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Effects of different salt concentrations and vacuum packaging on the shelf-stability of Russian sturgeon (*Acipenser gueldenstaedti*) stored at 4 °C

Yue-wen Chen^{a,b,*}, Wen-qiang Cai^{a,b}, Yu-gang Shi^{a,b}, Xiu-ping Dong^{c,d}, Fan Bai^e, Shi-ke Shen^{a,b}, Rui Jiao^{a,b}, Xiang-yu Zhang^{a,b}, Xuan Zhu^{a,b}

^a School of Food Science and Biotechnology, Zhejiang Gongshang University, Hangzhou, Zhejiang, 310035, China

^b Zhejiang Provincial Collaborative Innovation Center of Food Safety and Nutrition, Zhejiang Gongshang University, Hangzhou, Zhejiang, 310035, China

^c School of Food Science and Technology, Dalian Polytechnic University, Dalian, 116034, China

^d National Engineering Research Center of Seafood, Dalian, 116034, China

^e Quzhou Sturgeon Aquatic Food Technology Development Co. Ltd, Zhejiang, 324000, China

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ABSTRACT

The effects of different salt concentrations and vacuum packaging on the quality and microbiota dynamics of Russian sturgeon fillets stored at 4 °C were evaluated over a 12-day storage in this study. During refrigerated storage, The combination of 6% (w/v) brine salting for 2 h and vacuum packaging exerted beneficial effects on the quality of Russian sturgeon fillets, as demonstrated by affecting the sensory acceptability, retarding the production of total volatile basic nitrogen (TVB-N), delaying lipid oxidation measured by thiobarbituric acid reactive substances (TBARS) value. Meanwhile, the morphological changes in the muscle at different salt concentrations were observed by Scanning Electron Microscopy (SEM). Based on total viable counts (TVC), there was a lag phase of 3 days for the shelf life of 6% salting samples and vacuum packaging samples compared with the control. Moreover, the microbiota variability in different groups was investigated. The combination of 6% salting and vacuum packaging could effectively delay the growth of *Pseudomonas* which was the predominant microbiota in Russian sturgeon fillets during the final period of storage. The results indicated that 6% salting combined with vacuum packaging extended the shelf-life of Russian sturgeon fillets during storage, which was mainly due to their inhibition on dominant spoilage bacteria.

1. Introduction

China has already been the largest sturgeon producing country since 2000 and the production accounts for over 85% of the world (FAO, 2015; Wang et al., 2016; Wei, Zou, Li, & Li, 2011). Russian sturgeon accounts for 10% of the annual sturgeon production in China (Shen, Shi, Zou, Zhou, & Wei, 2014). However, compared to other fish commodities, sturgeon meat is highly perishable due to high moisture content and inevitable microbial activity by affecting the changes of pH, the generation of TVB-N, and the production of TBARS during storage (Fan, Luo, Yin, Bao, & Feng, 2014; Liu et al., 2017; Ozogul, Polata, & Ozogul, 2004; Shakhtour & Babji, 2013). The sturgeon processing plant normally marinates them for 12 h with 10% salt concentration to extend their shelf life. However, there is limited research about how to extend the shelf life of Russian sturgeon fillets.

Salting is a traditional process used for the fish preservation due to

decrease the water activity, and inhibit bacterial growth (Hong, Luo, Zhou, & Shen, 2012; Yanar, Celik, & Akamca, 2006). High concentrations of salts contacted with fish alter the balance of protein-protein interactions and promote fat oxidation (Jittinandana, Kenney, Slider, & Kiser, 2002). Medical studies show high-salt intake as an independent factor determining high blood pressure, and kidney problem (Liu, Xu, He, Wang, Hu, Li, & Jiang, 2017a). The pathway for the movement of salt ions from the brine into the cell is via diffusion caused by concentration gradient (Inguglia, Zhang, Burgess, Kerry, & Tiwari, 2017). However, NaCl migration is normally quite slow (Cárcel, Benedito, Bon, & Mulet, 2007; Gou, Comaposada, & Arnau, 2003). Thus it is meaningful to establish a suitable salt concentration and salting time under which the bacterial growth can be effectively inhibited and the quality changes of Russian sturgeon fillets are acceptable.

Oxygen in food packaging can reduce the shelf life of Russian sturgeon fillets by promoting lipid oxidation (Ozogul et al., 2004;

* Corresponding author. School of Food Science and Biotechnology, Zhejiang Gongshang University, Xiasha University Town, Xuezheng Str. 18, Hangzhou, 310018, China.

E-mail address: chenyw@zjgsu.edu.cn (Y.-w. Chen).

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Serdaroglu & Felekoglu, 2005). It has been proven that vacuum packaging could delay quality changes of fish products through reducing the deleterious effect of oxygen, and it has good market prospects due to consumer demands for lightly processed products (Manju, Mohan, Mallick, Ravishankar, & Srinivasa Gopal, 2008; Noordin, Shunmugam, & Huda, 2014). However, there is scarce information about the role of vacuum packaging in the microbiota changes of Russian sturgeon fillets.

