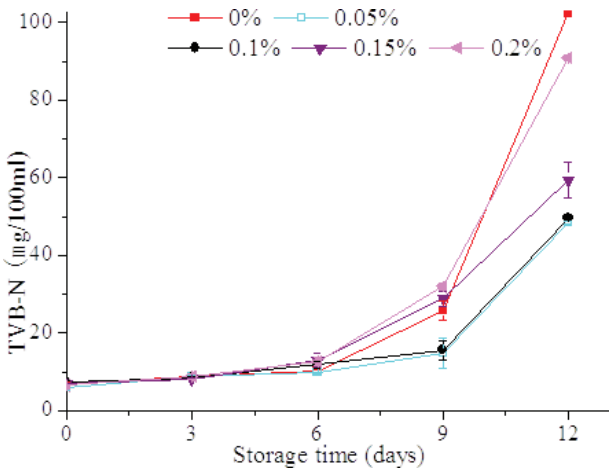

Figure 2. Changes in TVB-N contents of sturgeon fillets during storage at 4 °C.

Table 1. Comparison of alpha-diversity indices of the bacterial communities of six samples.

Sample	Observed species	Shannon	Simpson	Chao1	ACE	Good's coverage	OTUs
CK-0 d	2,128	7.476	0.966	2451.625	2590.673	0.985	3,188
CK-6 d	1,686	4.881	0.829	2169.136	2258.006	0.985	2,647
CK-12 d	1,708	5.404	0.875	2049.535	2234.787	0.985	2,777
0.05% AOB-0 d	1,849	7.768	0.983	2679.508	2338.469	0.986	3,062
0.05% AOB-6 d	1,605	5.005	0.842	2161.325	2275.242	0.984	2,601
0.05% AOB-12 d	1,492	4.616	0.829	1926.276	2053.445	0.986	2,374

OTU, operational taxonomic unit; AOB, antioxidants of bamboo leaves.
CK-0 d, CK-6 d and CK-12 d denote control samples at 0, 6 and 12 days. 0.05% AOB-0 d, 0.05% AOB-6 d, 0.05% AOB-12 d denote 0.05% AOB treatment samples at 0, 6 and 12 days.

Shannon index varied from 7.476 in the CK-0 d sample to 4.881, 5.404, 7.768, 5.005 and 4.616 on average in CK-6 d, CK-12 d, 0.05% AOB-0 d, 0.05% AOB-6 d and 0.05% AOB-12 d samples, respectively (Table 1). At 6d and 12d, alpha-diversity of the control samples was higher than that of the 0.05% AOB treatment group. Overall, the results show that treatment with AOB inhibited growth and reproduction of some microorganisms, and changed the microflora of sturgeon fillets during storage. At day 0, the alpha-diversity values of the 0.05% AOB treatment group (0.05% AOB-0 d) and control group (CK-0 d) samples were higher than those of other samples, which may have been caused by the low temperature storage environment inhibiting microbial growth (Wu *et al.*, 2019a).

NMDS analysis

Non-metric multi-dimensional scaling (NMDS) based on the Bray–Curtis distance of species abundance was used to study the structure of microbiota between the control and the 0.05% AOB treatment (Figure 3). The microbial composition of the control group was similar in the middle and late storage period (CK-6 d, CK-12 d), but different from that of the 0.05% AOB treatment group (0.05% AOB-6 d, 0.05% AOB-12 d), which could have been caused by the antimicrobial properties of AOB.

Microbiological analysis

The shelf life of fresh fish is affected by the initial bacterial population, and packaging materials (Singh *et al.*, 2018). For chilled stored fish, *Pseudomonas*, *Achromabacter*, *Flavobacterium* and *Moraxella* species are the major microorganisms responsible for aerobic spoilage (Ojagh *et al.*, 2010; Tavakoli *et al.*, 2018). However, the fish species, season and storage environment have important implications for the bacterial spoilage (Dalgaard *et al.*, 2003). At the genus level, the relative abundance was different in the control and 0.05% groups (Figure 4).

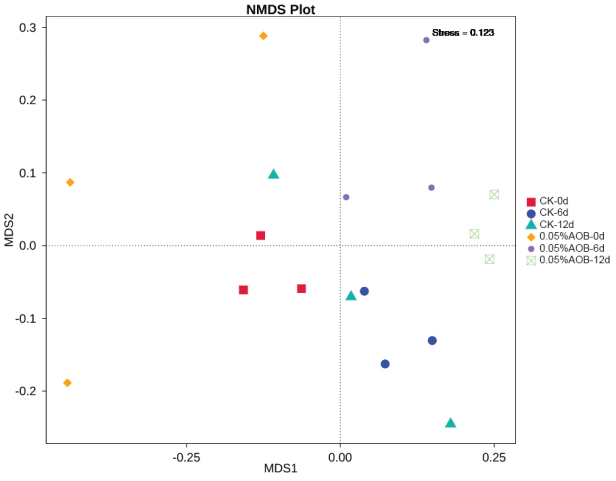

Figure 3. An NMDS plot with Bray–Curtis distances generated from species abundance data for different treatments.