In this study, the effects of different concentrations of salt and vacuum packaging are investigated on the quality alterations of Russian sturgeon fillets during storage at 4 °C including the physical properties, chemical quality and sensory acceptability, as well as microbiota composition. The morphological changes of the muscle after salting was observed using SEM.

2. Materials and methods

2.1. Sample preparation

Russian sturgeon (age of 3 years, weight of 3 ± 1 kg, length of 45 ± 5 cm) was obtained from Quzhou Sturgeon Aquatic Food Technology Development Co. Ltd, and transported to the laboratory in ice. After arrival, the fish was immediately filleted into steaks ($3 \text{ cm} \times 3 \text{ cm} \times 1 \text{ cm}$), and washed with sterile water. Then the fillets were randomly divided into five batches: control group (no treatment), T1 (fillets immersed in 2% salt solution (w/v) for 2 h), T2 (6% salt solution (w/v)), T3 (10% salt solution (w/v)), T4 (Vacuum packaging, vacuum degree 0.02 MPa). All samples were kept individually in polyethylene bags and stored in the refrigerator (4 °C) for 12 days. Three independent replicates were conducted for each group at 4-day intervals.

2.2. Measurement of salt content

Salt contents of Russian sturgeon fillets were estimated according to the procedures described by Hilderbrand (1991) with some modifications. Fish flesh (10 g) was homogenized with 90 mL of deionized water for 2 min by a homogenizer (Hand mixer 4192, De'Longhi Braun Household GmbH, Romania) at 500 rpm, and the solution was titrated with standard silver nitrate solution. The value was read from the burette. The salt content is calculated by the following equation: % Salt = $0.0355 \times V/m \times 100\%$. In this equation, 0.0355 means mass of chlorine equivalent to 1 mL of silver nitrate standard titration solution; V means the volume of silver nitrate standard titration solution consumed during titration of the sample solution; m means sample quality.

2.3. Sensory analysis

Sensory evaluation of the fish samples was done according to Zhang et al. (2017). Eight judges from the laboratory were trained to evaluate Russian sturgeon fillets for color, texture, odor, and overall acceptability, and scored from 1 to 10 points. The total scores less than 24 points were considered unacceptable.

2.4. Determination of pH

The pH was measured by the method described by Shi et al. (2019). Fish flesh (10 g) was homogenized with 90 mL of deionized water for 2 min, and then filtered with a qualitative filter paper. The pH of the filtrate was measured with a pH meter (Seven2Go pH meter, Mettler-Toledo Instrument Co., Ltd. China).

2.5. Measurement of color and textural characteristics

Changes in color of fish samples were determined as described by Wetterskog and Undeland (2004). Redness (a^*), lightness (L^*), and

yellowness (b^*) were measured by a colorimeter (Chroma Meter CR-400, Hangzhou Ke Sheng Instrument Co., Ltd. China), and then the data was calculated by the following equation:

$$W^* (\text{white}) = 100 - ((100 - L^*)^2 + b^{*2} + a^{*2})^{1/2}$$

Resilience was measured by a texture analyzer (TA.XT Express, Stable Micro System, UK) at a pressing distance of 5 mm and a speed of 2 mm/s (Cruz-Romero, Kerry, & Kelly, 2008).

2.6. Scanning electron microscopy analysis

The efficacy of salt on the morphological changes of Russian sturgeon fillets was carried out by scanning electron microscopy (SEM) as reported by Shi et al. (2019). The samples were cut into steaks of 0.5 cm^3 , and fixed with 2–3 mL 2.5% (v/v) glutaraldehyde at 4 °C for 3 d. Afterwards, washed three times with PBS (0.1 M, pH 7.0) for 0.5 h. Then used 1% OsO₄ to postfix for 1.5 h. Washed $\times 3$ with PBS for 0.5 h again. Fish samples were further dehydrated through gradient ethanol solutions (30%, 50%, 70%, 90%, 100%, 100%), coated with gold-palladium in Hitachi Model E-1010 ion sputter for 4–5 min Under critical point drying conditions, and then observed in Hitachi Model TM-1000 SEM (Hitachi, Tokyo, Japan).

2.7. Thiobarbituric acid reactive substances (TBARS) analysis

Thiobarbituric acid reactive substances (TBARS) value was estimated as described by Tarladgis, Watts, Yonathan, and Dugan (1960). 5 g of fish sample was homogenized and mixed with 25 mL trichloroacetic acid (7.5%, v/v). It was then filtered with a qualitative filter paper after shaking for 30 min. Added 5 mL thiobarbituric acid (0.02 mol/L) into the filtrate and immersed in a boiling water bath for 40 min. Then rinsed in cool distilled water for 10 min. The supernatant was harvested by centrifugation (5500 rpm, 4 °C) for 25 min. Read the optical density of the supernatant at a wavelength of 532 nm and 600 nm (UV-visible Spectrophotometer Model 2100, Unico, China). The malondialdehyde (MDA) content was calculated by the following equation:

TBA index (mg MDA/100 g meat) = $(A_{532 \text{ nm}} - A_{600 \text{ nm}}) / 155 \times (1/10) \times 72.06 \times 100$. In this formula, $A_{532 \text{ nm}}$, $A_{600 \text{ nm}}$ means the optical density of the supernatant read at 532 nm and 600 nm, respectively. Numerical value of 155 means the optical density of malondialdehyde solution (1 mmol/L) read at 532 nm. Numerical value of 72.06 means the molecular weight of malondialdehyde.

2.8. Total volatile base nitrogen (TVB-N) analysis

Total volatile base nitrogen (TVB-N) value was measured by the semi-micro kjeldahl method described by Hong et al. (2012). 5 g of minced sample was homogenized with 50 mL of the perchloric acid solution and stirred for 30 min. Then it was filtered and used for the measurement of TVB-N by a microtitration methodology.

2.9. Total viable counts (TVC) analysis

Total viable counts (TVC) was determined based on a method modified from Song, Liu, Shen, You, and Luo (2011). 10 g of fish sample was aseptically transferred to a stomacher bag, and then added 90 mL of 0.85% sterile normal saline and homogenized for 1 min by a stomacher blender (Clapping Stomacher SH-400A, Shanghai Hegong Scientific Instrument Co., Ltd. China). The homogeneous substance (0.1 mL) was dispersed on the surface of prepared plate count agar (PCA) plates after serially diluting in 0.85% sterile physiological saline (1:10), and incubated at 37 °C for 48 h (Townley & Lanier, 1981) to calculate total viable counts (TVC).

2.10. Characterization of the microbiota

2.10.1. Sample preparation and bacterial collection

Bacteria were collected from samples (Xu et al., 2018) which were prepared and randomly divided into 4 batches: Group A (no treatment), B (fillets immersed in 6% salt solution (w/v) with vacuum packaging), C (6% salt solution (w/v)), and D (Vacuum packaging). 10 g of Russian sturgeon samples were mixed with 90 mL of sterile normal saline (0.85%) and homogenized. Then the homogenate was centrifugated at 3000 rpm at 4 °C for 5 min. Bacterial cells were concentrated from the supernatant by centrifugation at 10000 rpm at 4 °C for 5 min.

2.10.2. DNA isolation and PCR amplification

Bacterial DNA was extracted using the MicroElute Genomic DNA Kit (D3096-01, Omega, Inc., USA) as reported by Jia et al. (2018). Bacterial 16S rRNA gene (V3–V4 regions) was amplified by PCR using primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACH-VGGGTWTCTAAT-3'). PCR reactions were done in triplicate in a 25 µL mixture that contained 12.5 µL Phusion® Hot Start Flex 2X Master Mixart 50 ng of template DNA, Version, 2.5 µL of each primer, and PCR-grade water. And it was performed in a L96G gradient PCR instrument (LongGene, China) with parameters setting described by Bao et al. (2018).

2.10.3. MiSeq sequencing and data processing

The PCR products were pooled and normalized according to standard protocols for sequencing by AxyPrep™ Mag PCR Normalizer (Axygen Biosciences, Union City, CA, USA). The OUT table was generated and used to select operational taxonomic units (OTUs) (Li & Godzik, 2006). Sequences were assigned to one unit when the similarity of OTUs is over 97% after using UCHIME to identify and remove the chimeric sequences. To reduce false positive rate, the singleton and low abundance were filtered which were controlled and confirmed by QIIME quality filters (version 1.17) (Bokulich et al., 2013). A heatmap was created with MeV 4.9.0.

2.11. Statistical analysis

The different analyses of Russian sturgeon samples were performed in triplicate and reported as the means \pm SD. Differences among storage time were performed on variance and Duncan's test using SPSS (version 20.0) for multiple comparisons. The value of $p < 0.05$ was considered in interpreting the significance of the difference.

3. Results and discussion

3.1. Salt content analysis

The different salt contents have a pronounced influence on the sensory quality, the microbial growth and the shelf life of the product (Kose & Hall, 2010). The salt content of the control group was $0.03 \pm 0.01\%$ (results are not shown). After 2 h of salting, the salt contents of T1, T2, T3 groups reached 0.75%, 2.18% and 3.87%, respectively, and then increased slowly (Table 1). The change of salt contents between different samples showed significantly different ($P < 0.05$). As salting time increased, protein denatures, cross-linking and shrinks which causes water loss from the muscle (Brás & Costa, 2010). These results indicated that 2 h of salting was more suitable for storage of Russian sturgeon fillets.

3.2. Sensory analysis

The sensory changes of Russian sturgeon fillets with different treatments were shown in Fig. 1. The fillets were considered as acceptable before the overall acceptability score reached 24 points (Zhang et al., 2017). The overall acceptability attribute score decreased

Table 1

Changes in the salt content of Russian sturgeon fillets treated with different salt concentrations and different salting duration.