At the beginning of storage, the main community components in the control sample (CK-0 d) included *Pseudomonas*, *Aeromonas*, unidentified *Clostridiales*, *Romboutsia*, *Janthinobacterium* and *Turicibacter*. *Pseudomonas* had the highest abundance of 15.46%; followed by *Aeromonas* (11.58%). In the control group CK-6 d, *Pseudomonas* and *Aeromonas* increased to 38.40% and 25.30%, respectively. Unidentified *Clostridiales* and *Romboutsia* decreased, and *Pedobacter* and *Janthinobacterium* increased. *Pseudomonas* was the most highly represented genus in the CK-12 samples (38.31%). *Janthinobacterium* and *Pedobacter* in CK-12 d increased to 13.17% and 5.97%, respectively, whereas *Aeromonas* decreased to 5.79%. In the early storage stage, the dominant taxa in the 0.05% AOB treatment group (0.05% AOB-0 d) included *Pseudomonas* (10.57%), *Aeromonas* (4.42%), *Phyllobacterium* (2.47%), *Acinetobacter* (2.08%), *Cetobacterium* (1.71%) and *Janthinobacterium* (1.71%). In the 0.05% AOB-3 d samples, *Aeromonas* had the highest abundance of 33.36%, followed by *Pseudomonas* (28.29%). In the 0.05% AOB-6 d samples, *Cetobacterium* and *Janthinobacterium*

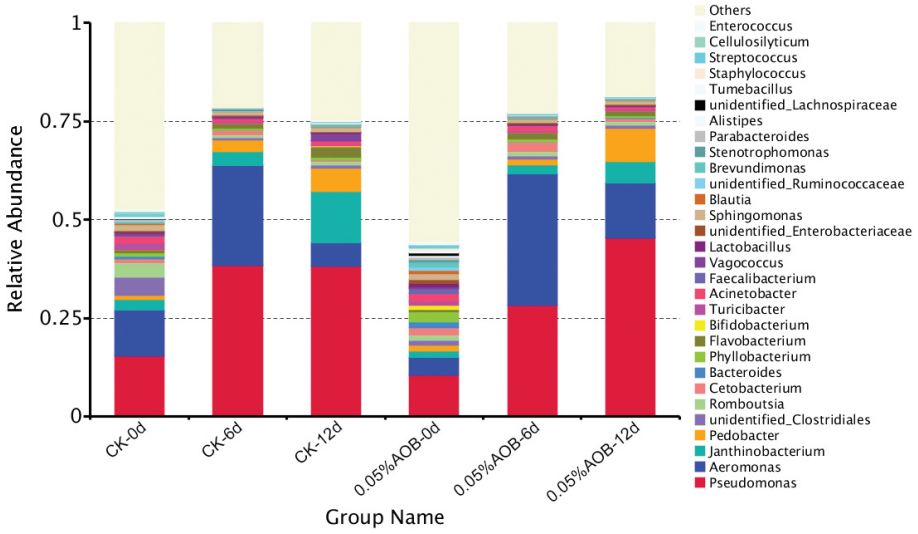

Figure 4. Bacterial distribution pattern at the genus level.

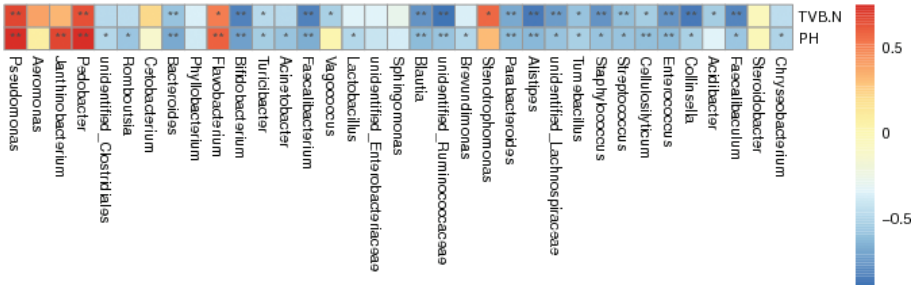

Figure 5. Spearman's rank correlation analysis heatmap.

increased to 2.51% and 2.34%, respectively. In the late storage stage, in the 0.05% AOB treatment (0.05% AOB-12 d) samples *Pseudomonas* had the highest abundance of 45.47%, *Janthinobacterium* and *Pedobacter* concentrations increased to 5.53% and 8.52% respectively, whereas *Aeromonas* decreased to 13.77%. The relative abundance of *Pseudomonas*, *Janthinobacterium* and *Pedobacter* in the 0.05% AOB treatment group was lower than that in the control group, which indicates that AOB can inhibit the growth of these three bacterial genera.

The microbiota of both control and 0.05% AOB treatment sturgeon fillets changed dramatically during the storage. *Pseudomonas* and *Aeromonas* were the dominant bacteria throughout the storage. This observation is in agreement with that of Liu *et al*. (2017). In previous studies, *Aeromonas* and *Pseudomonas* were identified as the main spoilage organism. Zhao *et al*. found that *Aeromonas veronii bv. veronii* is fairly efficient at producing TVB-N and putrescine (Zhao *et al.*, 2018). *Aeromonas sobria* was one of the dominant microorganisms isolated from spoiled Pacific white shrimp stored at 4 °C (Yang *et al.*, 2017). Huang *et al*. identified *Pseudomonas* as the main spoilage organism in the grass carp fillets treated with cinnamon bark oil (Huang *et al.*, 2017). At the end of the shelf life, *Pseudomonas* was the most common group in air-packed silver carp fillets (Li *et al.*, 2018a). In this study, the concentration of *Pseudomonas* increased gradually. We calculated the Spearman's rank correlation coefficient between the TVB-N, pH value and microbial community (Figure 5), and the results showed that *Pseudomonas* was positively correlated with TVB-N and pH. It can be proved that *Pseudomonas* plays an important role in the decay of sturgeon fillets.

Members of the genus *Janthinobacterium* are frequently found in freshwater (Zotta *et al.*, 2019). Møretrø detected *Janthinobacterium* spp. in salmon fillets stored for 10 days (Møretrø *et al.*, 2016). *Janthinobacterium* appeared in tilapia fillets at the end of the storage period (Duan *et al.*, 2018). In spoiled bighead carp meat samples, *Janthinobacterium* was the predominant genus (Liu *et al.*, 2018). In addition, *Janthinobacterium* can also be found in healthy Atlantic salmon (Wang *et al.*, 2018). The contributions of *Janthinobacterium* to the spoilage of cold stored fillets are unknown yet. In this article, *Janthinobacterium* was present throughout the entire storage period, but it proliferated greatly and became dominant at the end of the storage. The result of Spearman's rank correlation

analysis showed that *Janthinobacterium* was positively correlated with pH. However, its spoilage potential needs to be studied in the future.

Pedobacter was generally isolated from soils, water, compost, sludge, glaciers, chilled food, fish and other extreme environments (Viana *et al.*, 2018). *Pedobacter* could produce neutral phytases to improve nutrient digestibility and performance in crucian carp, whereas some *Pedobacter* are associated with soil or plant-pathogenic nematodes (Bahram *et al.*, 2018; Nie *et al.*, 2017). In freeze-dried *Agaricus bisporus*, *Pedobacter* was one of the dominant bacteria (Yang *et al.*, 2019). *Pedobacter* is prevalent in some fishes (Davis *et al.*, 2016; Rasheeda *et al.*, 2007), but there is no evidence to show that it is associated with the spoilage of fish. In this article, *Pedobacter* increased gradually, and it was positively correlated with pH and TVB-N. However, its spoilage potential needs to be studied in the future.

4. Conclusion

In this study, pH, TVB-N and microbial composition were detected in fresh sturgeon fillets after different AOB treatments. Compared with the control samples, 0.05% AOB treatment showed a better pH value, and a slower TVB-N increase. High-throughput sequencing analysis indicated that control samples and 0.05% AOB samples had a similar bacterial composition. *Pseudomonas* and *Aeromonas* dominated the indigenous bacterial flora at the beginning of storage, whereas *Pseudomonas*, *Aeromonas*, *Janthinobacterium* and *Pedobacter* dominated the flora at the end of the storage period. However, the relative abundance of *Pseudomonas*, *Janthinobacterium* and *Pedobacter* in the 0.05% AOB treatment group was lower than that in the control group. *Pseudomonas* and *Pedobacter* were positively correlated with pH and TVB-N. However, the spoilage potential of *Pedobacter* needs to be studied in the future.

Acknowledgements and Funding

This study was supported by the earmarked fund for Science and Technology Platform Construction Project of Guizhou Province (20165714), National Characteristic Fresh Water Fish Industrial Technology System (CARS-46), Guizhou Characteristic Aquatic Industrial Technology System (GZCYTX2013-01102) and Agricultural Development Project of Guizhou province (20182316).

Conflict of interest

The authors declare that there are no conflicts of interest with any financial organisation regarding the material discussed in this article.

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