Duration of salting (h)	Salt content (%)		
	T1	T2	T3
1	0.67 ± 0.01^a	1.92 ± 0.02^a	3.79 ± 0.03^a
2	0.75 ± 0.04^{ab}	2.18 ± 0.10^{ab}	3.87 ± 0.06^a
3	0.83 ± 0.05^{bc}	2.35 ± 0.08^{bc}	3.93 ± 0.11^a
4	0.90 ± 0.04^c	2.61 ± 0.11^c	4.43 ± 0.07^b
5	0.77 ± 0.02^{abc}	2.27 ± 0.05^b	4.27 ± 0.13^b

The different superscripts (āc) in the column denote significant differences ($p < 0.05$).

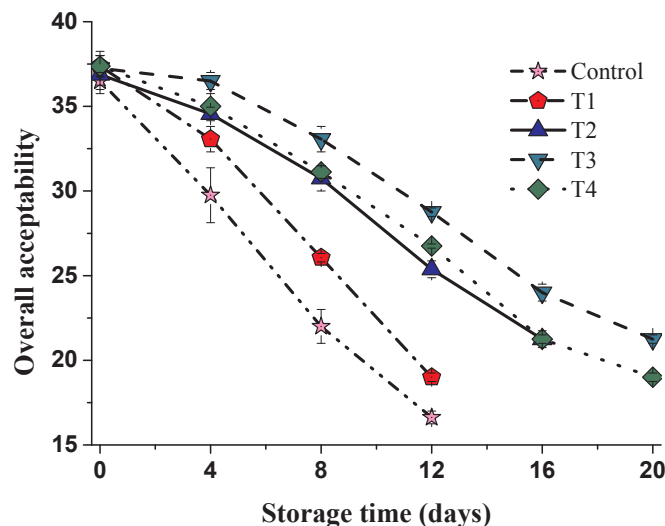


Fig. 1. Effect of different salt concentrations and vacuum packaging on the sensory quality of Russian sturgeon during storage at 4 °C. Control: no treatment; T1: 2% salt solution (w/v); T2: 6% salt solution (w/v); T3: 10% salt solution (w/v); T4: vacuum packaging. The bars indicated standard deviations.

significantly with increasing storage time in all samples. The shelf life of the control group was 7 d, and 9 d for T1 group due to sensory scores. There were no significant differences in the sensory scores between T2 and T4 groups, which still kept score of 21.25 at 16 d. As the salt concentration increased, the rate of decline in sensory scores had a greater slowdown. Similar findings have been also observed by Zhang, Qin, Luo, and Shen (2015). The efficacy of vacuum packaging can be attributed to the inhibition of microbial growth in samples (Gui et al., 2014).

3.3. Changes in the pH

The initial pH value was 6.32 for the control group, which was higher than that for T1, T2, T3 groups at 6.15, 6.18, 6.17 respectively (Fig. 2). This difference might be attributable to the formation of hydron and an unionized protein chloride caused by diffusion between haemoglobin solutions and salt solutions (Adair, 1928). The pH values decreased initially and then increased in different groups. This trend was due to the decomposition of glycogen in early stage (Song et al., 2011), and the production of volatile basic components by microbial proteolysis in late period (Goulas & Kontominas, 2005; Ocaño-Higuera et al., 2009). The pH changes of T2 and T4 groups were much lower than the control group during storage due to the inhibition of bacterial growth and the reduction in fat oxidation (Deumier, Trystram, Collignan, Guédider, & Bohuon, 2003; Remya, Mohan, Venkateshwarlu, Sivaraman, & Ravishankar, 2017).

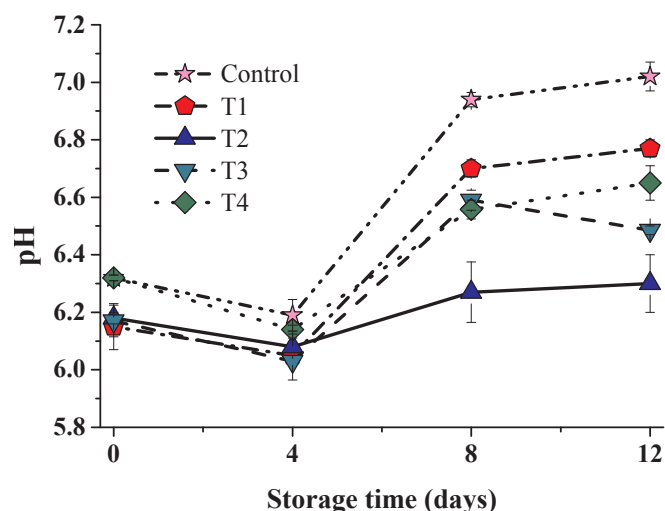


Fig. 2. Effect of different salt concentrations and vacuum packaging on the pH of Russian sturgeon during storage at 4 °C. Control: no treatment; T1: 2% salt solution (w/v); T2: 6% salt solution (w/v); T3: 10% salt solution (w/v); T4: vacuum packaging. The bars indicated standard deviations.

3.4. Color and springiness

The external quality criteria such as color and texture are related to the physiological, biochemical and microbiological changes of the muscle (Ruiz-Capillas & Moral, 2001). The changes in springiness and color for Russian Sturgeon fillets are shown in Table 2. Springiness is an important textural characteristic of meat, connected with muscle structure and biochemical components (Xiong et al., 2014). There was no significant difference in the initial physical characteristics changes of samples with increasing salt concentration, and T2 and T4 groups showed higher W^* values than the control group, indicating that salt mainly played a role in storage. The values of b^* were detected with a gradual increase at the beginning, and then decreased rapidly with increasing salt concentration. It showed the same tendency as TBARS values. The yellowness (b^*) has a close relationship with lipid oxidation (Hong et al., 2012).

3.5. Effect of salting on Russian Sturgeon muscle morphology

The microstructure alternations of Russian Sturgeon muscle when treated with different salt concentrations were investigated by SEM analysis to gain a better understanding of the effect of salt, and the fiber surfaces fractured perpendicular to the fiber axes were showed in Fig. 3. Untreated sample (Fig. 3(A)) contained muscle bundle wrapped in the perimysium, and the fibers wrapped in the endomysium. The perimysium and endomysium of muscle after salting (2% w/v NaCl) were separated with the muscle bundle and fibers respectively and form filamentous segments (Fig. 3(B)). As salt concentration increases, the

perimysium and endomysium were damaged severely (Fig. 3(C)). Besides, the muscle bundle and interfiber spaces became tighter (Fig. 3(C) and (D)). These results suggested that the structure of muscle was damaged by salting and positively correlated with salt concentration. Fox, Rorer, Fiddler, Carroll, and Wasserman (1980) reported the efficacy of nitrite and chloride on the microstructure of meat and showed similar results.

3.6. Lipid oxidation

Oxidative rancidity in Russian sturgeon fillets is a major quality problem during chilled storage because of the high content of polyunsaturated fatty acids (Ashie, Smith, & Simpson, 1996). TBARS value is a measure of oxidative rancidity and defines the second step of lipid peroxidation (Pérez-Villarreal & Howgate, 1991). The TBARS values in fish above 0.2 mg MDA/100 g meat are considered as the deterioration (Connell, 1990). The initial values of TBARS ranged from 0.01 to 0.03 mg MDA/100 g meat (Fig. 4). There was a significant difference between control, T2 and T3 groups at the end of storage, and the TBARS value in the T3 group reached 0.2 mg MDA/100 g meat on the 8th day. The results indicated that salting promoted lipid oxidation, which was consistent with pH and structure changes. It may be due to the effect of chloride ion and decreased activity of antioxidant enzymes (Vaz-pires, Capell, & Kirby, 1994).

3.7. Changes in TVB-N

TVB-N values are commonly measured to evaluate the chemical spoilage of fish, and an upper limit of 20 mg nitrogen/100 g meat is suggested (Kolodziejewska, Niecikowska, Januszewska, & Sikorski, 2002; Lu, Liu, Ye, Wei, & Liu, 2009). It was attributable to the autolytic degradation of free amino acids and nucleotides (Gill, 1990). The TVB-N values of samples increased over storage time and showed significant differences after 8 days (Fig. 5). At day 12, the TVB-N content in control group (22.70 mg nitrogen/100 g meat) was significantly higher than T1, T2, T3, and T4 groups (18.73 mg nitrogen/100 g meat, 11.47 mg nitrogen/100 g meat, 9.20 mg nitrogen/100 g meat, and 12.73 mg nitrogen/100 g meat respectively). These results revealed that salting (6% and 10% w/v NaCl) and vacuum packaging can keep the TVB-N values in Russian Sturgeon fillets at a relatively low level.

3.8. Changes in total viable counts (TVC)

The microbiological limit proposed by ICMSF (1986) for human consumption is 7 lg CFU/g in aerobic plate count analysis. The initial TVC for all samples (3–4 lg CFU/g) revealed the good quality of Russian sturgeon fillets (Fig. 6). The decreasing of TVC in T2 and T3 groups during the first 4 days was caused by the inhibitory action of NaCl on the spoilage bacteria (Hong et al., 2012). T2 (6.57 lg CFU/g), T3 (4.74 lg CFU/g) and T4 (7.62 lg CFU/g) groups showed a lower TVC than control (8.90 lg CFU/g) and T1 (8.10 lg CFU/g) groups after storing for

Table 2
Changes in the color and springiness of Russian sturgeon fillets during storage at 4 °C.

Parameter	Days of storage	Control	T1	T2	T3	T4
W^*	0	66.76 ± 1.35 ^a	62.67 ± 4.71 ^a	62.75 ± 6.00 ^a	64.67 ± 0.50 ^a	66.76 ± 1.35 ^a
	4	55.36 ± 2.03 ^{ac}	54.48 ± 0.47 ^{ac}	51.30 ± 0.89 ^a	46.19 ± 0.38 ^b	58.83 ± 1.91 ^c
	8	37.50 ± 0.99 ^a	56.58 ± 0.58 ^b	48.17 ± 0.01 ^{cd}	44.73 ± 0.41 ^c	49.60 ± 2.22 ^d
	12	35.52 ± 0.43 ^a	54.33 ± 2.21 ^b	47.26 ± 0.88 ^c	46.32 ± 0.46 ^c	37.52 ± 1.02 ^a
b^*	0	21.10 ± 1.33 ^a	21.73 ± 1.25 ^a	24.92 ± 1.62 ^b	25.06 ± 0.26 ^b	21.10 ± 1.33 ^a
	4	20.07 ± 0.74 ^c	17.53 ± 1.58 ^b	10.51 ± 1.81 ^a	9.84 ± 0.87 ^a	21.15 ± 0.58 ^c
	8	17.28 ± 2.98 ^c	12.35 ± 1.69 ^b	8.80 ± 0.73 ^a	7.07 ± 1.41 ^a	18.77 ± 1.66 ^c
	12	13.88 ± 1.36 ^{bc}	10.24 ± 2.14 ^b	7.95 ± 0.74 ^b	5.08 ± 0.36 ^a	15.38 ± 1.08 ^c
Springiness	0	0.95 ± 0.05 ^{ab}	0.81 ± 0.05 ^a	0.83 ± 0.04 ^{ab}	0.92 ± 0.02 ^{ab}	0.98 ± 0.04 ^b

The different superscripts (ad) in the column denote significant differences ($p < 0.05$).

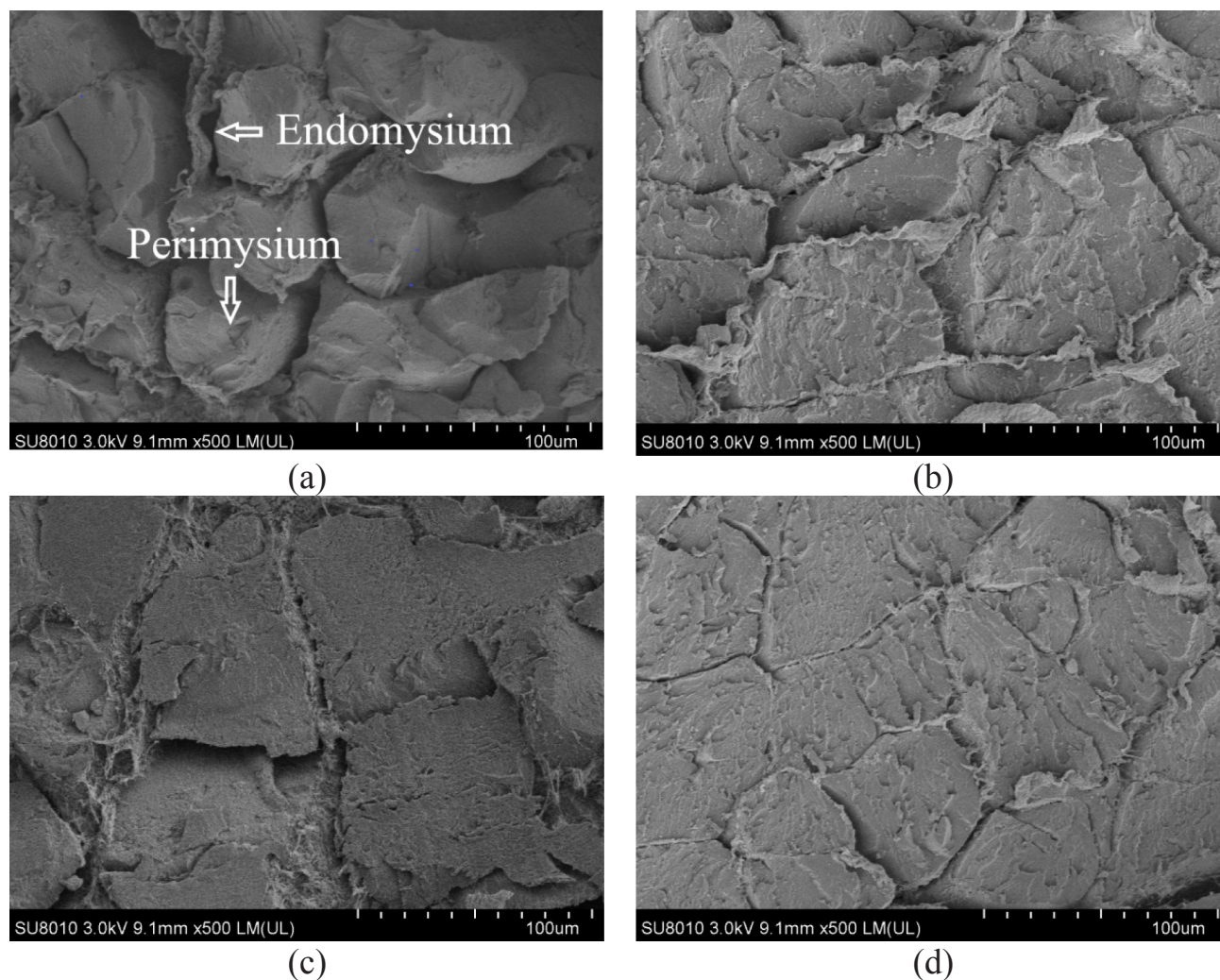


Fig. 3. SEM of Russian sturgeon muscle treated with different salt concentrations. (a): no treatment; (b): 2% salt solution (w/v); (c): 6% salt solution (w/v); (d): 10% salt solution (w/v).

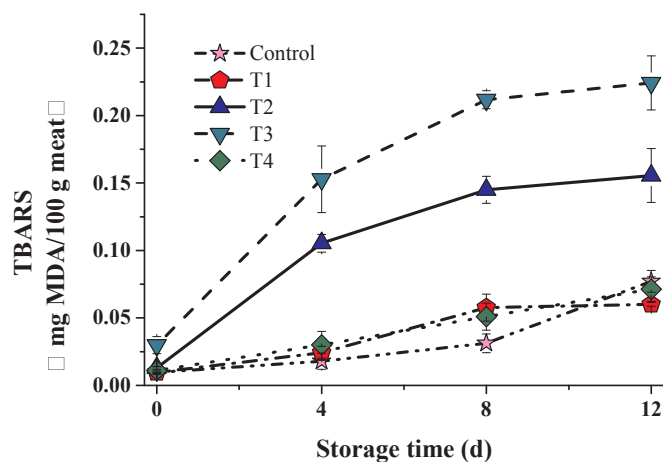


Fig. 4. Effect of different salt concentrations and vacuum packaging on the TBARS of Russian sturgeon during storage at 4 °C. Control: no treatment; T1: 2% salt solution (w/v); T2: 6% salt solution (w/v); T3: 10% salt solution (w/v); T4: vacuum packaging. The bars indicated standard deviations.

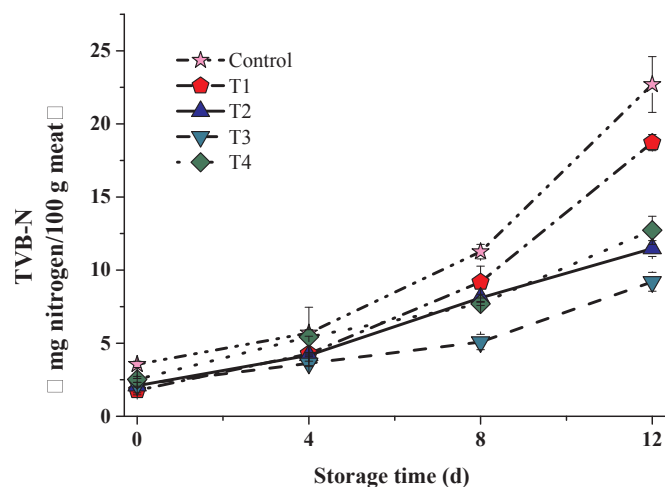


Fig. 5. Effect of different salt concentrations and vacuum packaging on the TVB-N of Russian sturgeon during storage at 4 °C. Control: no treatment; T1: 2% salt solution (w/v); T2: 6% salt solution (w/v); T3: 10% salt solution (w/v); T4: vacuum packaging. The bars indicated standard deviations.

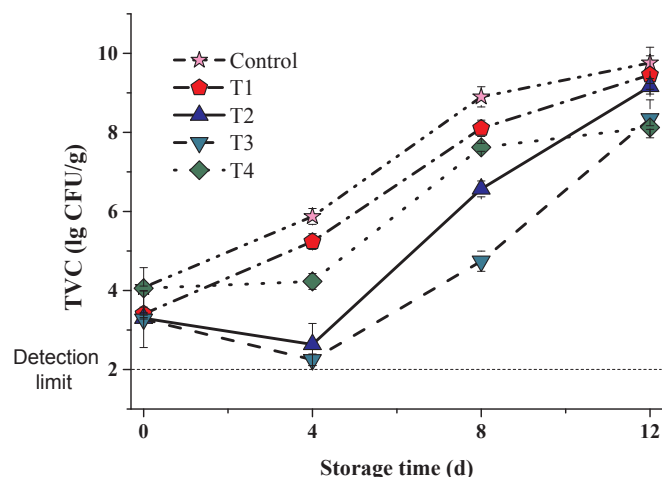


Fig. 6. Effect of different salt concentrations and vacuum packaging on the TVC of Russian sturgeon during storage at 4 °C. Control: no treatment; T1: 2% salt solution (w/v); T2: 6% salt solution (w/v); T3: 10% salt solution (w/v); T4: vacuum packaging. The bars indicated standard deviations.

8 days, indicating that salting and vacuum packaging can restrain microbial growth and extend preservation of Russian sturgeon.

3.9. Microbiota analysis

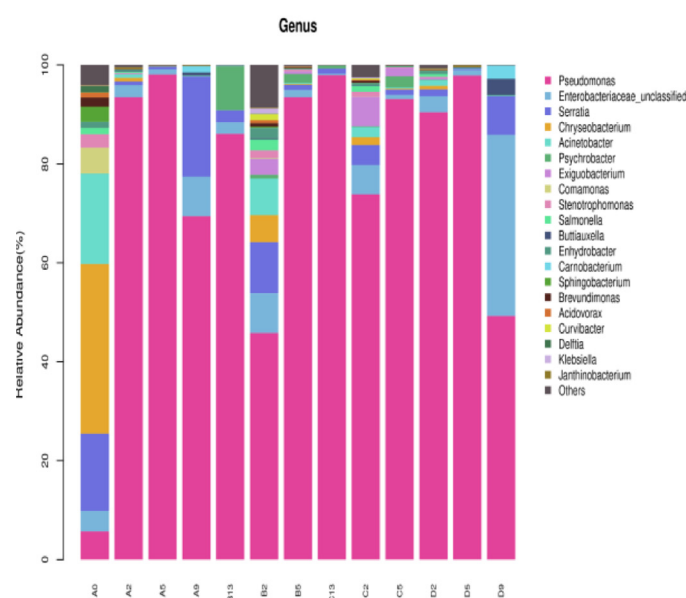
The high-throughput sequencing was used to detect microbiota composition. Fig. 7a showed the relative abundances of the top 20 genera for different storage periods. The bacterial diversity decreased with increasing time. The most abundant genera at the initial storage were *Chryseobacterium* (34.3%), *Acinetobacter* (18.2%), *Serratia* (15.63%), and *Pseudomonas* (5.69%). Compared with the A and D groups in which *Pseudomonas* (93.5%, and 90.5%, respectively) dominated the microbiota at day 2, *Chryseobacterium*, *Acinetobacter*, *Serratia*

still remained a fraction of the microbiota in the B and C groups. A high proportion of *Pseudomonas* (more than 90%) was detected from all samples by high-throughput sequencing after 5 days of storage. However, the relative abundances of *Serratia* and *Enterobacteriaceae* increased to 20.3% and 36.6% in the A and D groups at day 9, respectively, and the TVC was higher than 7.00 lg (CFU/g) (data not shown). Furthermore, the relative abundance of *Pseudomonas* still dominated the microbiota in the B and C groups at day 13. These results revealed the inhibitory effect of salting and vacuum packaging on the growth of *Pseudomonas* which was the predominant bacterial flora during the final storage of Russian sturgeon fillets.

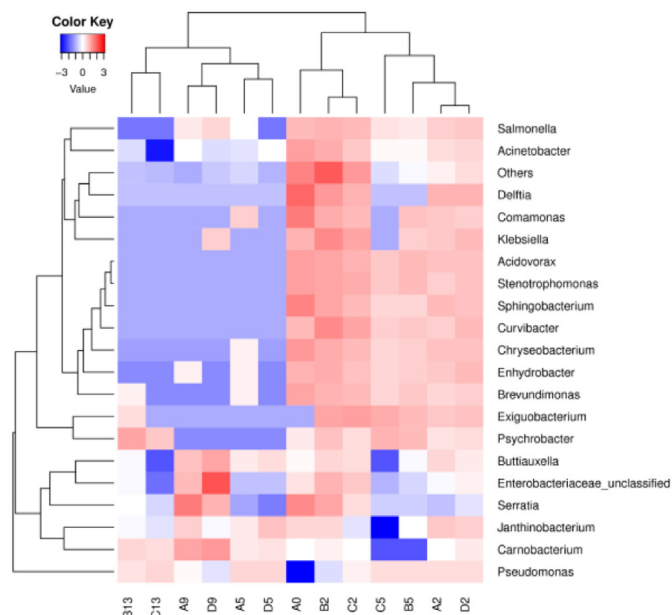
The microbiota composition in different samples at different times of storage was analyzed by constructing heat-map (Fig. 7b). In the heat-map, different genus-level phylotypes were represented by rows, and different Russian sturgeon samples were represented by columns (Jittinandana et al., 2002). The abundance of genera could be observed by the color intensity in each sample. The diversity of microbial was highest at the initial time according to the heat-map. With increasing time, the communities of microbiota became less diverse, and the relative abundances of *Chryseobacterium*, *Acinetobacter*, and *Serratia* decreased, while *Pseudomonas* became the predominant bacterial group.

4. Conclusions

The present work investigated the effect of salting and vacuum packaging on storage of Russian sturgeon fillets. 2 h of salting was more suitable, which maximized the salt concentration in fish muscle without causing serious damage. 6% salt concentration and vacuum packaging can inhibit bacterial growth, maintain or even improve the sensory quality, and delay chemical changes without affecting its lipid oxidation to prolong the shelf life of Russian Sturgeon fillets. In addition, the combination of salting and vacuum packaging exhibited significant growth inhibitory of *Pseudomonas*. More broadly, we expect this study to provide a scientific basis for optimization of salting conditions and packing in sturgeon farms and promote the development of Russian sturgeon products.



(a)



(b)

Fig. 7. Composition of microbiota (a), Heat-map on the genus level (b) in Russian sturgeon fillets during storage at 4 °C. Group A: no treatment; B: 6% salt solution (w/v) with vacuum packaging; C: 6% salt solution (w/v); D: Vacuum packaging. The digital represented days of storage.

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Abbreviation

pH	Potential of hydrogen
TBARS	Thiobarbituric acid reactive substances
SEM	Scanning electron microscopy
TVB-N	Total volatile base nitrogen
TVC	Total viable counts
MDA	Malondialdehyde

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